5th World Congress on Electroporation
& Pulsed Electric Fields in Biology, Medicine
Food and Environmental Technologies
Rome - Italy 15-19 September 2024

5th World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine, and Food & Environmental Technologies

Rome, Italy
September 15-19, 2024

Organised by
The International Society for Electroporation-Based Technologies and Treatments
and
Sapienza University of Rome,
Faculty of Civil and Industrial Engineering

Programme and Book of Abstracts

5^{th} World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine, and Food & Environmental Technologies

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Programme and Book of Abstracts

Organised by:

The International Society for Electroporation-Based Technologies and Treatments

&

Sapienza University of Rome, Faculty of Civil and Industrial Engineering

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Caterina Merla, Francesca Apollonio and Samo Mahnič-Kalamiza

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Welcome Notes

Dear Colleagues and Friends,

Welcome to the 5th World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine, Food, and Environmental Technologies!

We are thrilled to be part of this unique platform that brings together experts from diverse disciplines—biologists, medical professionals, engineers, and physicists—all united by a common goal: advancing the science of electroporation. This multidisciplinary approach is essential as we tackle critical challenges in areas such as cancer therapy, cardiovascular interventions, angiological malformations, DNA-based vaccines, and the development of innovative treatments. Beyond healthcare, our discussions will extend to pioneering applications in food technology, biotechnology, and environmental sustainability. Electroporation's versatile capabilities offer new ways to enhance food safety and processing, optimize biotechnological processes, and address environmental challenges, demonstrating its profound impact on creating a better, more sustainable future. By exploring these diverse applications, we aim to unlock the full potential of electroporation, advancing science and technology for the benefit of society and the planet.

This 5th edition brings several exciting features, including: i) the Industry Panel bridging the gap between academia and industry, with a focus on cardiac ablation using Pulsed Field Ablation; ii) the 3-Minute Thesis competition, where Ph.D. students present their thesis in three minutes in a clear and engaging way with the goal to enhance communication and presentation skills for a non-specialist audience; iii) the Senior2YS Meeting Corner, offering a unique opportunity for emerging researchers to connect with senior experts and industry leaders in the field of electroporation.

The Educational Session, now in its second edition, proves to be a valuable part of the conference; newcomers to the field may find prior to the Congress useful and worthwhile introduction to electroporation and pulsed field technology, given by great lecturers.

None of this would have been possible without the invaluable support of our sponsors, exhibitors, foundations, local and international committees, and, finally, the dedicated work of the scientific program committee. Each of these groups has played a crucial role in making this event a success. We are profoundly grateful for their support, that have made possible to realize all the initiatives and in particular the 34 Travel Awards for students and prizes for the best oral and poster presentations, recognizing outstanding work from both students and young scientists.

It goes without saying that we extend our gratitude to all participants for their contributions and enthusiasm. The future will undoubtedly benefit from the work done here, as well as the new ideas and collaborations that will emerge from this congress.

Welcome to Rome!

The Local Organizing Committee,

The Congress Chairs: Francesca Apollonio and Caterina Merla

General Information

Congress website
wc2024.electroporation.net

Congress venue
Faculty of Civil and Industrial Engineering
Sapienza University of Rome
Via Eudossiana 18, 00184 Rome, Italy

Congress Secretariat
Ega Worldwide congresses&events
Viale Tiziano 19,00196 Rome, Italy
mail: secretariat.wce2024@ega.it

Badges
The congress name badges must be worn during the congress. Access to the congress will not be granted without a name badge issued by the congress secretariat.

Certificate of Attendance
A certificate of attendance will be sent to participants by email after the conference.

Information for speakers
Guidelines for oral and poster presentation are available to download from the congress web-site. ***Important:*** **personal laptops cannot be used for presentations.**
At the end of the congress, all presentations will be deleted to ensure that no copyright issues will arise.

Wi-Fi
Free access to the Wi-Fi at the congress venue is provided. Personal access code will be given to each participant.

Journal
We have made an agreement with **Bioelectricity,** the official ISEBTT journal, for a special issue dedicated to the conference, more information will be provided on the congress web-site.
Further special issues are foreseen nearly 6 months after the end of the conference and info will be provided to the congress presenters by email and on the congress web-site.

Social Events

Welcome Ceremony

Date: Sunday, 15 September 2024
Time: 17:30-21:00 (see schedule below)
Places: San Pietro in Vincoli Basilica and Faculty of Civil and Industrial Engineering, University Sapienza of Rome

All the congress participants are invited to join the Welcome Ceremony and the Welcome Reception. The Welcome Ceremony will start with the welcome of authorities and will end with a vibrant string concert by the “Kepos” quartet.
After the concert, a welcome cocktail will be served in the Cloister of the Faculty.

Tours of Rome

Dates: Monday 16 and Tuesday 17 September 2024
Time: at the end of the Scientific Sessions
Duration: 1h30”
Departure place: Faculty of Civil and Industrial Engineering, University Sapienza of Rome

All the congress participant registered to this event are invited to reach the departure point a 18:00 and enjoy the visit of the most beautiful places of the ancient Rome and the wonderful company of colleagues from all over the world.

YS Night

Date: Monday, 16 September 2024
Time: 18:30-22:30
Place: The Sanctuary
Via delle Terme di Traiano, 4

All the students post-docs, and early carrier researchers registered to the congress are invited to reach the YS Night and enjoy a drink and glass of wine with food altogether in a very nice place in the heart of Rome. It will be great occasion to meet each other and to make new friendships.

Senior2YS Meeting Corner

Date: Tuesday 17 September 2024
Time: 18:30-20:00
Place: Faculty of Civil and Industrial Engineering, University Sapienza of Rome

This event offers an exceptional opportunity for young scientists to connect with senior researchers and industry leaders in the field, gaining advice from experts, while enjoying a fresh free beer!

Congress Dinner

Date: Wednesday, 18 September 2024
Time: 19:30-24:00
Places: Palazzo Brancaccio
Viale del Monte Oppio, 7

Join the Congress Dinner in the magnificent location of Palazzo Brancaccio. An Italian style menu will be served accompanied by excellent Italian wine. After the dinner there will be a DJ set, music and dancing.
N.B. If you have not yet purchased a congress dinner ticket (25 euros), you can do it at the registration desk by Monday 16. (subject to availability).

Floor Plan: First Floor

FIRST FLOOR

- Registration
- Speaker Ready Room
- Catering Area
- Scientific Sessions Halls
- Stand
- Lift
- Board Meeting Room
- Branded Panel
- Toilet

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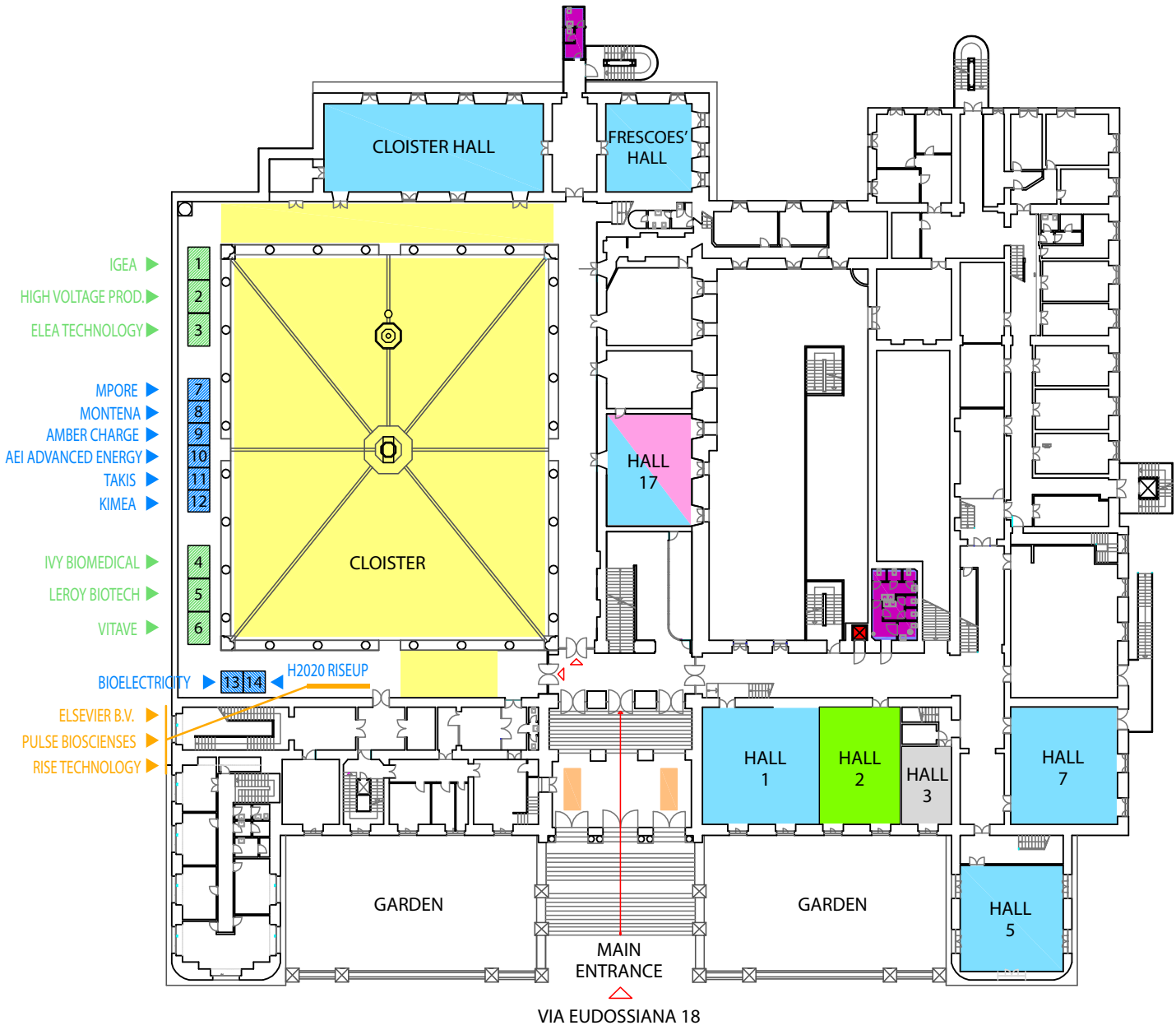

Floor Plan: Second Floor

SECOND FLOOR

Scientific Sessions Rooms

Poster Area

Social Space

Catering Area

Lift

Toilet

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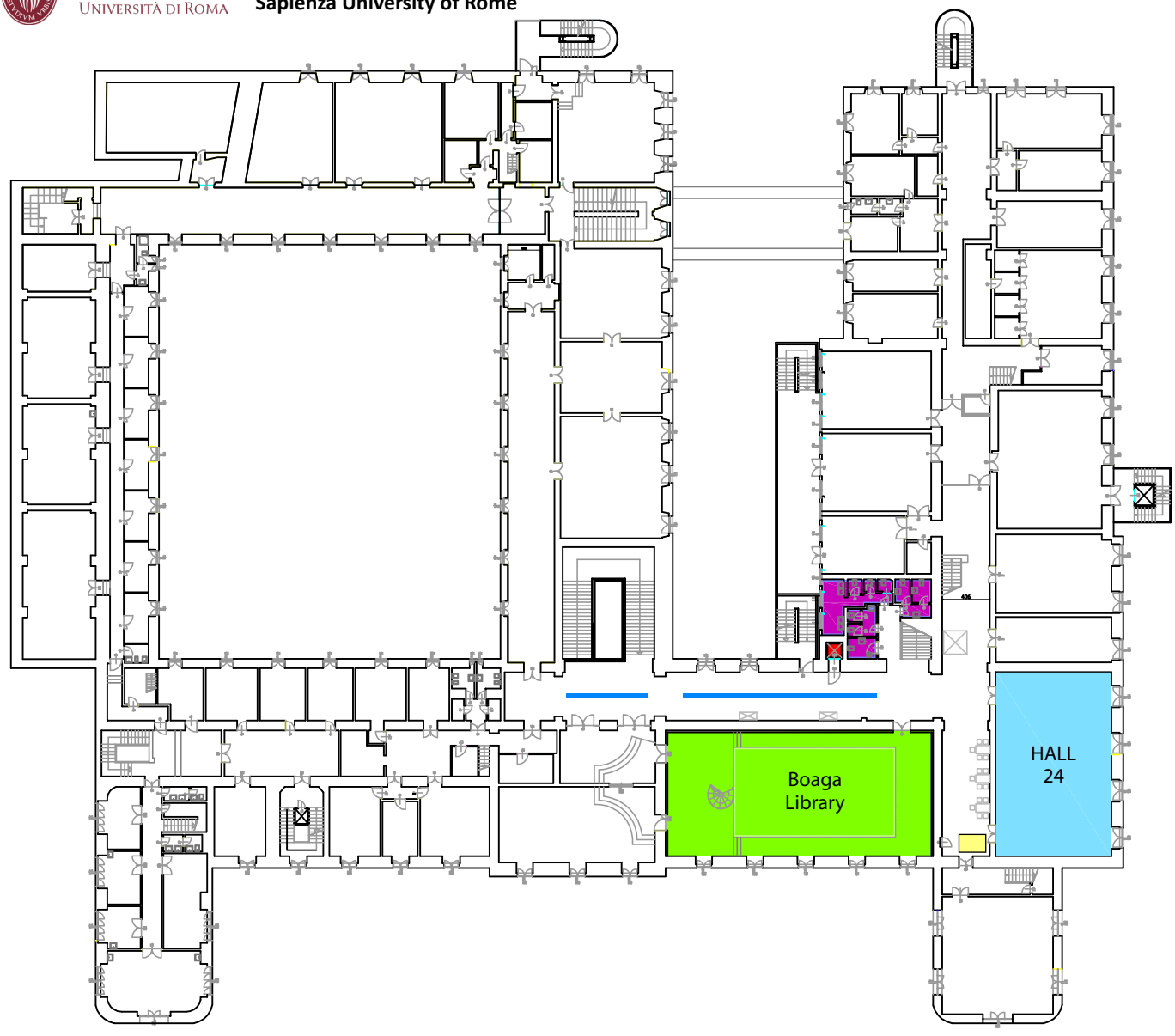

Programme Overview

	SUNDAY 15		MONDAY 16	TUESDAY 17	WEDNESDAY 18	THURSDAY 19
7:30-18:15	*Registration (12:00 - 17:00)*		*Registration*	*Registration*	*Registration*	*Registration (07:30 -17:00)*
8:30-8:50			Opening Session	**Plenaries: Hall_1**	**Plenaries: Hall_1**	Hall_1: S24 Hall_5: S07 Hall_7: P12 Cloister Hall: S12
8:50-9:50			**Plenaries: Hall_1** *Chairs: R. Davalos, E. Signori* A. Pakhomov S. Katsuki	*Chairs: M.P. Rols, R. Cadossi* A. Ivorra L. Rems V. Reddy	*Chairs: S. Mahnič-Kalamiza, J.* I. Oey R. Soliva J. Impellizeri	
9:50-10:00						
10:00-10:10			COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK
10:10-10:30		ISEBTT Council (Hall_17)				
10:30-10:40						
10:40-12:00			Hall_1: S21 Hall_7: P05 Hall_17: S05 Cloister Hall: S12	Hall_1: P13 Hall_5: S20 Hall_7: P05 Cloister Hall: P01	Hall_1: P07 Hall_5: S18 Hall_7: S16 Hall_17: S17 Cloister Hall: S04	**Plenaries: Hall_1** *Chairs: M. Cadossi, L.M. Mir* R. Heller F. Deschamps W. Wohlgemuth
12:00-12:10						
12:10-13:00	LUNCH		Congress LUNCH	Congress LUNCH	Congress LUNCH	Congress LUNCH
13:00-13:10			Young initiative 3MT (Hall_1)			
13:10-13:20						
13:20-13:30				Industry panel on PFA (Hall_1)	ISEBTT General Assembly (Cloister Hall)	J. Teissié Award (Cloister Hall)
13:30-13:40						
13:40-14:00	Educational session (Hall_1)		Hall_5: P03 Hall_7: S01 Hall_17: S03 Cloister Hall: S08			
14:00-14:10				*-> -> 10 min change room -> ->*	*-> -> 10 min change room -> ->*	*-> -> 10 min change room -> ->*
14:10-14:20						
14:20-14:30				Hall_1: S21 Hall_5: P11 Hall_7: S09 Cloister Hall: S08	Hall_1: S21 Hall_5: S13 Hall_7: S01 Cloister Hall: S11	Hall_1: S06 Hall_5: S14 Hall_7: P06 Hall_17: S15 Cloister Hall: P08
14:30-14:40						
14:40-15:00			COFFEE BREAK & POSTER SESSION			
15:00-15:10						
15:10-15:20	COFFEE BREAK					
15:20-15:30						
15:30-15:40				COFFEE BREAK & POSTER SESSION	COFFEE BREAK & POSTER SESSION	
15:40-15:50						COFFEE BREAK
15:50-16:00						
16:00-16:10	Educational session (Hall_1)	Bioelectric consortium (Hall_17)				
16:10-16:20						Awards & Closing Ceremony
16:20-16:30						
16:30-16:40			Hall_1: P13 Hall_5: P03 Hall_17: P10 Cloister Hall: S22			
16:40-16:50						
16:50-17:00						*-> -> 10 min change room -> ->*
17:00-17:10				Hall_1: P07 Hall_5: S07 Hall_7: S09 Cloister Hall: S23	Hall_1: S10 Hall_5: P11 Hall_7: S19 Hall_17: S15 Cloister Hall: S11	ISEBTT council
17:10-17.20						
17:20-17:30						
17:30-17:40	WELCOME CEREMONY					
17:40-17:50						
17:50-18:00						
18:00-18:10						
18:10-18:20						
18:20-18:30						
18:30-18:40				SENIOR2YS meeting corner		
18:40-18:50						
18:50-19:00						
19:00-19:30			YOUNG NIGHT			
19:30-20:00					CONGRESS DINNER	
20:00-20:30						
20.30-21:00						
21:00-21:30						
21:30-22:00						
22:00-22.30						
22:30-23:00						
23:00-23:30						
23:30-24:00						

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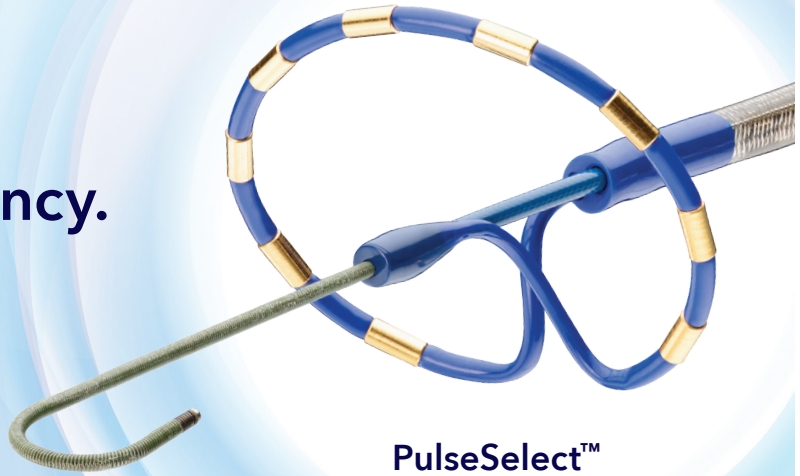

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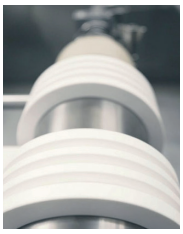

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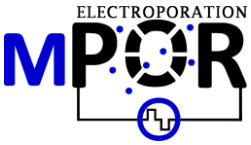
ELECTROPORATION
MPOR


RISEUP

P R O G R A M M E

Plenary Lectures

Thursday morning plenaries, Thursday, Sep 19 2024, 10:40-12:10
Location: Hall 1
Session: **Plenary talks** **73**
Chairs: Matteo Cadossi and Lluis Mir

Oral Presentations

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Sunday Educational Session part 1, Sunday, Sep 15 2024, 13:00-15:00
Location: Hall 1
Session: **Educational Session** **77**
Chair: Delia Arnaud-Cormos
Info: Presentations Schedule

13:00-13:30	Luis M. Mir	Words, concepts and facts: from the delivery of pulsed electric fields to electroporation? electropermeabilisation? reversible? irreversible? or even electropulsation?
13:30-14:00	Muriel Golzio	Visualization of the delivery of molecules by electroporation: a step towards understanding the mechanisms involved
14:00-14:30	Maja Čemažar	Electrochemotehrapy in human and veterinary oncology
14:30-15:00	Philippe Leveque	Electroporation technologies

Sunday Educational Session part 2, Sunday, Sep 15 2024, 15:30-17:00
Location: Hall 1
Session: **Educational Session** **77**
Chair: Delia Arnaud-Cormos
Info: Presentations Schedule

15:30-16:00	Michal Cifra	Biophysical bases of PEF effects on proteins: modeling and experiments
16:00-16:30	Rafael Davalos	Modeling of tissue electroporation and recent advances in irreversible electroporation
16:30-17:00	Samo Mahnič-Kalamiza	Non-biomedical applications of electroporation: Traps for young players?

Monday morning Track A, Monday, Sep 16 2024, 10:30-12:00
Location: Hall 1
Session: **S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)** **77**
Chairs: Damijan Miklavčič and Ana González-Suárez
Organizers/Conveners: D. Miklavčič, A. Ivorra

Monday morning Track B, Monday, Sep 16 2024, 10:30-12:00

Location: Hall 7

Session: **P5 - Electroporation and cellular processes and pathways** **81**

Chairs: Andrei Pakhomov and Anna Bulysheva

Monday morning Track C, Monday, Sep 16 2024, 10:30-12:00

Location: Hall 17

Session: **S05 - Intensification of fermentation processes by pulsed electric fields 85**

Chairs: Felix Schottroff and Javier Raso

Organizers/Conveners: F. Schottroff, J. Raso

Monday morning Track D, Monday, Sep 16 2024, 10:30-12:00

Location: Cloister Hall

Session: **S12 - Numerical modelling as an essential tool in electroporation research 88**

Chairs: Bor Kos and Clair Poignard

Organizers/Conveners: B. Kos, M. Casciola, C. Poignard

10:30 OR-18	**Estimation of spatial conductivity distribution within a tumor from impedance measurements and imaging following a simulated electroporation therapy protocol** *Damien Voyer*, Olivier Sutter, Clair Poignard	88
10:45 OR-19	**Finite Element evaluation of the electric field distribution in a cell-aggregates** Patrizia Lamberti, Michele Forzan, Stefania Romeo, *Elisabetta Sieni*	89
11:00 OR-20	**Finite element analysis model to predict electroporation of adherent cells in round, flat bottom wells** *Patrizia Lamberti*, Nicolas Mattei, Maria Evelina Mognaschi, Jelena Kolosnjaj-Tabi, Muriel Golzio, Marie-Pierre Rols, Elisabetta Sieni	90
11:15 OR-21	**Microdosimetric Study to Calibrate µsPEFs Application in Two Electrodes Technology for RISEUP Project** *Sara S. Fontana*, Noemi Dolciotti, Amir Ghassabi, Laura L. Caramazza, Irene Cuenca Ortolá, Micol Colella, Alessandra Paffi, Victoria Moreno, Luis M. Mir, Franck M. Andre, Claudia Consales, Francesca Apollonio, Micaela Liberti	90
11:30 OR-22	**Quantifying uncertainty of the numerical model of irreversible electroporation in the liver** *Helena Cindrič*, Damijan Miklavčič, Bor Kos	91
11:45 OR-23	**Simulation Investigation of Pulsed Magnetic Field-Induced Cell Permeabilization Using the Coupled Magnetic-Electric-Force Pore Energy Equation** *Chi Ma*, Yan Mi	92

Monday young initiative 3MT, Monday, Sep 16 2024, 13:00-13:40
Location: Hall 1

Session: **Three Minute Thesis (3MT)** **92**
Chairs: Micol Colella and Mariangela De Robertis
Info: Finalists:

Author	Affiliation	Title
Berzosa Alejandro	University of Zaragoza	“Valorization of Spent Yeast from the Brewing Industry Using Pulsed Electric Field (PEF) Processing”
Fontana Sara	Sapienza University of Rome	“Electric Pulses to Awaken Stem Cells: A Breakthrough in Injured Spinal Cord Regeneration?”
Gomez-Barea Mario	Universitat Pompeu Fabra	“Can you guess which electrical parameter can trigger different cell death mechanisms?”
Innamorati Giorgia	University of Rome ”Tor Vergata”	“Effects of microsecond electrical pulses on the inflammatory response”
Lanssens Lize	Katholieke Universiteit Leuven	“PEF sensory boost: steering vegetable aromas”
Walder Kate	BOKU University	“Power up your plate: Electrifying ways to optimize vegetable cooking”

Monday afternoon Track A, Monday, Sep 16 2024, 13:40-14:40
Location: Hall 17
Session: **S03 - Microalgae biorefinery** **92**
Chairs: Nabil Grimi and Urszula Tylewicz
Organizers/Conveners: N. Grimi, U. Tylewicz

Monday afternoon Track B, Monday, Sep 16 2024, 13:40-14:40
Location: Cloister Hall
Session: **S08 - Public health risks and pulsed electric fields in the food industry** **95**
Chairs: Ignacio Álvarez and Juan Manuel Martínez
Organizers/Conveners: I. Alvarez-Lanzarote, J. M. Martinez

Monday afternoon Track C, Monday, Sep 16 2024, 13:40-14:40
Location: Hall 5
Session: **P3 - Irreversible electroporation** **98**
Chairs: Rafael Davalos and Tomas Garcia

Monday afternoon Track D, Monday, Sep 16 2024, 13:40-14:40
Location: Hall 7
Session: **S01 - Medical applications of nsPEFs** **100**
Chairs: Richard Nuccitelli and Olga Pakhomova
Organizers/Conveners: R. Nuccitelli, O. Pakhomova

Monday late afternoon Track A, Monday, Sep 16 2024, 16:10-17:40
Location: Cloister Hall
Session: **S22 - New waveforms and electric field management strategies for electroporation-based therapies** **103**
Chairs: Antoni Ivorra and Micaela Liberti
Organizers/Conveners: A. Ivorra, M. Liberti

Monday late afternoon Track B, Monday, Sep 16 2024, 16:10-17:40
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Session: **P10 - Electrofluidic, microfluidic and lab on a chip** **107**
Chairs: Mihaela Moisescu and Hamid Hosano

Monday late afternoon Track C, Monday, Sep 16 2024, 16:10-17:40
Location: Hall 1
Session: **P13 - General applications of electroporation for food processing** **110**
Chairs: Gianpiero Pataro and Indrawati Oey

Monday late afternoon Track D, Monday, Sep 16 2024, 16:10-17:40
Location: Hall 5
Session: **P3 - Irreversible electroporation** **114**
Chairs: Saulius Šatkauskas and Muriel Golzio

16:55 OR-60 **Investigation of rabbit heart electrical activity changes after electroporation using combined optical and transmural microelectrode technique** 116
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17:10 OR-61 **Electrical conductivity in human liver tissue: In vivo Assessment on normal vs. tumor** 117
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Tuesday morning Track A, Tuesday, Sep 17 2024, 10:40-12:10
Location: Hall 5

Session: **S20 - PhD Students as important bricks in the wall of funded projects and basic research** **117**
Chairs: Sara Fontana and Giorgia Innamorati
Organizers/Conveners: S. Fontana, G. Innamorati

10:40 OR-62 **Gene electrotransfer of plasmid encoding Interleukin-12: off-target effects in murine cancer cells in vitro** 118
Ajda Medved, Maja Čemažar, Tanja Jesenko

10:55 OR-63 **Randomised Controlled Clinical Trial Investigating the Effect of Reduced Bleomycin in Electrochemotherapy Treatment on Patients with Cutaneous Malignancies** 118
Marie Tolstrup, Julie Gehl

11:10 OR-64 **Validation of in situ electroporation performed within a single cell microwave biosensor** 119
Anne A. Calvel, Olivia Peytral-Rieu, David Dubuc, Katia Grenier, Marie-Pierre Rols

11:25 OR-65 **Empowered Cellular and Subcellular Modeling for Microdosimetric Investigation of PEF Exposure** 120
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11:40 OR-66 **Effects of Intense Electric field on TRPV4 ion channel: a Molecular Dynamic study** 121
Carmen Pisano, Laura L. Caramazza, Paolo Marracino, Federico Del Signore, Micaela Liberti, Francesca Apollonio

11:55 OR-67 **Anti-tumor efficacy of new high-frequency electrical protocols on in vitro three-dimension colorectal cancer model** 121
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Tuesday morning Track B, Tuesday, Sep 17 2024, 10:40-12:10
Location: Cloister Hall
Session: **P1 - Electroporation and immune response** **122**
Chairs: Lluis Mir and Marco Benazzo

Tuesday morning Track C, Tuesday, Sep 17 2024, 10:40-12:10
Location: Hall 7
Session: **P5 - Electroporation and cellular processes and pathways** **126**
Chairs: Olga Pakhomova and Marie-Pierre Rols

Tuesday morning Track D, Tuesday, Sep 17 2024, 10:40-12:10

Location: Hall 1

Session: **P13 - General applications of electroporation for food processing** **130**

Chairs: Federico Gómez Galindo and Luis Redondo

PFA Industry Panel Track, Tuesday, Sep 17 2024, 13:20-14:10

Location: Hall 1

Session: **PFA Industry Panel** **133**

Chair: Damijan Miklavčič

Info: *Panelists:*

Ruggero Cadossi, IGEA, Italy;
Maura Casciola, FDA, USA;
Brendan Koop, Boston Scientific, USA;
Steve Miller, Abbott, USA;
Vivek Reddy, Mount Sinai Hospital, New York, USA;
Tushar Sharma, Biosense Webster, USA;
Daniel C. Sigg, Medtronic, USA;
Darrin Uecker, Pulse Biosciences, USA.

Tuesday afternoon Track A, Tuesday, Sep 17 2024, 14:20-15:20
Location: Hall 1
Session: **S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)** **134**
Chairs: Tomás García-Sánchez and Tugba Kumru
Organizers/Conveners: D. Miklavčič, A. Ivorra

Tuesday afternoon Track B, Tuesday, Sep 17 2024, 14:20-15:20
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Chairs: Ignacio Álvarez and Juan Manuel Martínez
Organizers/Conveners: I. Alvarez-Lanzarote, J. M. Martinez

14:50 OR-92	**Rapid Recovery of Bacterial Membrane Following Exposure to Pulsed Electric Fields** *Bingyu Yan*, Ryuya Kimura, Misato Kikuchi, Shoko Ishikawa, Kazuhiro Inobe, Ryo Sasahara, Taiga Kajiwara, Sunao Katsuki	138
15:05 OR-93	**Limitations of PEF for Food Pasteurization: role of membrane resealing in the microbial inactivation kinetics** *Carlota Delso*, Juan Manuel Martinez, Ignacio Álvarez, Javier Raso	139

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Location: Hall 7

Session: **S09 - Treatment of spinal cord injury: novel strategies and updates from the RISEUP project** **140**

Chairs: Claudia Consales and Micol Colella

Organizers/Conveners: C. Consales, M. Colella

14:20 OR-94	**Sensorimotor contributions to human cognition and emotion: clinical neuroscience clues for optimizing engineering approaches to functional restoration in people with spinal cord lesions** *Salvatore M. Aglioti*, Valentina Moro	140
14:35 OR-95	**Mechanisms of spinal cord regeneration** *Mark Anderson*, Jordan Squair, Alexandra de Coucy, Matthieu Gautier, Zhigang He, Bernard Schneider, Michael Sofroniew, Jocelyne Bloch, Gregoire Courtine	140
14:50 OR-96	**Towards neuronal reconnection after a spinal cord injury using graphene-based nanocomposites – The NeuroStimSpinal project** *Paula Marques*	141
15:05 OR-97	**Neuroprotective effect of Pulsed Electromagnetic Fields after Acute Ischemic Stroke** *Simona Salati*, Micol Colella, Micaela Liberti, Ruggero Cadossi	141

Tuesday afternoon Track D, Tuesday, Sep 17 2024, 14:20-15:20
Location: Hall 5
Session: **P11 - Electroporation modeling and mechanisms** **142**
Chairs: Damien Voyer and Daniela O. H. Suzuki

Tuesday late afternoon Track A, Tuesday, Sep 17 2024, 16:50-18:20
Location: Cloister Hall
Session: **S23 - Electroporation-based treatments in veterinary medicine** **145**
Chairs: Maja Čemažar and Nataša Tozon
Organizers/Conveners: M. Čemažar, N. Tozon

Tuesday late afternoon Track B, Tuesday, Sep 17 2024, 16:50-18:20
Location: Hall 5
Session: **S07 - Potential applications of PEFs technology in vegetable and fruit processing** **148**
Chairs: Marianna Giancaterino and Claudia Siemer
Organizers/Conveners: C. Siemer, M. Giancaterino

17:20 OR-110	**Seaweed processing with pulsed electric fields: from batch to continuous process development for functional ingredients production** *Alexander Golberg*	150
17:35 OR-111	**Optimizing valuable compound recovery from food side streams and microbial Biosynthesis through PEF-Induced Extraction and Stress Strategies** *Robert Sevenich*	150
17:50 OR-112	**Increasing the yield of juice and bioactive compounds extracted from blueberries using pulsed electric field** Shao-Keng Tai, Farzan Zare, Joseph Nastasi, *Nidhi Bansal*	151

Tuesday late afternoon Track C, Tuesday, Sep 17 2024, 16:50-18:20
Location: Hall 7
Session: **S09 - Treatment of spinal cord injury: novel strategies and updates from the RISEUP project** **151**
Chairs: Micol Colella and Claudia Consales
Organizers/Conveners: C. Consales, M. Colella

16:50 OR-113	**Boosting the development of Electro Pulsed Bio-hybrid implantable devices through advanced modelling in vitro and in vivo** *Micol Colella*, Francesca Apollonio, Marco Balucani, Laura L. Caramazza, Noemi Dolciotti, Sara S. Fontana, Paolo Marracino, Alessandra Paffi, Micaela Liberti	151
17:05 OR-114	**Materials solutions for an electrostimulable device for use in spinal cord injury model in rat** *Fernando Gisbert Roca*, Sergiy Ivashchenko, Francisco Navarro Páez, Cristina Martinez, Jorge Más Estellés, Manuel Monleon	152
17:20 OR-115	**Electromanipulation of calcium oscillations in Mesenchymal Stem Cells, a control of cell fate?** *Leslie A. Vallet*, Marina Sanchez Petidier, Romain Samiaa, Nataliia Naumova, Claudia Consales, Giorgia Innamorati, Caterina Merla, Franck M. Andre, Luis M. Mir	153
17:35 OR-116	**Effects of microsecond electrical pulses on the inflammatory response** *Giorgia G. Innamorati*, Francesca Camera, Fernando Gisbert, Sergiy Ivashchenko, Romain Samiaa, Sara S. Fontana, Noemi Dolciotti, Micol Colella, Alessandro Zambotti, Caterina Merla, Franck M. Andre, Victoria Moreno, Paolo Marracino, Claudia Consales	154
17:50 OR-117	**Emerging Approaches to Neural Tissue Regeneration: Electrical Stimulation of Stem Cells** Marina Sanchez Petidier, *Romain Samiaa*, Leslie A. Vallet, Giorgia Innamorati, Claudia Consales, Caterina Merla, Victoria Moreno, Fernando Gisbert, Sergiy Ivashchenko, Manuel Monleon, Micaela Liberti, Franck M. Andre, Luis M. Mir	154

18:05 OR-118 **Regeneration of Injured Spinal Cord by applying subdural electro pulsed stimulation and stem cell bio-hybrid approach** 155
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Tuesday late afternoon Track D, Tuesday, Sep 17 2024, 16:50-18:20
Location: Hall 1
Session: **P7 - Electroporation for clinical use** **156**
Chairs: Michael Sano and Julita Kulbacka

16:50 OR-119 **Optimization of Bipolar Microsecond Electric Pulses for DNA Vaccine Delivery** 156
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17:05 OR-120 **Keloid treatment with Electrochemotherapy** 157
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17:20 OR-121 **Development of a specific gel for skin cancer electrochemotherapy.** 158
Antonella María Cilio, Ana Campastri, Jesica Rodríguez Miranda, Ximena Manglano, Sebastian D. Michinski, Felipe Maglietti

17:35 OR-122 **The Synergy of Conductive Nanoparticles with Nanosecond and Microsecond Pulse Bursts for Bleomycin-based Electrochemotherapy** 158
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17:50 OR-123 **Development of novel genetic vaccine platforms: from the idea to GMP production** 159
Luigi Aurisicchio

18:05 OR-124 **Low-Dose Electrochemotherapy Enhances DNA Damage and Overcome Resistance through Synergistic Drug Delivery** 160
Vaishali Malik, Laurien G. P. H. Vroomen, Masashi Fujimori, Emma Gerace, Jaad Ismail, Govindarajan Srimathveeravalli

Wednesday morning Track A, Wednesday, Sep 18 2024, 10:40-12:10
Location: Cloister Hall
Session: **S04 - Advanced applications of PEF for food quality enhancement, food component modification, and structural alterations** **160**
Chairs: Samo Mahnič-Kalamiza and Jessica Genovese
Organizers/Conveners: S. Mahnic-Kalamiza, J. Genovese

Wednesday morning Track B, Wednesday, Sep 18 2024, 10:40-12:10
Location: Hall 17
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Chairs: Federica Castellani and Manfred Lindau
Organizers/Conveners: M. Lindau, F. Castellani

Wednesday morning Track C, Wednesday, Sep 18 2024, 10:40-12:10
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Session: **S18 - Bridging the gap between experimental and modeling studies in PEF electroporation: a Young Professional's perspective** **169**
Chairs: Laura Caramazza and Rosa Orlacchio
Organizers/Conveners: L. Caramazza, R. Orlacchio

11:25 OR-141 **Nanosecond pulsed electric fields and gold nanoparticles for cancer treatment** 171
Rosa Orlacchio, Jelena Kolosnjaj-Tabi, Nicolas Mattei, Lionel Michard, Hafsa Tjiou, Léna Serradeil, Isabelle Lagroye, Florence Poulletier de Gannes, Yann Percherancier, Philippe Leveque, Marie-Pierre Rols, Delia Arnaud-Cormos, Muriel Golzio

11:40 OR-142 **Deciphering the behavior of multicellular 3D spheroids exposed to high-intensity pulsed electric fields by a mathematical modeling approach** 172
Annabelle Collin, Jelena Kolosnjaj-Tabi, Muriel Golzio, Marie-Pierre Rols, Clair Poignard

11:55 OR-143 **On the complementarity of modeling and experimentation in the study of biological effects of subnanosecond pulsed electric fields** 173
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Wednesday morning Track D, Wednesday, Sep 18 2024, 10:40-12:10
Location: Hall 7
Session: **S16 - Electroporation in veterinary and translational medicine** **174**
Chairs: Felipe Horacio Maglietti and Joseph Impellizeri
Organizers/Conveners: F. Maglietti, J. Impellizeri

10:40 OR-144 **Chimeric DNA vaccination against the Chondroitin Sulfate Proteoglycan 4: a potential allied in combinatorial approaches for the treatment of melanoma and osteosarcoma** 174
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10:55 OR-145 **Electrochemotherapy plus IL-2+IL-12 gene electrotransfer in spontaneous inoperable stage iii-iv canine oral malignant melanoma** 175
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11:10 OR-146 **Adjuvant Electrochemotherapy and/or Radiotherapy in Feline Injection Site Sarcoma** 175
Matias N. Tellado, Franco Portillo, Vanda Guillen, Tadeo Sabella, Maura Diaz, Felipe Maglietti

11:25 OR-147 **Retrospective analysis of the outcome and survival time of dogs with mast cell tumors with different degrees of malignancy treated with electrochemotherapy** 176
Javier Ojeda, Paulina Sandoval

11:40 OR-148 **Evaluation of the safety and feasibility of electrochemotherapy with intravenous bleomycin as local treatment of bladder cancer in dogs** 177
Marcelo Monte Mor Rangel, Lais Calazans Menescal Linhares, Daniela Ota Hisayasu Suzuki, Krishna Duro de Oliveira, Felipe H. Horacio Maglietti, Andrigo Barbosa De Nardi

Wednesday morning Track E, Wednesday, Sep 18 2024, 10:40-12:10
Location: Hall 1
Session: **P7 - Electroporation for clinical use** **177**
Chairs: Yan Mi and Govindarajan Srimathveeravalli

10:40 OR-149 **Enhancing sensitivity to radiation therapy using electroporation in a radioresistant model of oesophageal cancer** 177
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11:10 OR-151 **Intraoperative electrochemotherapy of the posterior resection surface after pancreaticoduodenectomy: Preliminary results of a hybrid approach treatment of pancreatic cancer** 179
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11:25 OR-152 **Novel Synergistic Electric Pulses and First Human Cancer Clinical Trials: Towards the Balance between Negligible Muscle Contraction and Enhanced Ablation** 179
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11:40 OR-153 **Bleomycin based electrochemotherapy with standard electrodes for advanced stage, recurring vulvar/cervix carcinomas** 180
Aurel Ottlakan, Marton Vas, Gyorgy Lazar, Judit Olah, Gabor Vass, Mario Vincze, Erika Gabriella Kis

11:55 OR-154 **Electrochemotherapy: from palliation to important player in the multidisciplinary management of the cancer patient** 181
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Wednesday afternoon Track A, Wednesday, Sep 18 2024, 14:20-15:20
Location: Hall 1
Session: **S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)** **181**
Chairs: Lakshya Mittal and Antoni Ivorra
Organizers/Conveners: D. Miklavčič, A. Ivorra

Wednesday afternoon Track B, Wednesday, Sep 18 2024, 14:20-15:20
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Session: **S01 - Medical applications of nsPEFs** **184**
Chairs: Richard Nuccitelli and Olga Pakhomova
Organizers/Conveners: R. Nuccitelli, O. Pakhomova

Wednesday afternoon Track C, Wednesday, Sep 18 2024, 14:20-15:20
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Session: **S13 - High voltage electrical discharges: principles and applications 187**
Chairs: Nadia Boussetta and Eugene Vorobiev
Organizers/Conveners: N. Boussetta, E. Vorobiev

Wednesday afternoon Track D, Wednesday, Sep 18 2024, 14:20-15:35
Location: Cloister Hall
Session: **S11 - In vivo delivery of genetic medicine through gene electrotransfer 190**
Chairs: Kevin Hollevoet and Emanuela Signori
Organizers/Conveners: K. Hollevoet, E. Signori

15:20 OR-237 **Development of in vivo-launched synthetic DNA-encoded antibodies employing CELLECTRA® electroporation technology** 192
Trevor Smith, Paul Fisher, Ami Patel, Elizabeth Parzych, Kevin Hollevoet, David B. Weiner, Laurent Humeau

Wednesday late afternoon Track A, Wednesday, Sep 18 2024, 16:50-17:50
Location: Hall 5
Session: **P11 - Electroporation modeling and mechanisms** **193**
Chairs: Jelena Kolosnjaj-Tabi and Patrizia Lamberti

16:50 OR-170 **AC electrodeformation studies on Compound Giant Unilamellar Vesicle as a model of eukaryotic cell** 193
Rupesh Kumar, Rajarshi Chakrabarti, Rochish Thaokar

17:05 OR-171 **Suitability (and not) of Giant Unilamellar Vesicles in electroporation studies for biological applications** 193
Rochish Thaokar, Mohammad Maoyafikuddin

17:20 OR-172 **Correlation between numerical simulations and clinical outcomes of irreversible electroporation for hepatocellular carcinoma** 194
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17:35 OR-173 **Simulation study on waveform characteristics of measuring bio-impedance using pulse frequency response method** 195
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Wednesday late afternoon Track B, Wednesday, Sep 18 2024, 16:50-17:50
Location: Hall 17
Session: **S15 - Advanced imaging techniques for visualizing the mechanisms of pulsed electric field interactions** **195**
Chairs: Joel Bixler and Bennett Ibey
Organizers/Conveners: J. Bixler, B. Ibey

16:50 OR-174 **PEFfect Illumination: Observing Protein Oxidation Effects of Pulsed Electric Field Through Monitoring (Bio)Chemiluminescence** 196
Kateřina Červinková, Petra Vahalová, Michaela Poplová, Tomáš Zakar, Daniel Havelka, Martin Paidar, Viliam Kolivoška, Michal Cifra

17:05 OR-175 **Electric field effects on human skeletal muscle-derived mesenchymal stem/stromal cells investigated by scanning electrochemical microscopy** 196
Inga Morkvenaite-Vilkonciene, Tomas Mockaitis

17:20 OR-176	**Effects of Nanosecond Pulsed Electric Field on Cancerous and Normal Cells — Fluorescence Microscopy and Autofluorescence Lifetime Imaging** *Nobuhiro Ohta*	197

Wednesday late afternoon Track C, Wednesday, Sep 18 2024, 16:50-18:05
Location: Cloister Hall
Session: **S11 - In vivo delivery of genetic medicine through gene electrotransfer 197**
Chairs: Kevin Hollevoet and Emanuela Signori
Organizers/Conveners: K. Hollevoet, E. Signori

16:50 OR-177	**Immunomodulatory effects of plasmid DNA following gene electrotransfer in colon cancer utilizing different electric pulse protocols** *Tim Bozic*, Mariangela De Robertis, Iva Santek, Flaviana Marzano, Boštjan Markelc, Alessandro Silvestris, Apollonia Tullo, Graziano Pesole, Maja Čemažar, Emanuela Signori	197
17:05 OR-178	**Calcium electroporation and interleukin-12 gene electrotransfer** Barbara Lisec, Boštjan Markelc, Katja Ursic Valentinuzzi, Gregor Serša, *Maja Čemažar*	198
17:20 OR-179	**Unraveling a multifactorial host immune response to intramuscular electrotransfer of dna-encoded antibody therapy** Debby Thomas, *Jenny Sprooten*, Jannes Govaerts, Pascal Merchiers, Maarten Dewilde, Kevin Hollevoet, Abhishek Garg, Nick Geukens	199
17:35 OR-180	**Enhancing the Therapeutic Benefits of Proteins with Short Half-Lives: Delivery of G-CSF and GLP-1 with DNA-Based MYO Technology** *Debnath Maji*, Andrew D. Cameron, Linda Sasset, Sayantani Sinha, Andy Thompson, Carleigh Sussman, Delcora A. Campbell, Robert Miller, Marek M. Drozdz, Rachel A. Liberatore	200

Wednesday late afternoon Track D, Wednesday, Sep 18 2024, 16:50-17:50
Location: Hall 7
Session: **S19 - Pulsed electric fields in meat and fish and their by-products processing** **200**
Chairs: Urszula Tylewicz, Pietro Rocculi and Silvia Tappi
Organizers/Conveners: U.Tylewicz, P. Rocculi, S. Tappi

Wednesday late afternoon Track E, Wednesday, Sep 18 2024, 16:50-18:20
Location: Hall 1
Session: **S10 - Electrochemotherapy of cutaneous tumors** **202**
Chairs: Giulia Bertino and Julie Gehl
Organizers/Conveners: G. Bertino, J. Ghel

Thursday morning Track A, Thursday, Sep 19 2024, 8:30-10:00
Location: Hall 1
Session: **S24 - Emerging role of Electrochemotherapy in the treatment of GastroIntestinal cancer** **207**
Chairs: Luca Tagliaferri and Roberto Iezzi
Organizers/Conveners: L. Tagliaferri, R. Iezzi

Thursday morning Track B, Thursday, Sep 19 2024, 8:30-10:00
Location: Hall 7
Session: **P12 - Biomass transformation and biocompounds** **208**
Chairs: Samo Mahnič-Kalamiza and Wolfgang Frey

Thursday morning Track C, Thursday, Sep 19 2024, 8:30-10:00

Location: Hall 5

Session: **S07 - Potential applications of PEFs technology in vegetable and fruit processing** **211**

Chairs: Marianna Giancaterino and Claudia Siemer

Organizers/Conveners: C. Siemer, M. Giancaterino

Thursday morning Track D, Thursday, Sep 19 2024, 8:30-10:00
Location: Cloister Hall
Session: **S12 - Numerical modelling as an essential tool in electroporation research** **215**
Chairs: Bor Kos and Clair Poignard
Organizers/Conveners: B. Kos, M. Casciola, C. Poignard

Thursday afternoon Track A, Thursday, Sep 19 2024, 14:10-15:40
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Session: **P6 - Calcium electroporation** **217**
Chairs: Boštjan Markelc and Luigi Aurisicchio

Thursday afternoon Track B, Thursday, Sep 19 2024, 14:10-15:40
Location: Cloister Hall
Session: **P8 - Electroporation in veterinary oncology** **220**
Chairs: Urša Lampreht Tratar and Maja Čemažar

Thursday afternoon Track C, Thursday, Sep 19 2024, 14:10-15:40
Location: Hall 17
Session: **S15 - Advanced imaging techniques for visualizing the mechanisms of pulsed electric field interactions** **222**
Chairs: Joel Bixler and Bennett Ibey
Organizers/Conveners: J. Bixler, B. Ibey

Thursday afternoon Track D, Thursday, Sep 19 2024, 14:10-15:40
Location: Hall 5
Session: **S14 - Electromagnetic modelling for pulsed electric fields** **225**
Chairs: Elisabetta Sieni, Raji Sundararajan, Stefania Romeo and Patrizia Lamberti
Organizers/Conveners: E. Sieni, R. Sundararajan, S. Romeo, P. Lamberti

Thursday afternoon Track E, Thursday, Sep 19 2024, 14:10-15:40
Location: Hall 1
Session: **S06 - Electrochemotherapy in treatment of vascular malformations 228**
Chairs: Tobian Muir and Gregor Serša
Organizers/Conveners: T. Muir, G. Sersa

Poster Presentations

Coffee Break and Poster Session, Tuesday, Sep 17 2024, 15:20-16:50
Location: Coffee break area (2nd floor)
Session: **Poster session** **259**
Chairs: Olga Zeni, Alexander Mathys and Óscar Lucía

Coffee Break and Poster Session, Wednesday, Sep 18 2024, 15:20-16:50
Location: Coffee break area (2nd floor)
Session: **Poster session** **283**
Chairs: Rosa Orlacchio, Anna Szewczyk and Olga Martín-Belloso

PLENARY LECTURES' ABSTRACTS

Plenary talks

Monday morning plenaries
Sep 16, 8:50 - 9:50

PL-01

Visualizing Electropore Dynamics in Live Cells

Mantas Silkunas, Olga N. Pakhomova, Giedre Silkuniene, *Andrei G. Pakhomov*

Old Dominion University, United States

Discerning individual membrane lesions in electroporated cells has been a challenge due to their size, which falls below the resolution limit of optical microscopy. Nonetheless, we have succeeded in tracking individual electropores by imaging Ca2+ entry with total internal reflection fluorescence (TIRF) microscopy. TIRF imaging is restricted to an extremely shallow (˜100-nm) subplasmalemmal layer, which facilitates the observation of Ca2+ fluxes through individual pores instead of seeing a diffuse fluorescence cloud. Measuring pore currents simultaneously with imaging revealed different types of lesions and the dynamics of pore creation and resealing.

A whole-cell voltage clamp configuration was established in human embryonic kidney cells placed on glass coverslips with an indium tin oxide (ITO) layer as electrical ground. Voltage steps, 1 to 25 ms, applied between the pipette and the ITO, induced lesions in the membrane portion adjacent to the ITO. Loading cells with the Ca2+ sensitive dye CAL-520 enabled dynamic visualization of Ca2+ fluxes through these lesions. These experiments provided the first evidence of electropore persistence for at least a minute in the absence of an applied membrane potential, as well as direct measurements of single pore electrical conductance.

Hyperpolarizations to about -100 mV induced a diffuse zonal electropermeabilization, manifested by momentary and subtle Ca2+ fluorescence upticks (scintillas) over large membrane areas. The scintillas lacked definitive brighter spots which would indicate a pore opening, and were accompanied by a modest (<100 pS) increase in the whole-cell conductance.

Charging the membrane to -200 mV and beyond produced one or several focal fluorescence transients. Their brightness decreased sideways from the center, consistent with Ca2+ entry through a pore followed by a radial diffusion. These transients disappeared within 10-20 ms after the voltage step and marked the formation of short-lived electropores with 50- to 200-pS conductance. Larger voltage steps increased the brightness, the size, and the number of transients, which did not necessarily appear at the same locations as with the smaller steps. This pattern indicates that pre-existing lesions, by increasing local membrane permeability and reducing charging efficiency, could make the membrane locally less vulnerable to charging. At -240 mV we observed high-conductance pores (> 1 nS) that persisted for seconds after the voltage step. Attempts to charge the membrane beyond the limit of about -260 mV increased pore conductance in an adaptive manner, “clamping” the induced membrane potential at this limit or even depolarizing it. These observations suggest possible paradigm shift in recognizing electroporation not merely as a membrane injury but also as a potential protective mechanism.

Supported in part by NIH NEI R21EY034258 and R21EY034803 (to A.G.P).

PL-02

Ion ion/protein mobilization following PEF application

Sunao Katsuki

Kumamoto University, Japan

No abstract was provided.

Plenary talks

Tuesday morning plenaries
Sep 17, 8:30 - 10:00

PL-03

Lethal and non-lethal perturbation of cells by electroporation

Antoni Ivorra

Universitat Pompeu Fabra, Spain

It has often been stated that irreversible electroporation kills cells by disrupting cellular homeostasis. This vague statement, while true, implicitly acknowledges our ignorance regarding cellular death mechanisms after non-thermal large electric field exposure. Since early observations, it is understood that, unless field exposure is extreme, both in magnitude and in time, cell death does not occur immediately via necrosis. Delayed cell death, via regulated cell death mechanisms such as necroptosis, pyroptosis, and even apoptosis, has been reported for more moderate exposures [Bioelectrochemistry, 2021, 141:107871]. We also now know that extracellular calcium (Ca2+) uptake probably has a major role in cell death by electroporation [Bioelectrochemistry, 2020, 131:107369; Bioelectrochemistry, 2021, 142:107927]. In hindsight, this is unsurprising given the large difference in calcium concentration between the cell exterior (˜2 mM) and the cell interior (0.2 μM) and that calcium participates as a second messenger in several cellular signaling processes. That is, it is reasonable to assume that, if the plasma membrane is made much more permeable to calcium by electroporation, the inflow of extracellular calcium can severely interfere with cellular processes to the point of triggering cell death. And it is also reasonable to hypothesize that the calcium uptake by electroporation may have a profound impact on cellular processes such as gene expression and cell proliferation for non-lethal exposures; particularly if the insult is continuous or repeated over time. In my view, non-lethal electroporation using low fields and long exposures represents an opportunity for developing new therapeutic modalities. Electroporation is frequently depicted as a phenomenon that cannot be observed for low field magnitudes, even if the exposure is very long. However, recent experimental observations challenge such depiction. In the context of explaining the long-term neuromodulatory capabilities of pulsed radio-frequency currents (ac 500 kHz), it has been demonstrated that, albeit mildly, cell permeability does increase for calcium when relatively long exposures (˜ 2 seconds) of low magnitude (<200 V/cm) are applied [Bioelectrochemistry, 2020, 136:107624]. And, remarkably, researchers in the field of tumor treatment fields (TTFields), using alternating fields (200 kHz) with extremely low electric field magnitudes (4 V/cm) applied for days, observed an increase in cell membrane permeability to relatively large molecules (> 4 kDa) [Cell Death Discov, 2018, 4:113] which might also be due to calcium uptake.

PL-04

Mechanistic insights into the effects of electroporation on excitable cells

Lea Rems

University of Ljubljana, Slovenia

Understanding electroporation of excitable cells is becoming increasingly important due to the rapid advancement of applications targeting excitable tissues, including but not limited to electroporation-based cardiac tissue ablation, gene electrotransfer of nucleic acids into muscle tissue, irreversible electroporation of brain tumors and epileptic zones, as well as electrostimulation using nanosecond electric pulses. Excitable cells (muscle, nerve, and neuroendocrine cells) are characterized by expression of voltage-gated ion channels that enable the cells to trigger electrical signals called action potentials. This lecture will provide new insights from experiments, continuum modeling, and molecular dynamics simulations on how electroporation can affect the electrophysiological response of excitable cells and discuss the open questions relevant to the above-listed medical applications.

PL-05

EP for Cardiac ablation

Vivek Reddy

Icahn School of Medicine at Mount Sinai, United States

No abstract was provided.

Plenary talks

Wednesday morning plenaries

Sep 18, 8:30 - 10:00

PL-06

Pioneering sustainable food production with pulsed electric field technology

Indrawati Oey

University of Otago, New Zealand

No abstract was provided.

PL-07

Unleashing the potential of pulsed electric fields in food processing: from techno-functional improvement to health-boosting innovations

Robert Soliva-Fortuny, Pedro Elez-Martinez, Olga Martín-Belloso

University of Lleida, Spain

Applications of pulsed electric fields (PEF) in food processing are continuously expanding, demonstrating its capability to enhance the sustainability of food processes as well as the quality of the obtained products. Beyond its remarkable impact on food structure and functionality, PEF can be strategically used to preserve, and even enhance, the health-related content of plant-based products. Recent applications of mild PEF treatments emerge as a strategy to stimulate the production of secondary metabolites in fresh commodities, thereby increasing their antioxidant potential. PEF also stands as a potent tool to improve the bioaccessibility of desirable compounds, introducing a health-centric dimension to food processing.

Recent studies unveil the ability of PEF in elevating the techno-functional and health-related properties of food macromolecules, such as polysaccharides (e.g. dietary fiber, starch) and proteins. This lecture will explore the multifaceted role of electroporation for producing enhanced food ingredients, simultaneously addressing sustainable valorization, technological innovation, and health-boosting applications in plant-based products.

PL-08

Electroporation - The U.S. Veterinary Experience with ECT and GET

Joseph A. Impellizeri

Veterinary Oncology Services, United States

Electroporation as a therapeutic modality for cancer treatment is still “novel” in veterinary oncology especially in the United States and is predominantly dominated by ECT. This presentation will discuss the limited history of usage, misconceptions regarding the technology, the challenges faced with education of new and interested specialists and pet owners, the availability of generators and electrodes, the awareness and limitations with immunotherapy delivered via GET vs. traditional methods and finally, discuss future concepts for solutions on improving these current limitations and challenges.

Plenary talks

Thursday morning plenaries

Sep 19, 10:40 - 12:10

PL-09

Enhanced Delivery of Plasmid DNA Encoding Therapeutic Agents as a Means to Induce a Robust Anti-Tumor Immune Response

Richard Heller, Loree Heller, Guilan Shi, Jody Synowiec, Julie Singh, Alex Otten, Mark J. Jaroszeski

University of South Florida, United States

Gene electrotransfer (GET) has been used to successfully deliver plasmid DNA to multiple tissue targets. While there have been successes with GET, there is still additional hurdles that need to be overcome to advance the technology and enhance results. A key to enhancing effective gene-based immunotherapy is controlling the reproducibility and expression levels of the delivered transgene. To this end, our research group has modified the electrotransfer approach to enhance its utilization in multiple applications. One area that we are testing with this new approach is immunotherapy of solid tumors. The discovery and utilization of immune checkpoint inhibitors (ICIs) have been demonstrated to have positive clinical outcomes, but have been limited by both primary and acquired resistance. A major issue is the absence of T cells within the tumor microenvironment (TME). We previously demonstrated that the intratumor delivery of a plasmid encoding interleukin-12 (pIL-

12) using GET resulted in a significant increase in T effector cells and a reduction in T regulatory cells and myeloid derived suppressor cells within the TME. Adding ICIs together with pIL-12 GET induced a robust immune response leading to both local and systemic responses in preclinical and clinical studies. While these results were encouraging, there was still a need to enhance the delivery process. For example, the established GET technology requires high applied voltage for plasmid delivery and lacked a means to determine if delivery had occurred during the process. Our current research has focused on developing a GET system that could overcome these issues. A new system that incorporates a heat source and a tissue impedance measuring device as well as an array that incorporates independently addressable electrodes was developed and tested. A moderate elevation of the tissue temperature coupled with the electrode array enabled approximately a 60% reduction in the applied voltage. We tested the approach in the B16.F10 mouse melanoma model. Delivery of pIL-12 with the new system resulted in >80% long-term complete regression. In addition, monitoring impedance within each independent section of the electrode array revealed different pulse numbers were needed to achieve delivery in each section. The utilization of this enhanced approach enables a more controlled delivery and application of the pulse fields. Another aspect of the current research is to examine the ability to deliver plasmid DNA encoding checkpoint peptides, i.e., PD1 and combine that with pIL-12. In a multi-tumor model, the plasmids were delivered to a subcutaneous tumor; tumors generated with an intraperitoneal injection were left untreated. This new therapy induced complete regression of the subcutaneous tumor and blockage of peritoneal tumor growth as assessed via in vivo imaging. Work is ongoing to translate this approach to clinical evaluation.

PL-10

Clinical application in bone metastases compressing the spinal cord

Frederic Deschamps

Institut Gustave Roussy, France

Metastatic epidural spinal cord compression (MESCC) frequently results in severe pain and neurologic impairment such as paraplegia. Conventional external beam radiation therapy is the standard of care for frontline therapy. Unfortunately, recurrence is common and the option of re-irradiation is often limited out of concern for spinal cord damage from cumulative radiation dose. Electrochemotherapy is now an option to radiotherapy-resistant MESCC, providing rapid and durable pain relief and neurological improvement in the advanced cancer setting. However, neurological complications can occured, related to the inevitable technological reality that hyperthermia and irreversible electroporation occur in the immediate vicinity of the electrodes. Further research to tailor this technique is merited to improve the safety profile and risk/benefit balance.

PL-11

Vascular malformation ablation

Walter A. Wohlgemuth

UMH - Halle University Clinic, Germany

No abstract was provided.

ORAL PRESENTATIONS' ABSTRACTS

Educational Session

Sunday Educational Session part 1

Sep 15, 13:00 - 15:00

Info: This session is not supported by abstract submissions.

Educational Session

Sunday Educational Session part 2

Sep 15, 15:30 - 17:00

Info: This session is not supported by abstract submissions.

S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)

Monday morning Track A

Sep 16, 10:30 - 12:00

OR-01

In situ characterization of electroporation-dependent tissue properties for cancer and cardiac ablation

Edward Jacobs, Pedro Santos, Rafael Davalos

Georgia Tech, United States

Pulsed electric field (PEF) therapies deliver high-voltage, short electric pulses directly into tissue to permeabilizes cells through the generation of nano-scale pores (electroporation). The transitory formation of pores is called reversible electroporation and is used to deliver impermeable substances into cells. Larger and longer PEFs may induce cell death through loss of homeostasis, termed irreversible electroporation (IRE). IRE was considered the upper limit of reversible electroporation but has been developed as a standalone method for tissue ablation. PEF treatment efficacy depends on the application of a critical electric field over the targeted tissue volume, but the electric field distribution depends on the tissue-specific electrical properties, which both differ between patients in healthy and malignant tissues and change in an electric field-dependent manner from the electroporation process itself.

We use an in situ method, termed voltage ramp, that applies a series of increasing voltages across treatment electrodes and measures the resulting current. Due to the inherent non-linearity in the system, we develop a robust deep neural network, trained on finite element model simulations, to unravel the relationship between V/I characteristics and tissue properties. We found minimal test error ($p < 0.0001$), and our model was validated to correctly predict the complete dynamic conductivity curve in a previously characterized ex vivo liver model ($p < 0.0001$).

We believe this platform can be incorporated prior to treatment to rapidly ascertain patient-specific tissue properties paramount in electroporation treatment planning models and real-time treatment prediction algorithms, and this method can be used over current ex vivo methods for in situ tissue characterization. We characterized and validated the first reported electrical tissue properties of lung tumors from five canine patients. Further, we have characterized in vivo pancreatic cancer tumors in both mice and immunocompromised swine. Lastly, we have characterized the first-reported properties for cardiac tissue from for pulse widths ranging from 500 ns to 100 us, using ex vivo porcine tissue within 5 minutes of removal from the animal and maintained at body temperature.

The nonthermal mechanisms for IRE are paramount for treating tissue near anatomically sensitive structures. Numerous thermal mitigation protocols have been proposed to minimize temperature rise, but intraoperative temperature monitoring is still needed. We demonstrate here that an accurate and robust temperature prediction machine learning model can be developed using estimated tissue properties (bulk and dynamic conductivity), known geometric properties (probe spacing), and easily measurable treatment parameters (applied voltage, current, and pulse number). We show that the model can predict temperature rise within ex vivo perfused porcine livers, with error <0.5 °C, and is shown to predict temperature rise in over 1000

unique computational test conditions with <1 °C error and no observable outliers.

OR-02

Characterization of Thermal Safety Profile of a Novel Balloon-In-Basket PFA System under Repeated PFA Therapy Applications: Insights from in vivo and ex vivo Studies

Lakshya Mittal[1], Ben Niemiera[1], Jed Overmann[1], Catherine Pipenhagen[1], L. Boyce Moon[1], Jeffrey Fish[1], Matthew Miller[1], Autumn Myhand[1], Taylor Spangler[2]

[1]Abbott laboratory, United States

[2]Bayside Preclinical Research Services, Inc, United States

Introduction: Pulsed field ablation (PFA) is an attractive alternative to thermal modalities for treating cardiac arrhythmias using electrical pulses due to an improved safety profile and ease of use. Optimization of electrical pulse parameters is essential for effective irreversible electroporation without a significant Joule heating. This study investigates the impact of repeated therapy application on thermal profile of a novel PFA system.

Methods: The VoltTM PFA system, comprised of novel balloon-in-basket catheter and generator, was studied.

An acute swine (N=6) cohort was evaluated using PFA applications applied 8 times, with rotation of the catheter between each application, at each pulmonary vein (RSPV, LSPV, CIPV). Gross and histopathological evaluations were performed to assess in vivo thermal safety.

To study thermal profile in worst-case heating scenario, an ex vivo bovine right-ventricular tissue ablation model was evaluated at 37°C. Multiple consecutive therapy applications were performed (8× and 16×), without catheter rotation and as fast as possible. Temperatures were recorded using Fiber Optic probes at electrode-tissue interface and at 3mm and 7mm depths to characterize bulk tissue heating.

Results: Acute in vivo gross and histopathological findings at PFA treatment sites showed no morphological features indicative of significant thermal injury in cardiac tissues. While key pathological features of thermal injuries were absent, minimal (microscopic) collagen denaturation was rarely noted on the endocardium only, possibly suggesting proximity to a device electrode. The interpretation of contraction band morphology, lack of overt or predominate thermal signatures and other features such as artery and nerve sparing strongly suggests that the lesions created were almost exclusively a response to PFA rather than thermal coagulation necrosis.

The Ex vivo studies indicated an initial temperature increase with 8× and 16× therapy applications, which plateaued well below 50°C. Heating was limited to electrode-tissue interface (8×=45.76°C; 16×=45.96°C max. temperatures at 95/95 confidence level) with minimal temperature rise at 3mm (8×=42.11°C; 16×=42.38°C max. temperatures at 95/95 level) and 7 mm (8×=39.83°C; 16×=40.74°C max. temperatures at 95/95 level) indicating an absence of bulk heating. These temperatures were not sufficient to create thermal damage, as also indicated by an absence of thermal lesion and/or char formation on tissue. Despite using non-perfused tissue and no direct saline/blood flow for heat dissipation, favorable thermal observations were recorded in this worst-case ex vivo model.

Conclusions: The in vivo and ex vivo results indicate that the Volt PFA system is thermally safe under repeated therapy applications in these models. The favorable thermal safety profile could be attributed to the carefully optimized catheter design and waveform selection.

OR-03

Isolated primary rat ventricular cardiomyocytes response to electroporation: action potential – Ca2+ release – contraction

Vid Jan, Marko Stručić, Tina Turk, Jernej Jurič, Monika Kos, Matej Reberšek, Martina Perše, Lea Rems, Damijan Miklavčič

University of Ljubljana, Slovenia

Atrial fibrillation (AF) is the predominant form of arrhythmia encountered in clinical practice. Some research estimate that 1 in 4 adults older than 40 years will experience a form of AF. Catheter ablation with pulmonary vein isolation is reported to be the most effective treatment of paroxysmal

AF. Pulsed Field Ablation (PFA) is a promising new ablative intervention which uses irreversible electroporation to isolate electric triggers in pulmonary veins from the heart tissue and thus stops the arrhythmic beating of the heart. Although reported to be comparably effective and safer than conventional thermal ablation methods, questions remain, how PFA/electroporation affects cardiac tissue on cellular level and why arrhythmias sometimes reoccur in procedurally successfully isolated pulmonary veins.

In muscle (and cardiac) cells contraction/sarcomere shortening is preceded by Ca2+ release, triggered by an action potential, i.e. cell depolarization. In our study we used optical electrophysiology to explore how electroporation with different pulse parameters affects action potentials (AP), calcium transients (CaT) and sarcomere shortenings (SS) in primary rat ventricular cardiomyocytes. During the regular/continuous pacing resembling physiological pacing the three monitored signals were well synchronized and consistent in amplitudes and dynamics. We have then exposed cells to either conventional eight 100 μs monopolar pulses, eight bursts of 25 × 2 μs bipolar pulses with interphase and interpulse delays of 2 μs, or eight bursts of 40 × 200 ns monopolar pulses with interpulse delay of 100 μs. Bursts and 100 µs pulses were applied at 1 Hz. Irrespective of the waveform used, we observed the uncoupling between AP-CaT-SS with pulses that were below the lethal threshold. Uncoupling was reached at lower electric fields in cells that were oriented parallel to electric field, compared to perpendicular ones, when using the longest 100 μs pulses. Opposite was observed when cells were exposed to either 2 μs or 200 ns pulses.

The results further show that contractions of electroporated cardiomyocytes can occur even in the absence of APs, due to the AP-CaT-SS uncoupling, since Ca2+ ions can enter the cardiomyocytes through the permeabilized sarcolemma instead of voltage-gated Ca2+ channels. The effects of supraphysiological electric pulses on AP and CaT were described well with increased conductivity of sarcolemma in the Luo-Rudy model. Results that sublethal electric pulses can lead to uncoupling of AP-CaT-SS are important as they demonstrate that the disappearance of intracardiac electrograms, observed in PFA, does not necessarily indicate the absence of contraction.

OR-04

Electrogram loss is neither sensitive nor specific for durable lesion formation in PFA

Harikrishna Tandri[1], Shunsuke Uetake[1], Saleheldin Alhawamy[1], Parag Karmarkar[2]

[1]Vanderbilt University Medical Center, United States

[2]Johns Hopkins University, United States

Background: Loss of local electrograms occurs frequently during pulsed field ablation and has been affirmed as a measure of success. Whether this correlates with durable lesion formation is unclear at present. Furthermore, the determinants of local electrogram loss have not been determined.

Objective: To investigate energy determinants of electrogram loss during PFA and correlate that with durable lesion formation in an in-vivo porcine right atrium.

Methods: A 9 French focal ablation PFA catheter with 5 different closely spaced bipoles was designed and tested in vivo in a porcine model. The ablation electrode was designed as an Omni directional antenna to assess for contact. High-frequency electrical properties were monitored and calibrated to confirm electrode tissue contact. Sequential PFA pulses were applied from 250 V to 1000 V to the right atrial myocardium and electrogram changes were carefully recorded. Lesions were carefully catalog using EnSite mapping system. Lesions were assessed by cross histology using TTC staining 12 to 14 hours post ablation.

Results: A total of 8 lesions were assessed and 40 bipolar electrograms were evaluated. All lesions were delivered with adequate contact. Electrogram loss was voltage dependent. Voltage below 500 V did not result in complete electrogram abolition. Voltages > 750 V resulted in electrogram dimunition within 2 bursts of PFA. Of the 8 lesions, only 3 were detectable on TTC staining (250 V 40 bursts, 750 V and 40 bursts, 1000 V 20 bursts). Although 500 V lead to electrogram dimunition no lesion was observed.

Electrogram loss is neither sensitive nor specific for durable lesion formation in PFA. Electrogram loss routinely occurs with PFA once the voltage is greater than 500 V, however, this may not ensure durable lesion. Durable lesion was observed even with 250 V despite lack of complete loss of electrogram. Higher voltages i.e ≥ 1000 V can result in durable lesions with good contact along with electrogram loss. Alternate methods of lesion assessment are need to ensure durable lesion delivery intraoperatively.

OR-05

Arrhythmogenicity of monophasic and biphasic PFA waveforms in a porcine model

Tugba Kumru[1], Lars M. Mattison[1], Atul Verma[2], Khaldoun Tarakji[1], Daniel C. Sigg[1]

[1]Medtronic , United States

[2]McGill University, Canada

Intorduction: Pulsed Field Ablation (PFA) has emerged as a novel energy modality for addressing cardiac arrhythmias through irreversible electroporation [1]. While PFA is widely used in pulmonary vein isolation and the treatment of atrial fibrillation, its applicability and arrhythmogenic potential in ventricles remains less explored. There has not been a direct comparison of the arrhythmogenic potential of biphasic and monophasic pulse wave deliveries. The aim of the present study is to compare the arrhythmogenic potential of an atrial loop catheter (PulseSelect™ pulse field ablation (PFA) system , Medtronic, USA) delivering either biphasic, bipolar or monophasic, bipolar PFA energy to porcine ventricles.

Methods: Two swine (68.1 kg and 65.4 kg) were anesthetized, and arterial pressures and ECG measurements were recorded after establishing vascular access. No anti-arrhythmic medication was given. After the PulseSelect™ catheter was advanced into the right ventricle (RV), two different matched PFA waveforms were tested: a monophasic and a biphasic waveform. PFA energy with a delivery sequence of monophasic-biphasic-monophasic (n=2 RV positions) or monophasic-biphasic (n=3 RV positions) was delivered relative after detection of the R-wave in 10 ms increments. The energy administration began before the onset of the T wave, extending through the duration of the T wave or until the onset of ventricular fibrillation (VF), depending on which occurred first. If VF was induced, the animal was resuscitated while keeping the catheter in place, utilizing external defibrillation through patch electrodes.

Results: In two RV positions, VF was induced during monophasic pulse wave deliveries but not during biphasic pulse wave deliveries 270/300ms and 310/320ms after R-wave detection respectively. And in 3 RV positions, VF was induced during monophasic but not biphasic PFA application. In summary, VF was induced in 7/7 monophasic PFA deliveries during the vulnerable period of the T-wave and 0/5 biphasic energy deliveries. Even though utilizing the consistent delivery parameters including identical voltage, pulse width, delivery location, and number of pulses for both monophasic and biphasic deliveries, delivering biphasic PFA applications did not result in VF, highlighting the notable effectiveness of the biphasic approach compared to monophasic delivery methods.

Conclusion: The objective of this study was to evaluate monophasic and biphasic PFA waveforms for arrhythmogenic risk. The tested waveforms had identical amplitude, pulse widths, delivery location, and number of pulses. Monophasic PFA waveforms consistently induce VF when applied in porcine ventricles during the T wave, while biphasic PFA waveforms do not. This emphasizes the distinct advantage of biphasic delivery over monophasic delivery, highlighting the significance of waveform selection.

REFERENCES:

[1] Verma, A. et al., Circ. Arrhythm. Electrophysiol. 15, e010168 (2022).

OR-06

Surgical Ablation of Cardiac Tissue with Nanosecond Pulsed Electric Fields

Christian W. Zemlin, Jakraphan Yu, Ralph Damiano

Washington University in Saint Louis, United States

Ablation of cardiac tissue creates nonconduct-

ing lesions that either disrupt common pathways of reentrant arrhythmias or electrically isolate known sources of arrhythmic activity, for example the pulmonary veins. In current clinical practice, cardiac ablation is performed thermally, either by heating tissue with radiofrequency (RF) currents or by freezing it with a cryogen. A rapidly developing alternative approach is pulsed field ablation (PFA) that relies on strong electric fields to disrupt cell membranes in order to ablate tissue.

We compared the results of several studies that we performed using nanosecond PFA to create lesions on porcine hearts (n=30 combined). All lesions were created with clamp electrodes with an open chest on the beating heart, similar to clinical practice for surgical ablation of cardiac tissue. In the atria, 5 to 8 lesions per animal were created using pulse amplitudes up to 15 kV; some of our acute studies also included ventricular lesions. Ablation time was 2.5- 6 s for atrial and 3-6 s for ventricular lesions. The study durations ranged from acute to 6 months survival. Some lesions were placed across the mitral and tricuspid valves in order to assess the effect of nanosecond PFA on valve tissue and valve function (using echocardiography) as well as coronary arteries. Transmurality of lesions was assessed using triphenyl tetrazolium (TTC) stains as well as histological trichrome stains on multiple sections per lesion. In one acute study (n=6) exit block testing was conducted to assess whether the lesions electrically isolated pulmonary veins and atrial appendages as intended, both immediately after lesion creation and following a delay of 2 hours.

Nanosecond PFA achieved excellent transmurality, with more than 99% of the (add # of histologic sections examined) atrial sections transmural even for the shortest ablation times (2.5 s). Lesion width was highly consistent across the myocardial wall. Even ventricular tissue up to 12 mm thick was reliably ablated. Exit block was confirmed for 96% of lesions at lesion creation and 96?ter a delay of 2 hours. Our preliminary results suggested no effect of PFA on valve function and no histological changes in the treated valves and coronary arteries. Hearts harvested at 4 to12 days post-ablation showed a strong inflammatory response with immune cell infiltration of the treated tissue. The safety profile was excellent with no arrhythmias induced during or following PFA application.

In summary, acute and chronic porcine studies have demonstrated the ability of nanosecond PFA to quickly and reliably create transmural lesions without inducing arrhythmias. This is a promising technology which may allow for more efficacious and less invasive surgical ablation.

P5 - Electroporation and cellular processes and pathways

Monday morning Track B

Sep 16, 10:30 - 12:00

OR-07

Facilitation of gelonin cytotoxicity with electroporation and its prospects for electrochemotherapy

Olga N. Pakhomova, Andrei G. Pakhomov

Old Dominion University, United States

Gelonin, derived from the plant Gelonium multiforum, is a ribosome-inactivating protein (RIP) known for its potent cytotoxicity. It functions enzymatically, requiring only a few molecules to kill a cell. At the same time, gelonin poorly permeates into intact cells. The combination of extreme intracellular cell killing efficiency with a low systemic toxicity makes gelonin a promising candidate for electrochemotherapy-type ablation treatments.

We compared the cytotoxic efficiency of electroporation with and without gelonin in the medium in cultured CT-26 cells (colon carcinoma) and T24 cells (urinary bladder cancer). The cells were suspended in the growth medium and exposed in electroporation cuvettes to different numbers of 9 us, 2 kV/cm, 10 Hz electric pulses. Cell survival was measured with the Presto blue metabolic assay at 24 and 48 hours after the EP treatment. In CT-26 cells, 100 nM gelonin reduced the EP number to kill 50% of cells (LD50) at least 20- and 30-fold for the 24- and 48-hr timepoints, respectively.

Cytotoxic efficiency of different gelonin concentrations was compared after electroporation with 10 EP (9 us, 2 kV/cm, 10 Hz), a low dose that had

no cytotoxic effect by itself. Gelonin concentration that reduced survival by 50% (IC50) was only 5 nM. Without electroporation, even the highest gelonin concentration we could test (1,000 nM) reduced cell survival by only 10-15% (not statistically significant).

These promising in vitro data justify further studies of electroporation-mediated gelonin delivery into tissues. Compared to the conventional electrochemotherapy with bleomycin and cisplatin, the cytotoxicity of gelonin does not rely on cell division. This could make it suitable for ablation of tissues and tumors which are poorly sensitive to the conventional electrochemotherapy. It could have a niche for ablation of slow-growing cancers, prostate hyperplasia, as well as for the cardiac pulsed field ablation. Gelonin toxicity can also be utilized to reduce the electroporation dose, thereby minimizing heat production and adverse neuromuscular side effects of EP treatments.

OR-08

Concomitant Electrotransfer of Small and Large molecules

Ruta Palepšiene, Salvijus Vykertas, Ernestas Urbanskas, Justinas Venckus, Martynas Maciulevičius, Paulius Ruzgys, Baltramiejus Jakštys, *Saulius Šatkauskas*

Vytautas Magnus University, Lithuania

It is well described that the electrotransfer of small and large molecules, like nucleic acids, into cells is governed by distinct mechanisms. In both cases, the critical steps are cell electroporation and electrophoretic forces that drag the molecules to or through the electroporated membrane. In the case of small nucleic acids, it is believed that electrophoretic forces drag the molecules to and through the membrane directly into the cytosol, while in the case of pDNA, electrophoretic forces drag the molecules to the electroporated membrane, where the molecules form pDNA aggregates. These aggregates are then uptaken by the cell, employing endocytotic pathways. What is impact of presence of other, especially large molecules on electrotransfer efficiency of other molecules remains unclear. Recently we showed that presence of pDNA in electroporation medium has a positive effect on efficiency of electrotransfer of bleomycin molecules. On the other hand, we showed that plasmid DNA inhibits the electrotransfer of labeled siRNA and oligonucleotides. The mechanism of this inhibition is not fully understood. Some hints can be extracted from the findings showing that inhibition of electrotransfer of oligonucleotides occurs on both sides of the cells. i.e., facing the anode and the cathode. Stronger inhibition occurs at the cell side facing the cathode. The analysis shows that this is related to the occlusion of the electroporated membrane with pDNA aggregates, which form predominantly on the cell side facing the cathode. The inhibition on the cell side facing the anode is presumably related to the aggregation of small oligonucleotide molecules and pDNA. In addition to these results, we performed an analysis of the concomitant electrotransfer of pDNA of different sizes as well as the impact of proteins on the delivery of nucleic acids.

OR-09

Extracellular DNA enhances cell membrane damage stimulated by electrical short-circuiting via an aqueous droplet in dielectric oil

Hirofumi Kurita, Yoshino Tsurusaki, Rika Numano

Toyohashi University of Technology, Japan

We have been investigating an electroporation method using electrical short-circuiting via a cell suspension droplet in dielectric oil. An aqueous droplet of a few microliters placed between a pair of electrodes can be deformed by an intense DC electric field depending on the electric field intensity. When a droplet containing suspended cells and plasmid DNA elongates during deformation and connects the electrodes, the resulting short-circuiting can cause successful gene electrotransfection into various mammalian cells. We also investigated the mechanisms of gene electrotransfection using short-circuiting [1] and the influence of the electroporation medium on membrane permeabilization [2]. The short-circuiting using a low-conductivity electroporation medium enhanced transient and irreversible membrane pore formation. Taking these investigations into account, we aimed to investigate the influence of

the conductivity of the electroporation medium on the gene electrotransfer. As a result, the short-circuiting using a low-conductivity medium with plasmid DNA succeeded gene electrotransfer and resulted in a significant decrease in cell viability compared to the high-conductivity medium with plasmid DNA. However, short-circuiting using the low-conductivity medium without plasmid DNA did not show a decrease in cell viability. Thus, we demonstrated the influence of extracellular DNA on cell membrane damage stimulated by droplet electroporation using a low-conductivity medium. The electrical stimulation with the combination of plasmid DNA and the low-conductivity medium resulted in tremendous membrane damage. Linearized plasmid DNA stimulated more significant membrane damage than circular DNA. However, the size of linear DNA did not influence the efflux of small intracellular molecules. Although further investigations are required to prove the detailed mechanism observed here, this study may have implications for understanding the transportation mechanism of electroporation.

[1] H. Kurita, et al., PLOS ONE, vol. 15, e0243361, 2020.
[2] Y. Watanabe, et al., Sensors, vol. 22, 2494, 2022.
[3] Y. Tsurusaki, et al., PLOS ONE, vol. 18, e0285444, 2023.

OR-10

The inhibition of electrotransfection caused by the simultaneous transfer of multiple types of plasmid DNA

Ernestas Urbanskas, Baltramiejus Jakštys, Paulius Ruzgys, Salvijus Vykertas, Justinas Venckus, Saulius Šatkauskas

Vytautas Magnus University, Lithuania

Over the past decade, there have been significant advancements in safely delivering therapeutic compounds to tissues and organs using electric pulses. Gene electrotransfer (GET) has emerged as an effective method for facilitating the transfer of naked plasmid DNA (pDNA). However, the need to transfer two different genes prompts the question of the most effective approach to achieve this. Various methods for GET exist for different genes, including using two pDNA simultaneously, using a single pDNA containing two different genes, or transferring pDNA separately. The primary objective of this research was to evaluate the effectiveness of gene electrotransfer (GET) by simultaneously transferring plasmid DNA (pDNA) of different sizes and comparing it with separate transfection using the same plasmids.

Experiments were conducted utilizing the Chinese hamster ovary (CHO-K1) cell line, employing 1.2 kV/cm electric field strength, 100 μs and 1 – 5 pulses to assess the GET, by using two sizes of pDNA. The efficiency of transfecting plasmids coding for Green Fluorescent Protein (pEGFP-N1, 4.7 kb) and Red Fluorescent Protein (mCardinal, 6.2 kb) was examined by quantifying the count of transfected cells through the employment of a flow cytometer (AccuriTM C6, USA) 24 hours after the application of high voltage (HV) pulses. Additionally, the viability of the cells after GET was determined using the MTS assay.

Applying 1.2 kV/cm, 100 μs 5 pulses reached the highest transfection efficiency for both used pDNA's. Although, pEGFP transfection was around 4 times higher than mCardinal. Using same pulse parameters for simultaneous transfer of both pDNA's resulted in drastic decrease of transfection efficiency. Upon the simultaneous cotransfer of both pDNA the pEGFP transfection reached 10 % (as compared to 62 % of transfection efficiency when single pEGFP electrotransfer was performed). mCardinal transfection dropped from 16 % to 8 % respectfully.

Our findings indicate that transfection of smaller pDNA is more effective. Transfection efficiency of both plasmids is decreased by transferring both pDNAs at the same time; however, transfection efficiency of small plasmids decreased by 6 times, whereas transfection of mCardinal decreases only by 2 times.

OR-11

Role of the actin cortex in intracellular transport of electrotransferred DNA cargo

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Genome editing methods can precisely alter mammalian cell genomes, driving cell biology advancement and promising treatments for congenital diseases. Efficient genome editing for therapy involves delivering cargo such as nucleic acids and editing enzymes to cell nuclei by breaching the cell membranes. Electroporation is a technique that can permeabilize and induce pores in the cell membrane using high-voltage pulses. Electrotransfer describes the translocation of cargo from outside the cell, into the cell after electroporation. However, clear understanding about the mechanism behind this transport is lacking, which hinders efficacy in clinical applications. While prior research studied the active and passive intracellular transport of genetic cargoes [1], this research highlights the overlooked impact of the DNA cargo size during gene electrotransfer.

There are multiple intracellular pathways for DNA cargoes to reach the nucleus [1]. Previous research has shown that internalized DNA cargoes undergo anomalous diffusion [2]. This work extends on testing the dependency of the diffusion coefficient and transport behaviour of electrotransferred DNA cargo on the DNA size for different clinically relevant cell types [2].

Additionally, this ongoing study aims to uncover how the actin cortex (a network of filaments that strengthens the cell membrane) contributes to the transport of nucleic acids in the cytoplasm after transfer across the cell membrane. This is pertinent due to the actin cortex's effect on the resealing dynamics of electropermeabilized membranes [3], and its unclear role in intracellular transport after electroporation.

Using inverted fluorescence microscopy, time-lapse images are taken to track the movement of fluorescently labelled pEGFP-C1 aggregates in Chinese Hamster Ovary cells [2]. Results show DNA aggregate complexes interacting with the cell membrane after a unipolar electric field has been applied. The intracellular transport has been analysed through single particle trajectories and mean squared displacement, and gives insights on the anomalous diffusion regimes involved.

Thus far, we can conclude that at short lag times, most of the DNA cargoes show caged or subdiffusive behaviour [2].

Future experiments will internalize larger plasmid DNA cargoes into mammalian cells. Of these cargoes, some will contain DNA segments encoding proteins with specific genetic functions. These nucleic acids will be electrotransferred into a range of cell types such as benign, malignant, and metastatic breast carcinoma cell lines.

[1] Rosazza et al. (2013). "Intracellular tracking of single-plasmid DNA particles after delivery by electroporation." Molecular therapy, 21(12), 2217-2226.

[2] Muralidharan et al. "Intracellular transport of electrotransferred DNA cargo is governed by coexisting ergodic and non ergodic anomalous diffusion." BioRxiv (2021): 2021-04.

[3] Perrier et al. (2019). Response of an actin network in vesicles under electric pulses. Scientific reports, 9(1), 81

OR-12

Research on the behavior of annexin A4 protein after cell electroporation: insights into the active membrane repair mechanisms

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Increase in cell membrane permeability after cell treatment with short, high-voltage electric pulses (electroporation) is related with the formation of hydrophilic pores and ROS induced phospholipid oxidation. In any case, cell plasma membrane is permeabilized and its recovery plays a crucial part to sustain cell viability. It is known that proteins of annexin family are responsible for cell plasma membrane reparation processes. Specifically, one of the most abundant and most interesting annexin proteins is the annexin A4 protein. The

activity of annexin A4 protein is ATP independent, while Ca2+ is its ligand and precursor leading to polymerization of the protein and fusion with the positive phospholipids in cell plasma membrane forcing the membrane to curve, bend, and seal. Back to electroporation, it is known, that Ca2+ worsens the effectiveness of electroporation by inducing faster cell plasma membrane recovery. Considering all above, we intended to investigate the involvement of annexin A4 protein in cell plasma membrane recovery after cell electroporation and correlate this data to cell viability, and pore resealing.

We used MCF7 wild-type cells with intact annexin A4 gene and MCF7-AnxA4-KO cells with blocked annexin A4 gene expression. To visualize the annexin A4 activity in cells, we restored annexin A4 protein by transfecting MCF7-AnxA4–KO cells with Anx-A4-GFP plasmid. The MTS assay was utilized to assess the vitality of the cells, and flow cytometry was employed to determine the dynamics of electropermeabilization and plasma membrane repair by quantifying the amount of propidium iodide permeable cells. Fluorescence microscopy was used to monitor the activity of ANX-A4-GFP protein.

Results revealed that MCF7-WT cells better withstood the negative effects of electroporation in comparison to MCF7-AnxA4- KO cells. Secondly, the amount of permeable cells 35 mins after electroporation decreased in both cell lines signifying active cell plasma membrane restoring processes, which were more pronounced in MCF-WT cell line. Microscopy image analysis indicated that calcium had a drastic impact on ANX-A4 activity causing much faster translocation from the cytosol to the plasma membrane. To continue, we observed that annexin A4 translocation speed increased with pulse intensity. More interestingly, visual data showed that annexin A4 translocated from cytosol to plasma membrane in Ca2+ absent medium, while electroporation of cells in Ca2+ caused annexing A4 translocation not only to plasma membrane but also to nucleus membrane.

S05 - Intensification of fermentation processes by pulsed electric fields

Monday morning Track C
Sep 16, 10:30 - 12:00

OR-15

The role of PEF in enabling biotechnological processes for sustainable food processing

Claudia Siemer

Elea Technology GmbH, Germany

These days, sustainable food production is indispensable. All manufacturers strive to produce less waste, achieve higher yields through more efficient production methods, save energy, and require fewer additives such as water or extraction agents. PEF, as an innovative process, is currently widely used in various food productions, such as vegetable processing to make the production of products like French Fries or canned carrots more sustainable. Many publications and inquiries from the industry demonstrate the potential of PEF technology for biotechnological applications. For fermentation, PEF can be applied in both upstream and downstream processing, making it a highly attractive technology for industrial use. The presentation provides an overview of the current state of the art and offers insights into current industrial implementation for the use of PEF in biotechnological applications. During upstream processing, PEF can be used to induce a stress response in cells. The resulting effect varies depending on the strain and the goal. On the one hand, fermentation can be accelerated, and on the other hand, metabolic processes can be accelerated to selectively enrich target components within the cell. One example is yogurt cultures. After PEF treatment, the pH value decreases significantly faster during the fermentation, resulting in improved yogurt quality, particularly regarding syneresis. But also, the treatment of starter cultures for salami production shows process advantages due to a faster decrease in pH value. In microalgae, increased formation of fats was observed, leading to higher yields. In downstream processing, PEF can be used as a cell dis-

ruption method. Current industrial efforts aim on extracting oil from yeast, which is intended as a substitute for palm oil. Traditional cell disruption methods yield in low extraction rates and/or negatively affect the quality of the oil. In the field of microalgae as well, PEF can be used for cell disruption. Depending on the type of microalgae and the composition of its membrane, high yields can be achieved. A classic example is the extraction of the pigment phycocyanin from the spirulina algae, where the pigment can be extracted with high yield and purity. In summary, PEF technology has great potential for biotechnological applications due to its versatility. It can save materials such as solvents, shorten fermentation times, and save energy.

OR-13

Use of PEF for the extraction of oenological compounds and pigments from yeasts

Alejandro Berzosa, Javier Marín-Sanchez, Juan Manuel Martínez, Ignacio Álvarez, Ana Cristina C. Sánchez Gimeno, Javier Raso

Universidad de Zaragoza, Spain

Yeasts are eukaryotic microorganisms extensively utilized in the production of a wide range of fermented products such as alcoholic beverages, dairy products, and bread. Additionally, yeasts biomass generated during fermentation processes or specifically cultivated in different industrial processes is a rich source of valuable compounds such as amino acids, glutathione, proteins, nucleic acids, vitamins, mannoproteins, or β-glucans. While these compounds have diverse applications in pharmaceuticals, cosmetics, and the food industry due to their antioxidant, nutritional, and functional attributes, their significance in the oenological industry is particularly noteworthy. Glutathione has been proposed as a promising alternative to sulfur dioxide, while amino acids and proteins could serve as nutrients for fermentations, and mannoproteins contribute to the sensory properties of wine. More recently, yeasts have been recognized as extraordinary alternative sources of natural carotenoids and pigmented terpenoids with multiple applications, including astaxanthin production from X. dendrorhous and β-carotene production from Rhodotorula species.

Obtaining bioproducts from yeasts for various applications requires a release step to remove them from the cell, posing challenges due to the barrier effect of the cell wall and cytoplasmic membrane. Implementing strategies that permit selective and efficient release of desired products to achieve high recovery and reduced contaminants represents a subject of considerable interest for exploiting the bioactive compounds produced by yeasts.

Pulsed Electric Fields (PEF) technology has emerged as a promising technology for improving the recovery of compounds from yeasts cells. Recent studies have shown the potential of PEF for the development of a cascade extraction process that allows sequential extraction of compounds of interest from yeasts biomass based on their molecular weight. Electroporation of the cytoplasmic membrane enables the extraction of soluble compounds present in the cytoplasm (glutathione, amino acids, and proteins) in the first step. Subsequently, cell wall compounds (mannoproteins and β-glucans) can be extracted due to the hydrolytic action of the yeast's own enzymes.

Triggering endogenous enzymatic activity through PEF treatment has also been shown to be beneficial in improving the extraction of lipophilic pigments from yeasts, such as carotenoids and terpenoids. A previous incubation of the yeasts biomass after PEF treatment allows the extraction of these pigments from both fresh or dried biomass using green solvents such as ethanol or eutectic mixtures.

The aim of this presentation is to demonstrate how PEF technology may represent a valuable tool for the biotechnological industry in designing efficient processes for extracting bioactive compounds from yeasts biomass with minimal environmental impact.

OR-14

Technical implementation of nsPEF in industrial biotechnological cultures

Marco Stefan Fluri[1], Katharina Übelhör[1], Lya Siegenthaler[2], Lukas Neutsch[1], Leandro Buchmann[2]

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When advancing beyond the proof-of-concept stage towards industrial use, typical challenges of new technologies in entering the bioprocessing sector involve the need for validated scale-up / scale-down systems, and multiple other aspects related to standardization, data and IoT-integration, or physical interfacing. Applicability to biotechnological cultures calls for a design that meets stringent hygiene requirements and is equally compatible with sensitive cells, as well as harsh cleaning and sterilization protocols. Moreover, the range of environmental conditions encountered in bioprocesses is broad, for example in regard to cell concentrations (viscosity), media pH and conductivity levels, aeration rates (air bubble presence) in fed-batch cultures with high oxygen demand, and a high general bioburden with dense organic matter that renders tubing, connectors and valves prone to fouling.

This work presents field-proven implementation concepts and application data from a nanosecond Pulsed Electric Field (nsPEF) ecosystem tailored to rapid optimization and scaling in industrial biotech. Engineering aspects like electrical shielding of sensitive in-process analytical equipment, sterile interfacing in bypass loop configuration, and accurate fluid processing of shear-sensitive cell suspensions have successfully been solved. Next to the comparability of technical parameters, biological key characteristics of diverse origin have been compared to prove the validity of scale-down setups.

Our results underline the need for accurate pulse control and methods for compensation of conductivity effects. Moreover, we illustrate how and why the seamless interfacing of industrial nsPEF systems with bioprocess control software is an important asset for robust and efficient applicability. In combination with appropriate PAT tools and analysis routines, a straightforward transfer from shake flask scale to industrial bioprocessing settings is possible in such settings.

OR-16

Industrial application of nanosecond PEF

Leandro Buchmann
Bühler Group, Switzerland

Climate change, biodiversity loss and social inequality are major challenges facing society today. Ensuring food safety and security for a growing world population, while mitigating the negative impacts of the above, is a daunting challenge. Bioprocessing solutions have great potential to reduce the land and CO2e footprint of food systems. However, major step changes are required to sustainably reduce the unit cost of production. Nanosecond PEF has the potential to address some of today's challenges in industrial bioprocessing. Industrial bioprocesses with higher cell concentration or total biomass and product titre yields will be critical to the required transformation of the food system in the future. Buhler AG, in close collaboration with ETH Zurich, has developed Stellar™ technology based on nanosecond PEF, which, unlike conventional pulsed electric field applications, facilitates only the mass transfer of ions and small molecules. When applied precisely, Stellar enables non-invasive process optimisation without altering the target organism, in addition to or as an alternative to other optimisation methods. Stellar™ technology combines in-depth scientific knowledge with industrial process expertise to push the boundaries of bioprocess efficiency. Applicable across biological kingdoms, Stellar™ technology enables process intensification for yeast, bacteria, microalgae, cyanobacteria, fungi, mammalian and plant cell-based bioprocesses at scale with minimal energy requirements <0.5kWh/m3. Stellar™ technology offers flexible process integration from laboratory (<1mL) to industrial scale (>50m3).

OR-17

Enhancement of protein extraction and growth stimulation of microbial cells by µs PEF

Felix Schottroff
Boku University, Austria

Foods and food constituents are increasingly produced through fermentation and biotechnological methods, owing to their significant potential in

the transformation process towards more sustainable food systems. In this context, the utilization of pulsed electric fields (PEF) treatment is a promising approach for process intensification and optimization. Hence, this talk will give an overview on the topic and provide selected examples from research, considering the enhancement of microbial growth by PEF, as well as the selective recovery of valuable target compounds from microbial cells.

Growth stimulation by mild PEF treatment (0.5-5 kV/cm, 5-50 kJ/kg, 3-5 µs pulses, batch and continuous: 1 L/h) was demonstrated to increase the rate of biomass formation as well as the production of microbial target substances. Growth stimulation was carried out using the yeasts Saccharomyces cerevisiae and Pichia pastoris. PEF treatment led to an acceleration of fermentation time of up to 14 %, whereas CO2 formation (S. cerevisiae) and production of recombinant food proteins (P. pastoris) was increased by up to 8 %.

Selective extraction of recombinant proteins by continuous PEF treatment (10-50 kV/cm, 50-300 kJ/kg, 3-5 µs pulses, 1-1.5 L/h) was investigated using E. coli and P. pastoris. Product release, yield, as well as purity of the extract and viability of the cells were evaluated. It was shown that electric field strengths on the lower end of the spectrum were sufficient to obtain protein release (up to 89 %), whereas energy input levels in the higher range (corresponding to pulse repetition frequencies of up to 1 kHz) were beneficial. However, viability of the cells was increasingly maintained for lower energy input levels. Therefore, if preservation of viability is targeted, several consecutive treatments with lower energy input levels may be beneficial.

In summary, PEF treatment can be considered a promising building block for future sustainable food production. The technology has the potential to contribute to exploit and optimize microbial fermentation processes, ultimately leading to higher yields and purity of extracts.

S12 - Numerical modelling as an essential tool in electroporation research

Monday morning Track D
Sep 16, 10:30 - 12:00

OR-18

Estimation of spatial conductivity distribution within a tumor from impedance measurements and imaging following a simulated electroporation therapy protocol

Damien Voyer[1], Olivier Sutter[2], Clair Poignard[3]

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[3]Univ. Bordeaux, France

Electroporation-based therapies are becoming increasingly popular for treating cancerous tumors. Successful treatment is highly dependent on the electric field generated by the electrodes. In this context, it is essential to have a numerical tool for predicting electric field distribution, particularly in the case of deep-seated tumors. Imaging tools make it possible to build a precise geometric model of the patient, including the various tissues. However, fixing the electrical properties of tissues, and in particular their conductivity, is much more delicate.

In a recently published paper [1], we have shown that it is possible to combine computed tomography scans with electrical measurements made by clinical electroporators to estimate the electrical conductivity of patient tissues. The technique is based on the concepts of impedance tomography. The inverse problem was successfully solved with clinical data, exploiting electrical measurements of pre-pulses performed prior to electroporation pulses. However, the conductivity of the different tissues was assumed to be constant, which is a simplifying assumption.

In this study, we propose to solve the inverse problem to calculate the spatial distribution of conductivity, considering that conductivity may not be constant in a tissue. The difficulty lies in the fact that the number of measurements is small (there is no more than six electrodes around the

tumor) whereas the description of a conductivity distribution requires a thousand elements in 2D modeling, and even more in 3D. The key point is the choice of the penalty term, introduced as in traditional tomography problem [2]. Imaging provides us with valuable information about tissues that we can exploit to set the covariance matrix related to the penalty term.
In the numerical experiments we will present, we aim to reconstruct the conductivity in a tumor, considering that it is higher in the center due to necrosis. Six electrodes are placed close to the tumor. The geometric model is known, along with the position and shape of the tumor and the exact position of the electrodes (this information would be obtained by imaging). The impedance measurements are calculated by solving the forward problem (this information would be provided by the electroporator). Different strategies, including Tikhonov regularisation and Total Variation denoising, have been tried for calculating the spatial distribution of conductivity from the impedances and the geometric model. Numerical results show that the inhomogeneity of conductivity within the tumor can be reproduced.

[1] O. Sutter et al., “How Impedance Measurements and Imaging Can Be Used to Characterize the Conductivity of Tissues During the Workflow of an Electroporation-Based Therapy”, IEEE Transactions on Biomedical Engineering, Vol. 17, No. 4, 2024
[2] M. Vauhkonen et al., “Tikhonov Regularization and Prior Information in Electrical Impedance Tomography”, IEEE Transactions on Medical Imaging, Vol. 17, No. 2, 1998

OR-19

Finite Element evaluation of the electric field distribution in a cell-aggregates

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Electroporation of cells is obtained by applying pulsed electric fields generated by a sequence of voltage pulses. In the past, the influence of the electrical characteristics of the medium and cell density was studied experimentally and with Finite Element simulation. In particular, the simulation of inhomogeneities in terms of electric conductivity in the treatment area showed that the electric field distribution around the cells is modified with respect to the use of a homogeneous media. Moreover, cell aggregation evidenced a local modification of the electric field and transmembrane potential. In particular, accurate simulations of the extracellular environment have been demonstrated to predict more accurately the electric field around the cell membrane. Moreover, the electric field distribution is correlated also to cell permeabilization. This paper evaluates numerically the electroporation of cells considering aggregates of 9 or 25 cells. The cells are modelized as circles, with a given diameter, surrounded by a membrane with a thickness of 7 nm. The electroporation effect was simulated modelling the membrane layer with the Smoluchowski equation that estimates the number of pores as a function of the electric field intensity.
The 2D geometry of the Finite Element Model includes a square with a side of 1 mm where the circles that represent the cells are in the center of this area. The time varying electric field is applied by a rectangular voltage to two parallel faces that represent the electrodes. The pulse is 100 μs long and it is characterized by a 10 μs rise/fall time. The amplitude of the voltage is set to 100 V that is equivalent to 1000 V/cm in the center of the pulse in homogeneous conditions. A conduction problem and an electromagnetic time transient problem was solved using COMSOL software.
By using the numerical model, it is possible to estimate the intensity of the electric field and transmembrane potential in the examined region with cells. The effect of cell aggregates on electric field distribution and transmembrane potential values is compared to the effect obtained in the case of a single cell. Moreover, the transmembrane potential and electric field distribution with and without the Smoluchowski equation in cell membrane is evaluated. Simulation results will be validated with a set

of experiments with cells in adhesion.

OR-20

Finite element analysis model to predict electroporation of adherent cells in round, flat bottom wells

Patrizia Lamberti[1], Nicolas Mattei[2], Maria Evelina Mognaschi[4], Jelena Kolosnjaj-Tabi[2], Muriel Golzio[2], Marie-Pierre Rols[2], Elisabetta Sieni[3]

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In laboratory settings, parallel plate electrodes are commonly used to electroporate adherent cells grown in round, flat-bottom culture plates. This configuration selectively permeabilizes cells between the electrodes, with the permeabilization extent depending on electric field intensity. The electroporation yield is subsequently assessed visually by counting cells that uptake a fluorescent probe upon membrane destabilization, and this is a time-consuming process. An alternative strategy to determine electroporated cells would be useful to experimenters. As fluorescent cell ratio correlates with applied field, finite element analysis (FEA) can predict electric field distribution and cell permeabilization. This study thus aims to identify a method predicting electroporation efficiency across varying cell densities and pulse intensities using parallel plate electrodes.

We evaluated the effect of electric pulses, applied to adherent cells in round flat 96-well plates using stainless steel 4 mm wide plate electrodes with a 3 mm fixed gap. The protocol consisted of ten 5 ms long electric pulses with variable amplitude up to 800 V/cm. Two different electroporation media were used to perform electroporation: cell culture medium, supplemented with 10 ?S, 1% penicillin/streptomycin, or a low conductivity phosphate buffer. Cell culture medium is more conductive and a higher electroporation yield is expected. However, electric field distribution between electrodes may be uneven, resulting in non-uniform cell permeabilization.

The proposed work presents a technical theoretic tool capable to estimate the electroporation area under different electric field strength conditions considering a rectangle englobing the area between the electrodes. The tool is validated by in vitro experiments using the green fluorescent protein (GFP) expressing cells (HCT-116 and HEPA 1-6), pulsed in presence of 50 μM propidium iodide (PI) to evidence cell permeabilization. After electroporation, the bright field micrographs as well as the green (GFP, excitation of 480/40 nm, emission of 527/30 nm) and red (PI, excitation of 560/40 nm, emission of 630/75 nm) fluorescence micrographs were visualized and quantified. Experimental data are first used to build the FEA model. To each micrograph, a field of interest is fitted, comprising of a rectangle representing the width of the electrodes and the gap between them, and the PI positive cells are enumerated. Subsequently the analyzed area is used to build a predictive computing tool, which predicts the percentage of permeabilized cells in respect to a desired cell density.

This modeling strategy will allow reducing and refining in vitro experiments, exploring broader parameters and obtaining results in a faster and more cost-efficient way.

OR-21

Microdosimetric Study to Calibrate μsPEFs Application in Two Electrodes Technology for RISEUP Project

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The application of highly intense (kV/m) pulsed electric fields with duration of μs (μsPEFs) can increase cellular membranes permeabilization, extending also to the endoplasmic reticulum (ER) and leading to the intracellular calcium fluxes regulation [1]. This phenomenon is being leveraged within the RISEUP project [2], to foster stem

cells' differentiation in neuronal lineage, through an Electro Pulsed Bio-hybrid (EPB) device for the regeneration of the Spinal Cord Injury (SCI). Preliminary in vitro experiments on stem cells have been carried out through an exposure system comprising titanium faced electrodes, to fine-tune a stimulation protocol suitable for the project targets. Nonetheless, the final EPB device will employ a technology of golden planar and interdigitated electrodes, that induces an heterogeneous electric (E-) field distribution [3], different from the homogeneous one induced inside the faced electrodes. Therefore a calibration procedure, providing numerical estimate of the voltage intensity to be applied on the final EPB, becomes mandatory to achieve same poration effects on cells exposed inside the two systems. Both faced electrodes (with an inter electrodes distance of 2 mm) and a simulative core of interdigitated electrodes (as proposed in [3]) are modeled in Comsol Multiphysics v. 6.0. A realistic 3D Mesenchymal stem cell model, including cytoplasm, ER and nucleus, is placed inside both electrodes models and oriented with its major axis parallel to the electrodes. The multiphysics problem is reproduced in Comsol linking the electric current module, to simulate the μsPEFs application on the active electrode, and the Boundary ODEs and DAEs to implement numerically the pore formation dynamics [4]. Results will show that, considering a homogeneous E-field of 30 kV/m within the faced electrodes, a voltage of 4.5 V is required to be applied to the interdigitated electrodes to obtain a mean E-field of 30 kV/m on the electrodes' plane, between the electrodes. Furthermore, the stimulation results are in line, in terms of transmembrane potential, pore density and conductivity induced on plasma and ER membranes. Different rotations of the same cell, as well as a different cell shape, were considered, to confirm that the calibration is robust to different cells dimension and dispositions. In conclusion, this numerical study provided the EPB calibration in order to be used readily once its manufacturing process will be finalized.

[1] H. Hanna et al., "Electropermeabilization of Inner and Outer Cell Membranes with Microsecond Pulsed Electric Fields: Quantitative Study with Calcium Ions," Sci. Rep., vol. 7, no. 1, 2017.
[2] https://www.riseup-project.eu/.
[3] S. Fontana et al., "Electric field bridging-effect in electrified microfibrils' scaffolds," Front. Bioeng. Biotechnol., vol. 11, 2023.
[4] A. De Angelis et al., "Confocal Microscopy Improves 3D Microdosimetry Applied to Nanoporation Experiments Targeting Endoplasmic Reticulum,", Front. Bioeng. Biotechnol. 8:552261, 2020.

OR-22

Quantifying uncertainty of the numerical model of irreversible electroporation in the liver

Helena Cindrič, Damijan Miklavčič, Bor Kos
University of Ljubljana, Slovenia

The success of electroporation-based treatments depends on the complete coverage of the target tissue with a sufficiently high electric field, i.e. above the threshold value. Numerical models are an indispensable tool for investigating the electric field distribution in the tissue and form the basis for computer-aided treatment planning. However, numerical models usually contain several parameters with varying degrees of uncertainty. All uncertainties in the model parameters lead to uncertainties in the model results and thus influence the treatment plan. Uncertainty quantification can be performed to assess the robustness of the numerical model used for treatment planning.

The aim of this study was to quantify the uncertainty associated with parameters governing the nonlinear conductivity function that is usually used to model the phenomenon of electroporation. In particular, we sought to evaluate the robustness of a numerical model employed for the planning of irreversible electroporation (IRE) treatments in the liver.

We used COMSOL Multiphysics to create a 3D numerical model of liver tissue undergoing IRE using two stand-alone needle electrodes. In parallel, a series of in vivo experiments were performed on porcine livers mirroring the setup of the numerical model to obtain data for model development and validation. The collected liver samples were his-

tologically analyzed to delineate the zones of cell damage induced by IRE. We correlated the histologic samples with the electric field distribution calculated with the 3D numerical model and identified the electric field isocontours that best matched the damaged cell zones, i.e., we determined the IRE threshold that can be used for treatment planning.

For the uncertainty analysis, we used the variance-based Sobol method with a polynomial chaos-expansion metamodel. We quantified the relative contribution of each uncertain model parameter to the total variance of three key model outputs – the electric current, the IRE threshold, and the extent of the IRE treated zone.

The analysis showed that the most influential parameter affecting the electric current was the baseline electrical conductivity of the tissue, while the size of the IRE treated zone was more influenced by the conductivity increase factor and the onset of the conductivity increase.

OR-23

Simulation Investigation of Pulsed Magnetic Field-Induced Cell Permeabilization Using the Coupled Magnetic-Electric-Force Pore Energy Equation

Chi Ma, Yan Mi

Chongqing University, China

Pulsed magnetic field treatment has the capability to enhance cell membrane permeability, enabling larger molecular substances that were previously unable to traverse the cell membrane to enter the cell. This non-invasive treatment holds substantial promise for biomedical applications and represents a forward-looking medical approach. Nonetheless, the mechanism behind pulsed magnetic field-induced cell permeabilization remains elusive. Given the intricate coupling of electromagnetic fields in space, designing experiments solely to investigate the effects of magnetic fields and induced electric fields on cell membrane permeability proves challenging. Consequently, there is considerable importance in constructing simulation models through theoretical analysis to elucidate the mechanism behind pulsed magnetic field-induced cell permeabilization. However, current simulation studies primarily rely on traditional electroporation models, focusing solely on investigating the transmembrane voltage generated by the induced electric field from pulsed magnetic fields. As a result, it becomes challenging to compare simulation results with experimental findings. Building upon this foundation, our study further incorporates the deformation of the cell membrane induced by the electromagnetic stress generated by the electromagnetic field, thereby expanding upon the traditional electroporation model. Specifically, we employed the Maxwell stress equations to compute the magnetic and electric stress resulting from the pulsed magnetic field and induced electric field at the cell membrane surface. Subsequently, accounting for the impact of this stress on the cell membrane's surface tension, we modified the pore energy equation, thereby refining the traditional electroporation model. The conclusive simulation results demonstrate that the electroporation model founded on the coupled magnetic-electric-force pore energy equation provides a more accurate depiction of pulsed magnetic field-induced cell permeabilization. This establishes a solid theoretical groundwork for investigating the mechanism behind pulsed magnetic field-induced cell permeabilization, signifying its profound scientific importance.

Three Minute Thesis (3MT)

Monday young initiative 3MT

Sep 16, 13:00 - 13:40

Info: Look for the abstracts of the finalists in their respective sessions.

S03 - Microalgae biorefinery

Monday afternoon Track A

Sep 16, 13:40 - 14:40

OR-24

Microalgae Biocompound Extraction: Simulation and Experimental Based Analysis of Residence Time and Cell Suspension Characteristics for Consistent and Scalable Continuous Flow PEF Processing

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The increasing global population and arable land degradation have intensified the search for more sustainable bioresources. Microalgae, such as Auxenochlorella protothecoides, have emerged as potential alternatives in the bio-based economy, towing their minimal land requirements, rapid growth and rich biocompound content. However, traditional mechanical extraction methods often result in excessive cell debris, hindering efficient biocompound recovery. Pulsed Electric Fields (PEF) are a promising mild extraction alternative due to their extraction selectivity mainly through cell electropermeabilization. Nevertheless, the understanding of PEF processing parameters' influence on microalgal electropermeabilization remains incomplete, and most of the data has been generated in small batch setups. This knowledge gap poses challenges in upscaling biocompound extraction by PEF in relevant continuous flow systems owing to the dependence of the treatment outcome on the residence time distribution in the treatment chamber. Additionally, the microalgae suspension rheological behavior is affected by the residence time determined by factors such as cell concentration and temperature. The aim of this study was to achieve consistent microalgae electropermeabilization and protein extraction across PEF processing parameters, cell concentrations and scaled flow rates in continuous flow systems. The study used a combination of experimental methodologies and COMSOL Multiphysics simulations to accurately determine the residence time based on the experimental microalgae rheological data, while flow cytometry was used to assess cell permeabilization. The findings indicated that variations in suspension concentration and temperature minimally impacted residence time, with laminar flow being predominant. The research successfully achieved 90±5% of electropermeabilization (PEF processing parameters: 25 kV/cm, 6x15 µs square pulses, 80.54±3.5 kJ/kgsus) across the different flow rates and microalgae concentrations, enhancing flow processing capacity from 1 to 18 L/h. It also highlighted that while consistent electropermeabilization is achievable across different flow rates and microalgae concentrations with precise control of residence time, protein yields are strongly influenced by cell concentration. The highest protein extraction yield was observed at a concentration of 10 g/L, with yields dropping 40-50% at microalgae concentrations of 50 and 90 g/L. To optimize extraction, further research on an active extraction strategy post-PEF treatment for high-concentration suspensions is required. In conclusion, this research established a solid foundation for further upscaling of PEF technology in microalgae biocompound extraction. It provides insights and tools for designing consistent processes across various microalgae concentrations and flow rates, paving the way for more sustainable bioresource utilization and more efficient biocompound extraction from microalgae.

OR-25

Plasma-based extraction of compounds from the extremophile microalgae Galdieria sulphuraria

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The utilisation of microalgae as biogenic resource in the sense of a viable bioeconomy has become a growing field of interest. Microalgal metabolites can serve e.g., as resource for biofuels, chemicals, pharmaceuticals, cosmetics, or nutraceuticals. Nevertheless, the extraction of these compounds is challenging due to their robust cell wall. Standard extraction methods, e.g., bead-milling, sonication, chemical, or enzyme

extraction suffer from several problems. They are often ineffective against the cell wall, inacceptable heat development occurs, long treatment times are necessary, or environmental harmful solvents are used. Moreover, scaling of these methods for industrial application is often difficult or uneconomic, which is why microalgal compounds are still an economical niche.[1]

Based on our previous studies [2] [3] [4], we developed a spark discharge treatment system for the extraction of Galdieria sulphuraria, which is an extremophile microalga, to address the aforementioned obstacles. A semi-automatic setup was developed, which can treat volumes up to 1 litre within 30 minutes. Extraction processes were conducted with a pulse amplitude of 35-40 kV at 11 Hz, and a pulse length of 100 ns. The algae suspension was moved through the system by a peristaltic pump, which ensured uniformly treatment of the bulk liquid and made external cooling unnecessary. The biomass with an average dry weight of 0.8 g/L and volume of 500 ml was treated for 30 minutes. During the extraction process, the bulk temperature did not exceed 25 °C, which is preferable for thermolabile extractives, such as proteins or pigments.

After plasma treatment, samples were prepared for profiling flavonoids, fatty acids and the pigment phycocyanin with LC-MS, GC-MS and UV/vis measurements. Interestingly, the flavonoids compartment revealed a high number of lipophilic flavonoids, but only minor amounts of hydrophile flavonoids. Also, the fatty acid profile shows a wide range of interesting substances, which can be of industrial interest. The pigment phycocyanin, in contrast, is only detectable in minor yields, which contradicts to our previous findings. It is assumed that the cultivation conditions of Galdieria sulphuraria with regard to phycocyanin yields is highly sensitive towards light exposure and temperature.[4]

lipids for biofuels: processes and specific energy requirements.

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OR-26

Pulsed electric fields for efficient lipid droplet extraction from cell-wall deficient microalgae

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Microalgae are considered an under-exploited sustainable source of valuable macro-and micronutrients. Due to their fast, soilless growth and great metabolic diversity, they bear the potential to produce large quantities of valuable lipids and proteins independent of season. Like seeds, microalgae can store neutral lipids in lipid droplets. Such pre-emulsified oleosomes can be directly integrated into food or cosmetic products without the need of solvent extraction. Whilst suitable oleosome extraction technologies have been developed for lipid droplets from seeds, they are lacking for microalgae. This research focused on two technologies to aqueously extract oleosomes from a cell-wall deficient Chlamydomonas reinhardtii strain: pulsed electric fields (PEF) and NaCl treatment. Microalgae were grown under photoautotrophic conditions and lipid droplet production was induced by nitrogen limitation. Continuous PEF treatments (4-15 kV/cm, 5-30 μs, 3-9 Hz, up to 30 kJ/L) with square-wave pulses were employed, and the optimal electric field strength, pulse number and pulse duration were chosen based on a parameter screening. Aqueous lipid droplet extraction was possible after both PEF and NaCl treatment. These two technologies were compared in terms of total lipid yield (measured by GC-FID), color (measured spectrophotometrically), and protein content (based on nitrogen content) of the extract. The NaCl treatment led to an extraction of up to 62 % of the total lipids, while with the PEF treatment up to 46 % of

the total lipids were extracted. The PEF treatment led to whitish extracts, as opposed to greenish extracts from the NaCl treatment. Both processes led to a co-extraction of proteins (up to 60 % of the total protein). The PEF-based process was furthermore scalable and cyclic lipid droplet extraction was possible. Both PEF and NaCl treatment therefore showed great potential as oleosome extraction techniques for cell-wall deficient microalgae, allowing for a solvent-free microalgal biorefinery approach.

OR-27

Biological Signalling Supports Biotechnology: Cell Death Triggers Protein Release from Chlorella vulgaris

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Karlsruhe Institute of Technology, Germany

Multiple studies have demonstrated that following Pulsed Electric Fields (PEF) treatment, an incubation period exceeding 6 h in buffer is necessary to enhance the bio-accessibility of cell components such as proteins and lipids. This enhancement is primarily attributed to enzymatic processes activated during incubation, which facilitate the release of proteins and promote the solvent extraction of lipids from various microalgal species. The induction of this autolytic process can be triggered by various means, including dark anoxia, where microalgae are incubated in darkness for 24 to 48 h, or even by PEF treatment with very low specific energies (< 5 J/g). Notably, in C. vulgaris, there's evidence suggesting the presence of a specific cell death inducing factor (CDIF) of protein origin, which triggers cell death in intact cells. The prevailing concept suggests that CDIF plays a pivotal role during the algae incubation period post-PEF treatment, prompting cell death and subsequent autolytic processes, thereby enhancing the extraction process. The search for the identity of the CDIF led to the assumption that radical oxygen species (ROS) may play a decisive role as signalling molecules involved in many cell signalling pathways. To investigate this mechanism, we examined the intra- and intercellular ROS production during the incubation of fresh algal suspensions with algal extract containing CDIF, as well as with extract alone. While algal cell death induced by PEF treatment, even at low specific energies, resulted in a significant increase in intracellular ROS, no alterations in inter- and intracellular ROS were observed during cell incubation with CDIF-containing extract. This discrepancy may be attributed to the extract's antioxidant-rich composition, which not only contains CDIF but also suppresses ROS levels. This observation suggests that ROS depletion disrupts cell signaling pathways, ultimately leading to programmed cell death. To validate this hypothesis, we evaluated the cytotoxicity of antioxidants such as Trolox, glutathione, and ascorbic acid on algal cells at various concentrations. Surprisingly, ascorbic acid increased algal mortality over a 24 h incubation period and promoted the release of proteins, correlating with algal mortality. Based on these insights, we investigated the potential of enhancing the extraction process by combining PEF treatment with a subsequent incubation step in the presence of ascorbic acid. Our investigation unveiled a significant increase in protein yield compared to PEF treatment alone. This finding bears significance as the synergistic application of cell disruption and ascorbic acid not only enhances the yield of extracted cell components from algae but also shields them from oxidative degradation. This would allow for the development of new downstream processes that align with environmentally conscious practices, marking a significant step towards a more sustainable and efficient approach in biotechnological applications.

S08 - Public health risks and pulsed electric fields in the food industry

Monday afternoon Track B

Sep 16, 13:40 - 14:40

OR-28

Microbiological characterization of almond-based milk alternative and decontamination using pulsed electric fields (PEF)

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Background and objectives: While plant-based milk alternatives continue to grow in popularity, their microbiological quality and safety risks remain poorly understood. Furthermore, many plant-based beverages are processed at very high temperatures which could result in organoleptically and nutritionally inferior products. Pulsed electric field (PEF) is proposed as a gentle preservation technique that could help to retain heat-sensitive components while extending the product durability. The study aimed to identify the microbial contaminants in unprocessed almond beverage and evaluate the resistance of an indigenous lactic acid bacterial isolate in comparison to a laboratory-derived strain against PEF inactivation.

Materials and methods: Microbiological characterization was performed by plating the almond raw materials and unprocessed beverages on plate-count agar. After the isolation of phenotypically different colonies, the microbiota was identified via MALDI-TOF MS and/or 16S rRNA sequencing. Separate challenge tests were performed for model Lactiplantibacillus plantarum WCFS1 and indigenous Pediococcus pentosaceus to quantify the microbial resistance against PEF in almond beverage. Inactivation kinetic parameters were identified by using a Weibull type model.

Results: Pediococcus pentosaceus was the most frequently identified vegetative non-spore forming microorganism in the almond raw materials and unprocessed beverage. The microbial load and contaminants in almond beverage were dependent on the source materials, but also on the production practices prior to the decontamination step. PEF processing of almond beverage resulted in ≥ 5 log10 reductions of both model and indigenous lactic acid bacteria, with survival curves displaying downward concavity at similar processing conditions in terms of the electric field strength and specific energy at a defined inlet temperature.

Conclusions: The study offered insights into the type of microbial contaminants found in almond-based beverages. While PEF proved to be a promising alternative to thermal pasteurization, it will be crucial to define the target pertinent microorganisms as part of the risk assessment and process optimization, considering the species variability in microbial resistance.

OR-29

Electrochemical reactions as a side effect of Pulsed electric field treatment

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University of Salerno, Italy

Pulsed Electric Fields (PEF) is an advanced non-thermal processing method for achieving tailored cell disintegration of biological tissues or microbes. Various applications have been identified in several processes of food industry involving the inactivation of microbial cells in liquid foods (e.g., pasteurization), or mass transport phenomena of juice, water, and bioactive compounds from plant sources (e.g., extraction, expression, drying, osmotic dehydration, freeze-drying, freezing), or requiring structural modifications to facilitate some unit operation of food processing (e.g., peeling, cutting). The first industrial commercial applications have been achieved. However, several limitations still remain and have to be addressed prior to the full exploitation of PEF technology in food industry. Most of these limitations are related to the unavoidable occurrence of electrochemical reactions at electrode-food interface of a PEF treatment chamber, especially when typical PEF conditions (15-40 kV/cm) for pasteurization of liquid foods are applied. These reactions, especially those leading to corrosion and fouling of the electrodes, electrolysis of water, migration of electrode material components, and chemical changes of food product, must be minimized since may affect PEF commercialization through safety, quality, process efficiency, equipment reliability and cost aspects. In this frame, it should be also considered that the occurrence of these reactions is a very complex phenom-

ena, which is affected by several factors, such as circuit topology of the pulse generator, PEF chamber design and electrode material, PEF electrical parameters, as well as composition and chemical-physical properties of the treated products. In this work, the basic concept of electrochemistry along with a description of the electrochemical phenomena that occur at the electrode-food interface of a PEF treatment chamber are briefly described. Particular attention is devoted to the main side effects associated to the occurrence of electrochemical reactions and chemical processes. Finally, the main causes for electrochemical reactions are identified, and suggestions on how to limit the extent of electrochemical reactions and their side effects are also reported.

OR-31

Investigation of microbial strain variability resistance under PEF treatments and identification of the underlying molecular mechanisms

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Pulsed Electric Field (PEF) stands as an emerging technology, providing a compelling alternative to traditional thermal treatments. Typically, regardless of processing, microbial inactivation efficiency requires challenge tests employing selected target strains. In the realm of this study, our focus was twofold, firstly the identification of microbial targets resistant to PEF and secondly revealing the resistance molecular mechanisms for future optimization of the process.

This study commenced with a comprehensive investigation into the effects of PEF treatments applied with an electric field strength of 20 kV/cm across a range of total specific energies on microbial samples suspended in McIlvaine buffer at pH 7.0. A selection of 20 strains, originating from two pathogenic model microorganisms, Listeria monocytogenes and Escherichia coli, were systematically examined. Following this, RNA-Seq transcriptomic analysis was employed specifically for L. monocytogenes EGD-e with the primary objective of the analysis to discern the transcriptional profiles by comparing untreated and PEF-treated samples, and with the focused aim to elucidate the underlying mechanisms of microbial resistance.

The findings revealed that depending on the species and strain, microorganisms exhibited various resistances. PEF microbial resistance and strain variability data were correlated to the total specific energy applied. In general, L. monocytogenes strains identified more resistant compared to the E.coli strains in all the different PEF parameters, which may is related to the the thicker cell wall and stiffening lipoteichoic acids Gram positive bacteria have and the size of the microorganism. E. coli strains exhibited significant Log inactivation differences between the strains, under both 88 and 136 kJ/kg treatments with Log reductions ranging from 1.85 to 4.24 and from 2.19 to 4.59, respectively. In contrast, L. monocytogenes strains under both 88 and 136 kJ/kg treatments displayed no significant statistical differences and a <1.05 Log reduction. Under the highest PEF treatment parameter (184 kJ/kg), E. coli strains showed no statistical differences and a Log reduction ≥ 4.04 for all the strains. On the same condition, L. monocytogenes strains displayed significant statistical differences and L6 strain emerged as the most PEF-resistant strain. The transcriptomic analysis revealed a diverse array of genes as key players in the PEF stress environment, showcasing the dynamic up-regulation of glnR, lmo2419, tktB, and the synchronized down-regulation of rpmA and cspL.

Unraveling the molecular mechanisms responsible for the resistance under PEF treatments, particularly in the case of L. monocytogenes, can provide insightful information on developing customized Pulsed Electric Field (PEF) treatments. This could allow for better optimisation of processing parameters or identification of ideal hurdle combinations, whose synergistic effects can ultimately enhance strategies for elevating food safety standards while preserving the nutritional value of food products.

OR-32

Characterization of pulsed electric fields for in vitro tumor spheroids and metastatic invasion

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Georgia Institute of Technology , United States

Electroporation is a biophysical phenomenon where nanoscale hydrophilic pores are generated on a cell's plasma membrane when exposed to an applied external electric field. Irreversible electroporation (IRE) produces protracted nanopore formation, causing cell death [1]. A second generation of IRE, known as High-Frequency Irreversible Electroporation (H-FIRE), delivers shorter bipolar pulses in series of bursts to mitigate muscle contractions induced by IRE. Tumor spheroids, cancer cells cultured into a sphere (.01-2cm), can mimic in vivo conditions due to their spatial organization and cell-ECM interactions. Previously, tumor spheroids have been treated with electroporation to cause permeation for delivering chemotherapeutics [2] and high calcium concentrations [3] to assess cell death in a physiologically relevant model. Here, we aim to subject the tumor spheroids with lethal and sub-lethal pulse electric fields (PEFs) using various voltages and pulse durations to identify the extent of cell death and metastatic elimination/inhibition.

Human glioblastoma cells (U251) were used to form spheroids, seeded onto collagen hydrogels, and subjected to a uniform electric field. Day of treatment was denoted as day 0. A custom pulse generator was used to deliver 500-2000 V/cm of IRE or 1000-2500 V/cm of H-FIRE with 500 V/cm intervals. The biphasic PEFs had varying pulse widths of 1, 2, and 5 µs, but maintained similar interphase delays of 1 µs. The burst on-time and bursts delivered remained constant between IRE and H-FIRE at 100 µs over 100 bursts, respectively. Viability was measured using live/dead staining and an XTT viability assay at days 1, 3, and 5 post-treatments. Metastatic invasion from the tumor spheroids was tracked daily using Brightfield imaging and analyzed using ImageJ.

Tumor spheroids partially ablated during their initial treatment were found to gradually recover after 5 days during the study. The XTT viability assay revealed that the longer pulse durations, such as IRE and the 5-1-5, caused deeper penetration within the tumor spheroids producing immediate stark decreases in viability as the voltage increased. Shorter pulse durations such as the 2-1-2 displayed marginal cell death until 2500 V/cm, while the 1-1-1 presented minimal cell death across all PEFs applied. Only the longer pulse durations at the highest voltages applied were consistently successful in tumor spheroid elimination and complete metastatic invasion elimination. Metastatic elimination was consistently initially seen during treatment; however, metastatic inhibition varied depending on the level of penetration within the spheroids. Due to the presence of cancerous cells post-electroporation, adjuvant therapies such as chemotherapeutics or immunotherapies are optimal solutions to eradicate remaining malignancies.

OR-33

Study of irreversible electroporation-induced cell death in a 3D spheroid hepatocarcinoma model

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While radiofrequency ablation has been the main method to thermally destroy deep-seated solid tumors such as hepatocellular carcinoma, it cannot be used when tumors are located close to vital vessels or bile ducts. Alternatively, irreversible electroporation (IRE), consisting of the application of about eighty 100-µs long, high intensity pulsed electric fields (PEFs), has emerged as an athermal ablation method. However, anatomical barriers can hamper an optimal placement of the electrodes, yielding to inefficient IRE and consequent cancer relapse. In order to evaluate can-

cer cell potential to trigger an immune response at sub-therapeutic IRE pulses, we herein study the immunogenicity of IRE-induced cell death. If immunogenic, therapeutic immunostimulants could therefore be used to prevent relapse. In this study we assessed the growth of 3D multicellular spheroids after PEFs exposure. Then, we assessed the immunogenicity of the cell death by measuring apoptosis (through caspase 3/7 activation) and the release of major Damage Associated Molecular Patterns (DAMPs): ATP, HMGB1 and calreticulin (CRT).

Our IRE treatment consisted of eighty 100-µs long pulses with a repetition rate of 1000 Hz applied with two parallel plate electrodes, with PEF intensity varying between 0 and 2500 V/cm. Murine hepatoma-derived Hepa 1-6 cells (GFP-expressing or wild type) were used in this work.

Spheroids were treated with IRE and imaged with fluorescence microscopy for four days. Caspase 3/7 activation over time was assessed with a specific fluorescence kit. ATP, HMGB1 and CRT release were assessed by a bioluminescent assay, immunoblotting and immunostaining, respectively.

Partial IRE (transient loss of GFP and regrowth of residual cells) and complete IRE (full loss of GFP expression) were determined at 1500 V/cm and 2500 V/cm, respectively. An increased caspase activation was observed after treatment. Interestingly, the peak of activation occurred 6 hours after partial IRE treatment, and only 3 hours after complete IRE treatment. All DAMPs observed show an increased release correlating with the PEF intensity. HMGB1 was detected 6 hours but not 3 hours after 1500 V/cm treatment.

When the PEF intensity does not reach a sufficient level, residual cells are capable of regrowth, which might account for relapse in patients. Moreover, we saw that IRE induces ICD, and therefore has the potential to induce an immunogenic response. Notably, both caspase 3/7 activation and extracellular HMGB1 results highlight that the cell death is not only PEF intensity-dependent, but also time-dependent. Thus, the timing parameter should be considered in immunotherapy. Taken together, this work is in favor of the combination of IRE and immunotherapeutic agents. Additionally, these experiments serve as basis to develop mathematical models for prediction of spheroid growth and DAMPs release after IRE treatment.

OR-34

Acute efficacy and durability of in vitro pulsed field ablation in relation to the delivered energy impulse

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Background: Pulsed-field ablation (PFA) is a new method for catheter ablation of arrhythmias based on the destruction of cardiomyocytes by short, high-voltage electric pulses [1]. Electric pulse generators with different characteristics are now being developed for the PFA rate. The efficacy in terms of complete and durable tissue destruction may vary according to the intensity of the applied energy and the length and frequency of the pulses. Moreover, the durability of tissue destruction cannot be tested during in vivo human procedures.
Purpose: The aim of this study was to determine 1) the efficiency of inducing cell death in HL-1 cardiomyocytes inn relation to different PFA electric field strengths and 2) the durability of tissue desctruciton in 24 hours after the procedure.
Methods: A tumor line of murine cardiomyocytes was used for this study. PFA was conducted using a commercial TONAPULSE electrical pulse generator equipped with an electrode plate (TONAGENA, CZ). Cardiomyocytes were exposed to a burst consisting of 216 bipolar pulses lasting 2 μs, with 5 μs pauses in between of the pulses. Each burst was repeated 20 times with a 1 second pause between. Applied electric fields ranged from 250 V/cm to 1500 V/cm. The ratio of viable to non-viable cells was assessed at 1 hour and 24 hours post-electroporation with fluorescent microscopy.
Results: Electric fields ranged from 250 to 500 V/cm did not result in a any acute and sufficient rate cell death. Electric field of 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm induced cell death in 27 ± 6 %, 44 ± 6 %, 56 ± 7 %, and 75 ± 18 % of

exposed cardiomyocytes. After 24 hours and in relation to the PFA energy, the rate of destroyed cardiomyocytes was 16.5 ± 2.9 %, 37.3 ± 10 %, 50.3 ± 2.4 %, and 62.3 ± 15.3 ?ter exposure of 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm.
Conclusion: The minimum electric filed associated with any cardiomyocyte damage is 750 V/cm, but sufficient, acute myocardial damage is present after 1500 V/cm. The durability of cardiomyocyte death is very similar after 24 h confirmed the acute success rate, confirming minimal cell reparation, and excellent durability of acutely achieved lessions. The slight decrease of dead cells may be due to slight reparation of damaged cells or proliferation of cardiomyocytes due to their tumorigenic nature.

S01 - Medical applications of nsPEFs

Monday afternoon Track D
Sep 16, 13:40 - 14:40

OR-36

Nano-Pulse Stimulation initiates an immune response in several types of murine tumors

Richard Nuccitelli
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Nano-Pulse Stimulation therapy (NPS) applies electric pulses in the nanosecond domain to initiate regulated cell death in the treated tissues. This non-thermal therapy has been used to treat a wide range of murine tumors and has been shown to activate the immune system to inhibit the growth of rechallenge tumors as well as untreated, abscopal tumors when accompanied by the injection of some immune system stimulants into the treated tumors. Murine tumor types treated include melanoma, squamous cell carcinoma, lung carcinoma, breast carcinoma and pancreatic carcinoma. The energy required to ablate these tumors has been determined with pancreatic carcinoma and lung carcinoma exhibiting ablation with 240 mJ/mm3, lung carcinoma and squamous cell carcinoma requiring 360 mJ/mm3, and melanoma requiring 480 mJ/mm3. NPS therapy initiated a variable immune response as indicated by the rejection of injected rechallenge tumor cells with melanoma and hepatocellular carcinoma exhibiting the strongest response and lung carcinoma the weakest response.
The rejection of the rechallenge tumor indicates that an immune response specific to the tumor has been initiated, but ultimately, we want the immune system to eliminate metastases of the primary tumor. While metastases are difficult to routinely generate in mice, injecting a second, abscopal tumor at the same time as the primary tumor mimics the presence of a metastasis. We therefore studied the changes in an abscopal tumor following different treatments of the primary tumor. While NPS treatment alone does not affect the growth of the abscopal tumor, when it is combined with the injection of an immune system stimulant, we observe significant inhibition of abscopal growth. Our best result came from injecting the anti-OX40 antibody to agonize the function of the co-stimulatory T cell receptor, OX40. When we inject aOX40 into a Pan02 tumor following NPS treatment, up to 80% of untreated abscopal tumors were eliminated, suggesting that this immune response stimulation might be strong enough to go after metastases of the primary tumor.

OR-37

Distinct Tumor Immune Responses to Nanosecond Pulsed Electric Fields (nsPEFs) Determine Immunity

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Nanosecond pulsed electric field fields (nsPEFs) is a pulsed power technology that stores and then releases high-powered non-thermal electric pulses in nanosecond durations that induce in situ vaccination (ISV) after ablation of orthotopic rat N1-S1 liver (75-80%) and mouse 4T1-luc breast tumors (80-95%); however, not in the mouse melanoma (10-20%) models The studies here are designed to determine immune

mechanisms for nsPEFs in cancer models that do or do not readily induce immunity with ISV.

In 4T1-luc breast cancer, nsPEFs selectively targeted apoptosis-induction in activated T-regulatory cells (Tregs) and tumor-associated macrophages (TAMs) and eliminating myeloid-derived suppressor cell (MDSC) levels, showing relief of immunosuppression in local and systemic environments. There was a reduction in functional Treg suppression capacity, which is likely explained by decreases in activation markers (4-1BB and TGFβ) and a shift of Treg phenotype from predominantly activated (CD44+CD62L-) to naïve (CD44-CD62L+). This stronger nsPEF apoptotic bias for activated Tregs spared effector CD4+ and CD8+ T cells leading to a concomitant rise in effector CD4+ T cells and a 2.7-fold increase in the ratio of resident memory CD8+ CD103+ T-cells to CD4+ Tregs. These findings show nsPEFs effectively switch the TME and secondary lymphatic systems from immunosuppressive to immunoactive allowing cytotoxic T cell function and immune memory formation to eliminate cancer cells and account for the nsPEFs ISV.

Studies with nsPEF conditions that induce ISV in rat liver and mouse breast cancer models do not readily induce ISV in mouse melanoma. In the TME, there were increases in DCs expressing costimulatory molecules indicating the first step in an immune response, but memory T-cell numbers were low and likely anergic. Unlike the 4T1-luc tumors, Tregs, MDSC, and TAMs were not significantly increase on post treatment days 3 and 7. Overall, these responses allude to a narrow potential for immunity in the mouse melanoma model due primarily to immunosuppression. Studies indicate that nsPEF treated melanoma tumors co-incubated with carbon nanotubes (CNT) decreased the charging effect required for effective tumor elimination but had no effect on immunity.

OR-38

The effects of nanosecond pulses on cell growth and multi-drug resistance in pancreatic cancer cells

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Nanosecond pulsed electric fields (nsPEF) have been shown to exert anticancer effects. However, the mechanisms triggered in cancer cells by nanosecond-length pulses are not well known, especially when low sub-permeabilization voltage is used. In this study, various human pancreatic cancer cell lines were treated with nsPEF, and molecular changes at the cellular level were analyzed. We have used paclitaxel chemotherapy following nsPEF treatment and determined the correlation with the changes in the expression of multi-drug resistance (MDR) proteins. Finally, we examined the influence of nsPEF on the adhesive properties of cancer cells and the formation and growth of pancreatic cancer spheroids. Cell line response differed when applying a 200 ns, 100 pulses, 8 kV/cm, and 10 kHz PEF treatment. PEF treatment led to (1) the release of microvesicles (MV) in EPP85-181RDB cells, (2) electropermeabilization in EPP85-181RNOV cells, and (3) cell shrinkage in EPP85-181P cells. The release of MV's in EPP85-181RDB cells reduced the membrane content of P-gp and LRP, leading to a transient increase in the vulnerability of the cells towards paclitaxel. In all cell lines, we observed an initial reduction in the size of the cancer spheroids after the nsPEF treatment. Cell line EPP85-181RNOV exhibited a permanent reduction in the spheroid size after nsPEF. We propose a mechanism in which the surface tension of the membrane, regulated by the organization of actin fibers, modulates the response of cancer cells toward nsPEF. When a membrane's surface tension remains low, we observed some cells form protrusions and release MVs containing MDR

proteins. In contrast, the cell membrane is electroporated when cell surface tension remains high. The latter effect may be responsible for the reduced tumor growth following nsPEF treatment. Our findings underscore the clinical relevance of nsPEF as a novel therapeutic approach for pancreatic cancer, meriting further investigation into its role in modulating drug resistance and enhancing chemotherapy effectiveness. This study lays the groundwork for future clinical trials aimed at integrating nsPEF into existing treatment regimens, aiming to improve patient outcomes in pancreatic cancer care.

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OR-35

Are high-voltage nanosecond pulsed electric fields selective for cardiomyocytes?

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Nanosecond pulsed electric fields (nsPEFs) are an emerging cardiac ablation method based on electroporation. The nearly inevitable endothelial injury during the procedure may contribute to transient myocardial dysfunction, resulting in electrical and structural remodeling of the atria, a potential substrate for early arrhythmias recurrence.

Although studies suggest that cardiomyocytes are more sensitive to electroporation than endothelial cells, there is still a lack of data unequivocally confirming it. This study aims to compare the efficacy of nsPEFs on both cell types and explore the effect of fractionated nsPEFs on the therapy's selectivity.

We applied monolayers of HL-1 murine cardiomyocytes and MHEC 5-T murine endothelial cells and constructed a robotic system positioning stimulation electrodes orthogonal to the substrate. We measured cell viability and permeability using wide-field fluorescence microscopy and fitted the stained areas to a simulated electric field to obtain dose-response curves. Ca2+ transient imaging was performed to follow the permeabilization of internal cell membranes after exposure to nsPEFs. Furthermore, we validated the effect of nsPEFs fractionation in the ex-vivo murine model.

Our study showed that the permeabilization of plasma membranes for YO-PRO-1 dye is more efficient in endothelial cells than in cardiomyocytes. The calculated ED50 (electric field intensity which affects 50% of cells) for trains of 200, 300-ns pulses, 10 Hz was almost 50% higher for cardiomyocytes (8,05 kV/cm vs. 5.43kV/cm, p <0,0001). Differences in plasma membrane permeabilization did not reflect on ablation efficiency. ED50 for endothelial cells was only 8% lower (5,51 kV/cm vs. 5,98 kV/cm, p< 0,0001). The latter can be explained by the increase of cytoplasmatic Ca2+ after exposure in a Ca2+-free medium which was 2 times higher in cardiac cells, suggesting a superior efficiency of nsPEFs in intracellular compartment permeabilization.

Fractionation of nsPEFs (4 trains of 50 pulses, at 10 Hz, with 300-ns duration, each train separated by a 50-sec interval and 2 trains of 100 pulses with a 100-sec interval between trains) increased endothelial cell permeabilization. The ED50 was 12-13% lower after fractionated nsPEFs in comparison to a single train of 200 pulses, (p<0,0001). Consistently, the endothelial cell-killing effect increased by 6-11% (p<0,0001). Conversely, fractionation had neither an effect on the susceptibility of cardiomyocytes nor an effect on their plasma membranes permeability. Also, ex vivo studies showed no significant effect of fractionated exposure on the ablation area of the myocardium.

We have shown that myocardium and endothelium are similarly susceptible to nsPEFs, with a greater electroporative effect on cardiomyocyte intracellular substructures. Fractionation of exposure results in increased endothelial injury without altering cardiac ablation efficiency.

S22 - New waveforms and electric field management strategies for electroporation-based therapies

Monday late afternoon Track A

Sep 16, 16:10 - 17:40

OR-39

Fifty shades of Electroporation

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The range of pulse durations used in electroporation-based applications extends from a few nanoseconds (and even sub-nanoseconds) to tens of milliseconds. Traditionally, these pulses were used for specific applications: short, i.e. microseconds, to introduce small molecules (e.g. bleomycin and cisplatin) into cells in electrochemotherapy, and longer pulses in the millisecond range for gene electrotransfer. With the advent of nanosecond pulses arose the ambition to manipulate cells by electroporation of membranes of internal organs without damaging the plasma membrane. Later, high-frequency bipolar pulses came on to the stage with the promise of causing no muscle contraction nor pain.

It was shown that we can introduce small and large molecules in vitro with nano-, micro- (mono- and bipolar), and millisecond pulses [1,2]. A combination of electroporation with bleomycin and cisplatin was shown to be equally efficient by adapting pulse amplitude and/or number of pulses, and that the same number of molecules introduced by these various waveforms will result in a similar cell death. Gene transfer was also successfully performed with these waveforms, albeit with shorter pulses requiring higher pDNA concentrations. Irreversible electroporation by the same waveforms has also been demonstrated [3,4]. Similarly, DAMP release was obtained with these waveforms, suggesting that a similar immune response may be triggered in vivo – which was then also confirmed in vivo [5,6].

At the same time, claims (and proofs) are being made that delivery of high-frequency bipolar pulses is associated with less electrochemistry, nerve stimulation, and muscle contraction [7,8], although this has not been unequivocally demonstrated. With shorter pulses, and use of bipolar pulses, higher amplitudes and/or more pulses are required to compensate for less efficient electroporation by these new pulse waveforms. Higher amplitudes and a greater number of pulses can also significantly increase heating, so thermal damage can become significant.

To summarize, we can perform electroporation with the intention of treatment (ECT, IRE, GET) with nanosecond to millisecond pulses with monopolar and bipolar pulses, but with different pulse amplitude and number of pulses to achieve the desired degree of electroporation. However, other effects (electrochemistry, temperature, pain, contraction, …) will be different and, more importantly, they may not depend on the pulse parameters in the same way as electroporation. The choice of parameters thus requires more comprehensive optimisation than just the amplitude.

[1] https://doi.org/10.2478/raon-2024-0005
[2] https://doi.org/10.3390/app12168237
[3] https://doi.org/10.1007/s10439-020-02462-8
[4] https://doi.org/10.1016/j.bbamem.2016.06.024
[5] https://doi.org/10.3390/vaccines11061036
[6] https://doi.org/10.3389/fonc.2022.853779
[7] https://doi.org/10.1016/j.electacta.2020.137187
[8] https://doi.org/10.1038/s41598–022-12112-9

OR-40

Impact of pulse protocol parameters on the efficacy of electrochemotherapy in vitro

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Electrochemotherapy (ECT) is the combination of the electric field with a chemotherapeutic agent, usually bleomycin, or cisplatin, for the local treatment of cancer. The electrical protocol used in clinics (ESOPE) consists of the application of 8 mono-

polar 100 μs pulses at 1000 V/cm.
In clinical practice, the electric field amplitude fixed at 1000 V/cm could have some limitations, e.g., when the tissue resistivity is particularly low, current levels may exceed the limits of the device and treatment is interrupted, or when large tumour volumes are targeted, the electroporation (EP) can be achieved only by multiple electrodes repositioning.
In 2009, Ibey through in vitro experiments demonstrated that EP of the cell membrane depended on Absorbed Dose (AD) i.e., electric field amplitude and duration/number of applied pulses.
$AD = \sigma \cdot E^2 \cdot \frac{\tau(n,t)}{\rho}$
Where E is the local electric field, σ the conductivity of the tissue, ρ the density of the tissue, and τ the total duration of exposure to the electric field and a function of n and t (n, number of pulses and t, the duration of single pulse).
In this work, we aim at identifying the Reversible Electroporation Absorbed Dose (READ) value necessary to induce reversible electroporation of the cell membrane. The READ value could allow the use of different pulse protocols in which electric field amplitude, single pulse duration and number of pulses are combined to achieve reversible electroporation of the cell membrane and allow the diffusion of the anticancer drug into the cytoplasm.
The analysis of the impact that each component of the pulse protocol has on the effectiveness of ECT was performed as follows: first of all, N50, V50 and τ50, respectively the number of pulses, the electric field and the pulse duration resulting in a 50% reduction in the effectiveness of the ESOPE protocol, were identified. Then, the individual parameters of the pulse protocol were modulated to compensate for the loss of ECT effectiveness due to the application of V50, τ50 and N50. The experimental data collected were used to identify and validate the READ formula. Results from in vitro experiments will be further validated in relevant cancer animal models.
Based on the READ, pulse parameters will be modulated to identify new pulse protocols capable of covering larger tissue volumes and/or to reduce muscle contraction and pain associated with the delivery of electrical pulses.
This study will lead to the development of a new pulse generator that will integrate the READ formula for the identification of appropriate pulse protocols to obtain complete coverage of the target volume with sufficient energy to achieve effective electroporation. This technological advancement will increase ECT efficacy and flexibility of use, will optimize the number of electrodes needed to cover the tumour volume and will limit muscle contractions and pain reducing the invasiveness of the procedure.

OR-41

Voltage vs Current Control. Selecting the Best Delivery Strategy

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The outcomes of the electroporation-based therapies rely on the induction of the desired biological effect into the target area. For a defined electric waveform, the generated therapeutic effects are mainly dependent on the electric field magnitudes present at the tissue area. Proper control of the electric field magnitude distribution is crucial for the treatment successful, making the geometry, location, and energization strategies of the electrodes critical.

Since homogeneous effects over the therapeutic area are typically desired, confining the target tissue between parallel electrode plates at a constant voltage could be considered the optimal delivery approach. However, the inability to surround the target tissue between planar rigid electrodes limits this method to surface treatments. To overcome this limitation, alternative electrode geometries such as needles or endoluminal catheters have been used to place the therapeutic electrodes close to the target in a minimally invasive manner These electrode configurations often result in a non-homogeneous electric field distribution that requires additional consideration regarding delivery strategies. Specifically, the number of therapeutic electrodes (monopolar vs. bipolar) and the electric magnitude delivery strategy (voltage vs. current) are strategy approaches that plays a key role in the electric field distribution and should be care-

fully evaluated in relation to the different treatment environments.

Here, different electrode configurations and treatment environments will be defined to assess how various delivery strategies influence therapeutic outcomes. This analysis will underscore both the advantages and disadvantages of the different electric field management approaches across different scenarios. Such variability highlights the absence of a universally effective therapeutic strategy, emphasizing the importance of tailored approaches to optimize treatment effectiveness.

OR-42

Differentiating Electroporation Currents using Dynamic PEF Modeling

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Universidade Federal de Santa Catarina, Brazil

Distinguishing electroporation current from other macroscopic alterations is essential for advancing the understanding and control of electroporation in tissues. This study aims to identify this differentiation by employing a dynamic model of pulsed electric field (PEF). Tissue dielectric dispersion is described by a multipole Debye function, implemented in the time domain through auxiliary differential equations method. Furthermore, the electroporation effects were elucidated by establishing an electric field-to-transmembrane potential (TMP) relation. The time-dependent increase in tissue conductivity was assessed through extrapolation of a kinetic model of cell electroporation, while the conductivity-temperature dependency is modeled as a linear function. Experimental data collected from in vitro potato tuber (Solanum tuberosum) samples are utilized to implement the model using commercial finite element method (FEM) software. The results demonstrate the model's efficacy in distinguishing the electroporation currents from other macroscopic phenomena, thus offering a valuable tool for advancing the understanding and control of electroporation in tissue systems.

OR-43

Comparison of the Thresholds for Electroporation and Excitation for Pulses within Nanosecond–Millisecond Duration Range

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Exposure of cells with pulses of strong electric field can electroporate the cell plasma membrane, stimulate of excitable cells, or cause both effects. In some applications, e.g. non-thermal ablation of cardiac or tumour tissue with irreversible electroporation, excitation of muscle cells is not desirable, while in other ones, it would be better to avoid electroporation.

Theoretical analysis and experimental data available show that, depending on the membrane charging time constant, pulse strength and duration, the complex interplay of excitation and electroporation can be observed. Excitation with or without damaging of the cell plasma membrane due to electroporation can be achieved.

However, while carrying theoretical analysis, some minor simplifications were used. Meanwhile, these simplifications might be important for the detailed comparison of electroporation and excitation thresholds as for some pulse durations these thresholds are close to each other. The aim of this study was to analyse theoretically the dependence of the threshold for electroporation in more details within a wide range of pulse durations (nanoseconds–milliseconds) and compare these results with experimental data on excitation of neurons obtained earlier.

Electroporation of mouse hepatoma MH-22A cells was determined from the increase of the plasma membrane permeability to potassium ions. It was assumed that the threshold of electroporation corresponds to the formation of one pore. Theoretical dependence of the threshold of electroporation of a neuron on pulse duration were calculated based of the mechanism of electroporation. Parameters required were estimated from the experimental data obtained in mouse hepatoma MH-22A and Chinese hamster ovary cells. For the comparison with experimental data, the dependence of the threshold

for excitation of dissociated E18 rat hippocampal neurons, determined for single square-wave electric pulses with the durations from 100 ns to 1 ms obtained earlier was used.

The experimental excitation-duration curve can be approximated by the straight line in a log-log plot with the slope equal to -0.5, which remains the same for all range of pulse durations studied (from 100 ns to 1 ms). Meanwhile, the slope of the dependence of the electric field strength required to electroporate the cell on the pulse duration varies from -0.06 to -0.9 within the same range of pulse durations.

From this analysis, it can be concluded that the results of the exposure of neurons by a single square-wave pulse depend strongly on its duration:

1) For pulses longer than 10–20 μs the threshold for neuron excitation is much lower than that for electroporation.

2) Pulses with the durations from 200–300 ns to 10–20 μs, which cause neuron excitation, should also electroporate them.

3) Threshold for excitation becomes close to or even lower than the threshold for electroporation for pulses shorter than 200–300 ns.

OR-44

Study on Effect of Electroporation Using Amplitude Modulation Signal and Harmonic Addition

Borja López-Alonso[1], Tamara Polajžer[2], Matej Reberšek[2], Héctor Sarnago[1], Óscar Lucía[1], Damijan Miklavčič[2]

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The electroporation effects are highly dependent on the shape of the applied voltage signal. This shape can modify the transmitted power or the current path, affecting the distribution of the electric field or the temperature rise, among other effects. These effects, and their synergy with the phenomena of electroporation and electropermeabilization, have been studied in several areas such as the food industry, among others. However, in the medicine field, these effects have not been studied in depth, although there has been hypothesis about the effects of combining radiofrequency signals with traditional electroporation pulses. This is since the radiofrequency thermal ablative treatments use application systems, i.e., electrodes and generators like those of electroporation, and it is feasible to synthesize a combination of the voltage waves that are traditionally used in these treatments. In this way, these waveforms could improve the accurate application of treatments or to allow controlling the thermal distribution.

In this work, a proof of concept has been carried out by developing a power electronic system to study the effects of the combination of traditional radiofrequency and electroporation waveforms. For this purpose, printed electrodes have been used on slides with a separation of 100 μm, and a high frequency digital-to-analog converter together with a high-power operational amplifier have been used to generate the voltage signals. With this testbench, two strategies have been studied to combine the waves used in radiofrequency and electroporation: harmonic addition and amplitude modulation. The first is the direct addition of two pure harmonics, one with frequency in the electroporation range, and the other one with the frequency of typical radiofrequency treatments. The purpose of this signal is to increase the applied power with respect to a traditional electroporation signal to generate a controlled and homogeneous temperature increase that can improve the effectiveness of electroporation. The second strategy is the generation of an amplitude modulation wave by means of the interference of two harmonics with frequency in the radiofrequency range and amplitude in the electroporation range. In contrast to the previous strategy, this wave does not have the electroporation harmonic; however, it is thought that the membrane may demodulate the signal leading to the desired effects.

To investigate the proposed strategies, membrane permeabilization experiments were performed on CHO cells. Prior to the experiments, the cell suspension was mixed with propidium iodide (PI). 20 μl of the mixed cell suspension was then transferred to the electrodes, which were placed under the fluorescence microscope. Three minutes after electroporation, images of PI fluorescence were taken. The changes in PI fluorescence were stat-

istically analyzed to confirm or refute the initial hypotheses.
In conclusion a power electronics system has been proposed to perform a proof of concept to study the feasibility of combining electroporation and radiofrequency treatments.

P10 - Electrofluidic, microfluidic and lab on a chip

Monday late afternoon Track B
Sep 16, 16:10 - 17:40

OR-45

Microphysiological system for PEF treatment of mammalian cells with integrated oxygen and TEER sensors

Neringa Bakute[1], Eivydas Andriukonis[1], Kamile Kasperaviciute[1], Jorunas Dobilas[1], Martynas Sapurov[1], Gatis Mozolevskis[2], Arūnas Stirkė[1]

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Batch electroporation, a conventional method for PEF treatment, typically involves a cuvette with embedded electrodes. Although the use of commercially available cuvettes is a simple, routine procedure, it does have certain drawbacks, including distortions in the electric field, local pH variation, excessive heat generation, and the dissolution of metal ions leading to reduced electroporation efficiency and cell viability.
To address this, conventional batch pulsed electric field (PEF) technology has shifted toward the microscale and integration with microfluidic devices also known as 'lab-on-a-chip', 'organ-on-a-chip' and microphysiological system (MPS). In microfluidics, most soft-lithography microchips are made of polydimethylsiloxane (PDMS). An alternative to PDMS, an off-stoichiometry thiol-ene (OSTE), overcomes PDMS limitations while maintaining transparency, biocompatibility, and repeatability.
We present an MPS with an OSTE-based microfluidic chip for the PEF treatment of mammalian cells with the oxygen and trans-epithelial/endothelial electrical resistance (TEER) monitoring modules. A microfluidic channel is divided into two subchannels by the porous PC membrane and performs several functions in the microchip: (i) separates two flows in subchannels, (ii) serves as a substrate for cell adhesion, (iii) strengthens the electric field at the surface of the membrane.
We conducted the viability studies on rat glioma C6 cell in suspension using PEF treatment with various electric field strengths and 100 μs 1 Hz in our microchip. The PEF treatment in the microchip revealed a minor impact to the cells up to 10 kV/cm. For comparison, the viability after PEF treatment in a conventional cuvette decreased with increasing electric field, and the 5 kV/cm electric field was lethal. We assume the difference of PEF impact arises from Joule heating which is known to depend on the dimension of the electrodes. We have measured the permeability in microchip to DAPI and the saturation of permeability with 16 pulses at 1.8 kV/cm was observed. These results proved that the cells undergo membrane permeabilization during PEF treatment in the microchip with a very gentle impact on the viability of cells.
The performance of integrated sensors was studied with the application of an electric field. We monitored the oxygen concentration changes immediately after PEF treatment, and the oxygen difference between the two integrated sensors was evaluated. A slight change of oxygen concentration was captured with and without PEF treatment with cell suspension and not with buffer.
To conclude, our MPS for PEF treatment has several advantages: (i) a relatively easy soft-lithography process, which circumvents the shortcoming of PDMS; (ii) the electroporation of the cells ensures higher viability ((iii) monitoring of physiological parameters can be performed simultaneously. (iv) the same setup of our microfluidic chip can be used for electroporation of suspension.

OR-46

Study on the technique of inducing perforation of blood clots by pulsed electromagnetic fields

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While electroporation and magnetic perforation techniques are known for their ability to alter cell permeability, the current applications of this electromagnetic perforation technique are mainly targeted at biological cells or microorganisms, and few studies have applied this technique to thrombus therapy. In this study, we realized the perforation of blood clots in vitro by driving magnetic nanoparticles through a high-intensity pulsed magnetic field. First, we built a modular pulsed magnetic field generator with an energy feedback function, which consists of front-end energy-feeding circuits and discharge modules at various levels in cascade, and is capable of realizing flexible adjustment of the coil magnetic field. By utilizing the state change of the front-end feeder switch, the load coil energy can be fed back into the capacitors at all levels, thus outputting various forms of pulsed magnetic field waveforms such as long exponential waveforms and short triangular waveforms. Secondly, we built a three-stage modular pulsed magnetic field generator platform and carried out in vitro thrombolysis experiments using this platform. By outputting a 1T high-intensity, high-gradient pulsed magnetic field, magnetic particles were rapidly directed in the direction of the thrombus, thus generating magnetic force to induce perforation of the thrombus, which in turn changed the permeability of the blood clot. We documented this clot perforation and disruption phenomenon by white light microscopy and fluorescence imaging. Finally, pulsed magnetic force in vitro thrombolysis experiments were carried out, and by comparing the thrombolysis results of magnetic nanoparticles mixed with thrombolytic drugs under a pulsed magnetic field with those of a control group such as drugs alone, it was shown that the method of pulsed magnetic force-assisted thrombolysis can promote the thrombolysis efficiency, and it has a promising prospect for research and application.

OR-47

Dielectrophoresis – tool for analysis of mesenchymal stem cells differentiation

Ioan Tivig[1], Leslie A. Vallet[2], Romain Samiaa[3], Franck M. Andre[3], Luis M. Mir[3], Tudor Savopol[1], *Mihaela G. Moisescu*[1]

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[2]Université Paris-Saclay, France

[3]Centre National De La Recherche Scientifique, France

Electrical properties of mesenchymal stem cells (MSCs) along with their osteogenic and adipogenic progenies were evaluated at different stages of cells differentiation, using dielectrophoresis (DEP). The utilization of MSCs in therapeutic applications is increasingly significant due to their capabilities in cell replacement and their immunomodulatory effects. However, the differentiation protocols are protracted and might lead to incomplete or non-synchronous differentiation. It is essential to provide procedures which segregate during or post-differentiation, various cells for obtaining homogenous populations for clinical use.

In practice, a DEP force is obtained when applying a non-uniform AC electric field which manipulates a cell based on the polarizability differences between the cell and medium. The AC field frequency dependence of a factor called Clausius-Mossotti (which integrates geometrical and electrical properties of the cell) represents a DEP spectrum.

Human adipose-derived MSCs were subject to standard differentiation protocols towards osteogenic and adipogenic progenies. The assessment of differentiation was done weekly for 4 weeks, using Alizarin Red and Bodipy staining and biochemical tests (alkaline phosphatase and Oil Red O assays).

DEP spectra (20 frequencies between 10kHz and 40MHz) were acquired weekly during the differentiation process, using a 3DEP system (LABtech, UK). The DEP spectra were analyzed using an in-house developed program, OpenDEP (https://github.com/IoanTivig/OpenDEP_Compute.git) and electrical parameters of MSCs and their

progenies have been calculated. To simulate separations of MSCs from their progenies, Autodesk Fusion 360 was used to design a microfluidic channel (2 inlets and 2 outlets) embedding DEP electrodes, and COMSOL Multiphysics to simulate electric fields, fluid flow and cell tracking.

A notable reduction in both membrane permittivity and conductivity was observed after the initial week of differentiation for both progenies. These changes preceded the microscopical morphological modifications specific to differentiation and correlated with the biochemical tests results that confirmed the cells undergoing transformation. In subsequent weeks, the membrane permittivity continued to slowly decrease. Changes in cells' electrical parameters at the end of the first differentiation week indicate thus that the transformation process started.

Moreover, our simulations showed that these electrical changes enable the early-stage separation of differentiating cells using the DEP microfluidic channel, although those cells have sizes similar to those of MSCs. DEP separation facilitates two applications: purifying differentiated cells from mixed populations early in their differentiation process and isolating MSCs from other cells sources. This method is advantageous as it requires no cell labeling, utilizes low-cost lab equipment and consumables, and can begin in the first week of treatment.

OR-48

OpenDEP: A free-access platform for collecting and analyzing dielectrophoresis spectra

Ioan Tivig, Tudor Savopol, *Mihaela G. Moisescu*
Carol Davila University of Medicine and Pharmacy, Romania

Label-free living cell characterization procedures based on dielectrophoresis (DEP) offer significant advancements in cellular biology by preserving cell integrity. DEP is the phenomenon by which a dielectric polarizable object placed in an inhomogeneous AC field is experiencing a driving force exerted on the direction or in opposition of the electric field gradient (situations called positive or negative DEP, respectively). The AC field frequencies at which the DEP force is null, and a cell goes from negative to positive (or vice-versa) DEP behavior, are called crossover frequencies (fCO).

DEP leverages electric fields not only to analyze but to sort cells based on their electrical properties. These properties together with the geometrical ones are integrated into a Clausius-Mossotti factor, which is determined by cell and medium permittivities and conductivities. This factor's frequency dependence represents a DEP spectrum, crucial for identifying fCO. Cell sorting is enabled by creating conditions for different cells to be simultaneously either at fCO or under a DEP movement.

We propose a platform for DEP spectra acquisition and processing, named OpenDEP. It has two components: i/ a lab-on-a-chip (LoC) system integrating electrodes that allows the acquisition of microscopic images of cells distribution under AC field exposure, ii/ a software which, by images processing, computes DEP spectra and calculates cells electric parameters (https://github.com/IoanTivig/OpenDEP.git). The platform has been used for acquiring and processing DEP spectra of NIH3T3 and DC3F cells, and results were validated by comparison with those obtained with a commercially available device, 3DEP (LABtech, UK).

OpenDEP requires image acquisitions for each frequency to obtain a DEP spectrum. For the same number of AC frequencies, a spectrum acquisition by OpenDEP typically takes 30 min, compared to less than 1 min in the case of 3DEP. But the OpenDEP platform offers several significant advantages: a) Manufacturing Simplicity - the platform is produced using standard 3D printing techniques and features modular design, b) Optimized Chamber Design - allowing to focus under microscope simultaneously cells and electrodes, c) Robust Statistical Analysis - calculations are based on large cell populations, d) Effective Electrode Geometry - the indium tin oxide electrodes are design to generate a strong vertical DEP force, directing cells to the chamber's lower plane (artifacts due to gravitational deposition and light scattering are reduced), d) Sterile and Versatile Lab Conditions - the LoC may operate in a sterile environment and is compatible with various microscopy techniques

(e.g., fluorescence microscopy), e) Open-Source Software - facilitating broader accessibility and continuous customization.
The platform features collectively enhance the functionality and application scope of OpenDEP, making it a valuable tool for cellular experimentation.

OR-49

A Lab-On-Chip Based on Transferred Laser-Induced Graphene Electrodes and Machine Learning for Electroporation of Adhered Cells

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Lab-on-Chips paved the way for great improvements in biology research, thanks to integrating multiple facilities in a small environment. In this scenario, Laser-Induced Graphene (LIG) has recently allowed the generation of conductive geometries, which has been demonstrated to be an alternative solution for integrated metal electrodes in lab-on-chip experiments. In this work, we present a new Lab-On-Chip platform based on LIG electrodes, which were transferred on a biocompatible transparent polymer substrate (namely PMMA) for the electroporation of adherent cells detected by fluorescence and brightfield microscopy. The proposed platform was used to study the calcium intake and the pseudopods retraction on U-87 glioblastoma cell line after electroporation stimuli. Using a tailored machine-learning algorithm, the electrodes' design and the intensity of the applied electric field were adjusted to optimize the trends of calcium flow inside the single cell and its motility after the electroporation stimulus, demonstrating the potentiality and reliability of the proposed technique.

OR-50

Non-invasive vaccine/drug delivery and theranostics by microfluidics of shock waves

Nushin Hosano, Hamid Hosano

Kumamoto University, Japan

Recent progresses in nanosecond duration ultra-high voltage pulses provides exciting possibilities to produce direct intracellular effects. Meanwhile, we have been using ultrashort electrical pulses and miniature shock drivers to generate fine micro underwater shock waves, which can penetrate deep in soft tissue. Shock waves, as physical stimuli for cell manipulation, are of particular interest, as they can transiently increase the permeability of the cell membrane with less side effects. These unique characteristics make them appropriate for delivering energy for vaccine/drug delivery. Ultrasound and shock wave can be applied through totally non-invasive procedures taking advantage of focusing in soft tissue; appropriate for clinical therapy. We utilized micro-streaming of shock wave and cavitation to deliver liquid vaccine/drug to the depth of dermis. The paper will cover our recent progresses with shock waves and pulse ultrasound for minimally invasive pain-free needleless microfluidic vaccine/drug delivery.

P13 - General applications of electroporation for food processing

Monday late afternoon Track C

Sep 16, 16:10 - 17:40

OR-51

Validating pulsed electric field pasteurization of protein rich plant-based milk alternatives: a novel challenge trial approach

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Plant based milk alternatives (PBMA) are an emerging food category that suffers from technical challenges due to a tendency of plant protein (such as pea protein) to coagulate during thermal processing (e.g., UHT, HTST). Pulsed electric field treatment (PEF) with mild preheating has shown promise as an alternative pasteurization method to reduce these undesired thermal impacts. However,

the established procedures to validate this use of PEF through microbial challenge testing typically require a thermal presterilization of the product. PBMA containing heat-sensitive protein do not permit the application of sterilization, presenting a challenge to food producers looking to conduct such trials with PEF. As a solution, this work proposes an alternative approach that permits a reliable enumeration of inoculated surrogate organisms during microbial challenge testing of a PBMA without the need for presterilization.

An oat-based PBMA enriched with commercial pea protein isolate was prepared as a model food system and inoculated with two surrogate organisms (E. coli and Listeria innocua). Pasteurization was carried out by applying a preheating treatment (35-45 °C) followed by the continuous application of PEF at a constant field strength, pulse width, and flow rate (10 kV/cm, 20 μs, and 14 L/h, respectively). Pulse frequency was adjusted to modify the specific energy input to 97-245 kJ/L and the inactivation of inoculated surrogates was assessed using a combination of selective and non-selective plating techniques. Oxford and eosin methylene blue agar permitted a selective enumeration of E. coli and Listeria sp., respectively, despite the presence of native microbial contaminants, while total plate and spore counts on brain heart infusion, Luria-Bertani, and plate count agar indicated overall microbial reductions.

Using the selective enumeration approach, a minimum 5-log reduction in inoculated surrogates, required for treatment validation, was achieved via preheating to 35 °C followed by PEF treatment at 204 kJ/kg. L. innocua was more resistant than E. coli towards the effects of PEF, which demonstrated the importance of appropriate surrogate organism selection. No change in total plate and selective bacterial counts during a subsequent brief storage period (48h, 4 °C) was detected and indicated a limited occurrence of sub-lethal injury during PEF treatment. A significant presence of endospore forming bacteria was however detected and resisted PEF treatment, which will require attention during future investigations. Overall, our study showed that this unconventional approach can be successfully used to identify processing parameters for continuous PEF treatment combined with preheating to achieve a minimum 5-log reduction of inoculated surrogate microorganisms in PBMA containing heat-labile protein.

OR-52

Application of continuous pulsed electric field (PEF) treatment in human milk as an alternative pasteurisation technique

Yiting Wang, Farzan Zare, Negareh Ghasemi, P. Nicholas Shaw, Nidhi Bansal

The University of Queensland, Australia

Thermal processing, typically holder pasteurisation (HoP) has been used as a standard procedure to ensure the microbiological safety of donor human milk in numerous countries worldwide. The treatment involves heating the milk to 62.5°C for 30 minutes and often compromises the nutritional and antimicrobial components essential for newborn immunity. Hence, this study aims to explore the potential effects of pulsed electric field (PEF) treatment on donor human milk as an alternative pasteurisation technique. Donor human milk used in this study was sourced from anonymous consenting donors and stored in an ultracold freezer at -80°C. Before usage, milk samples from various donors were thawed in the refrigerator (4°C) overnight and pooled together. Escherichia coli JM109 bacterial culture was incubated for 24h to reach 10^9 colony-forming units per mL (CFU/mL) and was then subjected to two washes with sterile 0.85% (w/v) saline solution via centrifugation. The resulting E. coli suspension was then inoculated to either MilliQ water or donor human milk to achieve an initial count of approximately 8 log CFU/mL. Subsequently, 100 mL of raw human milk and E. coli inoculated samples were treated using a continuous PEF system with a flow rate of 100 mL per minute. This treatment involved the application of 240k pulses (60k pulses per step with a 5 min rest interval time between steps) at 20kV and 50Hz using a pulsed power generator capable of delivering energy up to 0.8 J. pulse-1 (with a 500Ω resistive load) and peak amplitude up to 30kV. Temperature measurements were conducted using an infrared thermal camera before and

after each pulsing step. The bacterial enumeration in the processed samples was performed using tryptone soya agar (TSA), with plates incubated at 37°C for a minimum of 48h before enumeration. Following the PEF treatment, the MilliQ water sample with an initial E. coli count of approximately 8 log CFU/mL exhibited a reduction of approximately 4.4 log. In comparison, the E. coli inoculated human milk with a similar initial bacterial count, showed a reduction of approximately 5.3 log. The raw human milk sample initially contained 10^1 CFU/mL and showed complete microbial inactivation post-PEF treatment. Throughout the process, all samples were maintained at a temperature below 35°C. This study explored the potential of PEF treatment as a non-thermal alternative for donor human milk pasteurisation while retaining its beneficial properties. The continuous PEF treatment effectively pasteurised 100 mL of raw human milk sample and achieved up to 5 log reduction in donor human milk inoculated with 10^8 CFU/mL E. coli at a significantly lower temperature compared to thermal treatment, potentially preserving the integrity of the essential milk.

OR-53

Comprehensive Analysis of Heat-Assisted Pulsed Electric Fields and Conventional Thermal Treatment for Orange Juice Pasteurization: Cost, Energy Efficiency, and Sustainability Assessment

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This study compared commercial heat-assisted Pulsed Electric Fields (PEF) and High-Temperature Short-Time Treatment (HTST) technologies for pasteurizing orange juice, focusing on cost, energy efficiency, and sustainability metrics. The investigation encompassed a broad range of thermal recovery efficiencies ranging from 0 to 65% for both the PEF and HTST processes. Additionally, the study examined the impact of varying inlet temperatures of the juice to the PEF chamber, ranging from 35°C to 55°C.

For the HTST process (90°C for 15 s), used as a benchmark, the study examined the influence of Waste Heat Recovery (WHR) efficiency on preheating temperature, thermal power, and steam flow rate. Results indicated that higher WHR efficiency resulted in elevated raw juice temperatures leaving the regeneration stage, thereby reducing the thermal power necessary to achieve the pasteurization target temperature of 90°C. Consequently, this led to reduced specific energy consumption, cost savings, and enhanced energy efficiency.

For the PEF treatment (E=36 kV/cm, WT= 40-200 kJ/kg), findings revealed that in combination with preheating at various inlet temperatures of the juice to the chamber, higher WHR efficiency was correlated with decreased electrical energy demand. Specifically, PEF demonstrated 30% lower electric consumption and less than 70% fuel gas usage compared to thermal processes, reducing greenhouse gas emissions by less than 40%. PEF treatment, with its lower energy requirements and reduced reliance on fossil fuels, exhibited lower CO2 emissions and comparable water usage compared to HTST, showcasing its environmental advantages.

The economic viability was assessed through a comprehensive cost analysis, encompassing capital investments, utility expenses, labour costs, and facility-related charges. The results highlighted that despite higher initial capital costs for PEF, its lower unit pasteurization cost and reduced annual expenses yielded significant cost savings compared to HTST. Additionally, the study explored payback periods under various energy cost scenarios, identifying optimal configurations for PEF plants with payback periods ≤ 5 years. Through the correlation of emissions and water consumption, the PEF plants with a WHR efficiency of 35% emerged as the most energy-efficient and sustainable solution, warranting its selection for optimal orange juice pasteurization capable of mitigating the impact of adverse energy scenarios. The preheating temperature of 45°C was chosen as the optimal solution due to its proven effectiveness in microbial inactivation compared to lower temperatures.

In conclusion, this comprehensive analysis

provides invaluable insights for stakeholders in the food processing industry, facilitating informed decision-making towards cost-effective, energy-efficient, and sustainable juice pasteurization technologies.

OR-54

Effect of pulsed electric field processing on functional properties of plant protein in yogurt alternative applications

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Plant proteins have gained popularity as a functional ingredient in yoghurt alternatives to provide texture by protein gelation while improving nutritional benefits of the product. Plant protein isolates are commercially produced from sources such as the yellow pea (Pisum sativum) but typically suffer from poor functionality. Thus, there is a need for processing methods that can induce targeted structural changes to improve their function as food ingredients. Pulsed electric field (PEF) treatment has shown promise in modifying plant protein functionality but has not been explored in yoghurt alternatives. Therefore, the aim of this study was to assess the effect of PEF with preheating on the protein gelation of yoghurt alternatives.

A model food system was prepared by hydrolysing oats (10% w/v) with α-amylase (60 °C, 60 min), followed by filtration and mixing with a commercial pea protein isolate (5% w/v). The resulting dispersion was pre-heated to 35 - 45 °C and exposed to PEF of bipolar square waveform using an EL-CRACK lab-scale PEF unit in continuous operation (14 L/h). A constant field strength and pulse width (10 kV/cm, 20 μs) were applied, while pulse frequency was adjusted to achieve targeted specific energies between 81 and 214 kJ/kg. Physicochemical properties (zeta potential, particle size, Brix) and protein solubility based on soluble nitrogen were measured after PEF, while yoghurt fermentation (35 °C, 10h) was applied to identify impacts on protein gelation via texture profile analysis (TPA). Thermal-treated samples (80 °C, 30 min) were included as a positive control for protein gelation whereas untreated samples without PEF were used as a negative control.

PEF at 88 kJ/kg with preheating to 45 °C permitted the formation of a gel during subsequent fermentation with similar hardness to the positive control. A further increase in gel hardness was observed with increasing specific energy (up to 21% at 214 kJ/kg) regardless of pre-heating temperature. Protein solubility increased with specific energy (up to 18% at 181 kJ/kg, 45 °C) compared to the negative control prior to fermentation, whereas zeta potential and particle size decreased. An enhanced protein solubilisation by PEF, based on particle size reduction and changes in net surface charge, could induce further cross-linking and stronger gel formation during fermentation. This demonstrated the potential of PEF as a suitable processing step to modify the texture of yoghurt alternatives. PEF could therefore play a significant role in the development of sustainable foods that satisfy ever growing consumer demands.

OR-55

Effect of pulsed electric fields on protein extraction of duckweed (L. minor and L. gibba)

Patricia Maag[1], Özlem Özmutlu[1], Cornelia Rauh[2]
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The Department of Food Technology and Horticulture at HSWT (University of Applied Sciences Weihenstephan-Triesdorf) is establishing a mild extraction process for optimized protein extraction using pulsed electric fields of moderate intensity (MIPEF), which is a research objective in the research project Smart Indoor Farming (”Science and the Arts in Bavaria”, STMWK), aiming to observe the effects on cell disruption and mass transfer, with a focus on the release of proteins from the plant cells of duckweeds (L. minor and L. gibba). The equipment used is a bipolar, square wave, pulse generator, model OmniPulser (Manufacturer: VITAVE) with an attached paralell plate batch treatment chamber.

In previous trials with duckweed, remarkably high protein contents of 35-40% (dry matter) were investigated and other research data has found a bal-

anced amino acid composition as well as valuable omega-3 fatty acids (α-linolenic acid) and antioxidants (lutein), offering excellent potential as a new healthy alternative protein source. Research into duckweed has therefore been intensified since its potential as a high-quality nutrient for food applications was recognized.

To pursue sustainable and mild extraction processes in which chemical solvents can be reduced, pulsed electric field (PEF) technology is used for cell disruption to pre-extract proteins in freshly harvested duckweed. Utilizing a batch processing approach, PEF of unipolar and bipolar types are applied to disintegrate the plant cell membrane. This method is directly applied to a pre-crushed duckweed cell system and subsequently compared to untreated samples to evaluate its effectiveness in enhancing protein extraction yield. PEF-Parameters such as electrical fields (1-5 kV/cm), number of pulses, and pulse width are considered. In addition, the stirring time after PEF treatment is considered a parameter for the optimized protein release, as well as having reversible or irreversible effects on cell opening are observed. Within the scope of current research, it was determined that the protein release of PEF-treated fresh duckweed at 3-5 kV/cm was 10-15% higher compared to the untreated reference. Demonstrating the effect of PEF-assisted protein release and mass transfer, the conductivity index before and after treatment, fluorescence microscopy to visualize the protein release from plant cells and protein measurement are presented. The effects of PEF on antioxidant capacities and polyphenolic content will also be evaluated through methodologies, including DPPH, FRAP, and Folin-Ciocalteu assays.

As a future objective, the study aims to isolate proteins using an optimized pre-extraction procedure. This will entail applying mild processing techniques such as ultrafiltration or diafiltration, with a primary focus on evaluating the techno-functional properties and nutritional profile of the concentrated protein for potential food applications.

OR-238

Pulsed electric field-assisted preparation of dialdehyde starch and its effect on structure and physiochemical properties

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In this study, dialdehyde starch was prepared using pulsed electric field (PEF)-assisted sodium periodate oxidation of starch, and its structure and properties were studied. The results showed that the aldehyde group content of DAS (12-DAS) prepared with the assistance of PEF of 12 kV/cm increased by 11.6%, compared with the dialdehyde starch (0-DAS) prepared without the assistance of pulsed electric field. And as the intensity of PEF increased, the molecular structure of the prepared DAS became more disordered. SEM results showed that the particle surface of DAS prepared with PEF assistance was rougher than that of 0-DAS. The rheological properties results showed that the viscosity of DAS decreases with increasing PEF strength. The decrease in viscosity means that the mobility of the molecular chains in the solution increases and the resistance to solution flow decreases, which is beneficial to the chemical reaction of DAS. In addition, the solubility of 12-DAS was significantly increased to 89.21% compared with the solubility of native starch (19.01%).In summary, PEF-assisted preparation of DAS has a more disordered molecular structure, lower viscosity, and a higher degree of damage to the surface of starch granules.

P3 - Irreversible electroporation

Monday late afternoon Track D

Sep 16, 16:10 - 17:40

OR-56

Study on Segmented Model of Tissue Conductivity Recovery after High Voltage Pulsed Electric Field Treatment

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Irreversible electroporation, induced by high-voltage pulses electric field, has shown tremendous application prospects in the fields of tumor treatment and cardiac ablation. The tissue conductivity would have a significant increase during treatment and recover to different extents after treatment. In the present study, the rabbit liver tissue is employed to investigate the conductivity change after treatment. Following the electroporation process induced by high-voltage pulses, a series of low-voltage pulses to monitor the change of tissue conductivity at different time points after treatment, finally, the conductivity model was built to reflect the recovery process. The results showed that there is an exponential decay phase in tissue conductivity within 1 ms after pulse treatment, which could be correlated to the recovery process of short-lived reversible electroporation. This is followed by a slow recovery process corresponding to the recovery process of long-lived electroporation. The tissue conductivity eventually stabilized to a value that remains higher than the initial value before treatment, indicating irreversible changes in tissue electrical characteristics after high-voltage pulse treatment, and this is in agreement with the characteristics of irreversible electroporation. With the increase in the number of pulses, the final stabilized value of tissue conductivity would increase first and then tend to saturate, indicating that the degree of irreversible electroporation caused by such conditions is saturating. Further increasing the number of pulses may not increase the ablation range but may instead cause tissue thermal damage. The above three stages of conductivity (rapid recovery, slow recovery, stability) are important references for establishing a dynamic model of tissue conductivity under irreversible electroporation, which would help us understand the dynamic development process of irreversible electroporation, and formulate dynamic treatment plans for irreversible electroporation, as well as predict the effect of irreversible electroporation ablation.

OR-57

Development of a Single Needle Electroporation Device Towards More Spherical Ablations

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Irreversible Electroporation (IRE) is a non-thermal focal ablation modality for the treatment of solid tumors by the application of high voltage pulsed electric fields onto undesirable tissue. Clinically, multiple monopolar 19-gauge needles (at least 2) are used to apply the necessary voltage across the target region. IRE treatment requires precise spacing and minimal angular deflection upon insertion of each probe for optimal electric field contours. Although IRE provides a viable treatment option for tumors situated in regions previously considered inoperable, the technical challenges introduced limit widespread adoption. Utilizing a single insertion bipolar electrode can induce clinically relevant lesions and simplifies the medical procedure compared to a traditional multiple needle array. However, containing several electrodes to a single probe amplifies preexisting limiting factors such as electrical arcing and irregular electric field contours. Electrical arcing presents an issue in the single needle electrode configuration due to the proximity of the cathode and anode to one another, encouraging a corona discharge across the insulative spacer. Irregular electric field distribution and the subsequent ablations are a product of the electrode geometry. Importantly, the non-spherical electric field contour is undesirable in the treatment of malignant tumors, as the irregular shape complicates treatment planning and incomplete ablations are susceptible to recurrence. The objective of this work was to optimize a single needle electroporation device towards a more complete ablation region. By using several independent electrodes of varying sizes on a single device, the electric field distribution can be controlled in multiple spatial directions through cycled pulsing. Here, through numerical and experimental investigations, we develop strategies for a single needle electroporation device for more spherical ablations.

OR-58

Production of Large Spherical Ablations Using Pulsed Electric Fields Administered Via a Single Applicator

Jewels L. Darrow[1], Callie Fogle[1], Robert H. Williamson[1], Alexia Cash[1], Kyle Mathews[1], Nate Nelson[1], Christopher Fesmire[1], Matthew Dewitt[2], Michael Sano[1]

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Traditional IRE treatments (NK-IRE) have demonstrated significant promise for the treatment of solid tumors; however, the monopolar pulses used induce intense muscle stimulation requiring the use of intraoperative paralytics. Additionally pulses must be delivered during the absolute refractory period of the heart to avoid potentially lethal cardiac complications. These challenges and instrumentation limitations effectively limit treatment zone sizes and can lead to long treatment times for large tumors.

Integrated nanosecond pulse irreversible electroporation (INSPIRE) continuously delivers ultrashort (250 – 2000 ns) paired positive and negative electrical pulses in conjunction with active temperature control. The ultrashort pulses used in INSPIRE significantly reduce muscle stimulation compared to traditional IRE protocols. This enables the use of an applicator and grounding pad approach as well as the use of greater voltages. This creates larger treatment zones than possible with a single NK-IRE treatment. However, these greater voltages lead to increased Joule-heating requiring active temperature control to dynamically adjust the rate at which pulses are delivered, which can be supplemented with applicator cooling to enable faster delivery rates.

This study investigated the safety and reproducibility of the INSPIRE approach in vivo. Healthy swine livers were accessed via an ultrasound-guided percutaneous approach with a temperature sensing internally cooled electrode applicator. Treatments consisting of various waveforms were administered with an amplitude of 6000V, a total dose of 0.02s, and temperature set points of 45ºC or 65ºC, with or without active applicator cooling. Blood based markers of cardiac damage (troponin) were monitored and treatments were imaged via CT one week following treatment prior to histological collection. Finite element analysis was used to model cell death processes.

The average treatment zone increased in size as pulse duration increased from 500ns (3.6cm3) to 2000ns (15.3 cm3). The temperature control algorithm successfully achieved and maintained target temperatures in vivo. Without cooling, the 65ºC temperature set point resulted in faster delivery (19.5 ± 6.8 minutes) compared to the 45ºC uncooled treatments (67.6 ± 27.3 minutes). Active cooling further reduced treatment times to 4.2 ± 1.2 minutes and 7.1 ± 2.2 minutes for 65ºC and for 45ºC set points, respectively. No evidence of cardiac damage was found following treatment and no other adverse events were observed during or after treatment.

This study indicates that INSPIRE can be safely administered via a single applicator approach with voltages up to 6000V. Active temperature control enabled rapid delivery of electroporation treatments without a priori knowledge of tissue electrical properties or local tissue perfusion conditions. Additionally, active cooling of the applicator significantly reduced treatment times.

OR-60

Investigation of rabbit heart electrical activity changes after electroporation using combined optical and transmural microelectrode technique

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Pulsed-field ablation (PFA) uses electrical pulses to induce nonthermal, irreversible electroporation (EP), causing cardiac cell death in specific tissue populations, with minimal damage to adjacent structures. Compared to the thermal ablation methods, PFA is a safer technique, but some major drawbacks hinder further development and trans-

lation of PFA procedure into clinical settings. We aimed to identify PFA parameters capable of creating controlled depth and size ablation zones and investigate changes of functional-electrical activity induced by PFA in the rabbit heart left ventricular tissue. We utilized an EP system with an electroporator and specialized electrodes (with 3 mm gap) to examine the area and depth of tissue ablation. We varied the duration (25-100 µs) and voltage (100-800 V) of applied pulses and used a series of 20 pulses in 900 ms intervals to investigate the effect of EP on electrical activity in Langendorff-perfused whole-heart and ventricular wall of cut-opened hearts; a cut was performed nearly through the center of the EP zone. Two additional stimulating pulses between every EP pulse were used to record action potential (AP) with 300 ms period, which allowed to follow changes of AP during the whole EP protocol. Optical action potentials were recorded using voltage sensitive NIR fluorescent dye Di-4-ANBDQBS. In addition, transmural AP were recorded using microelectrode technique. The results showed that EP slows down velocity of propagation of AP and induces partial or full conduction blocks. The depth of EP affected zones, with full inhibition of electrical activity, depending on EP parameters can reach up to 3 mm. In the zones close to EP, the resting potential, the velocity of upstroke and the duration of AP were reduced. In overall, our study reveals that our developed system is fully suitable to evaluate changes in functional activity of electrical signals in EP and proximal to EP zones.

OR-61

Electrical conductivity in human liver tissue: In vivo Assessment on normal vs. tumor

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Introduction: Spread hepatic tumours are not suitable to be treated with thermal ablation, conventional irreversible electroporation (IRE) or surgery. Unfortunately, tumour electrical conductivity is usually higher than hepatic conductivity, which impairs selective transhepatic IRE of tumour nodules. Even though there are several references of ex vivo conductivity measurements of liver and healthy hepatic tissue, in vivo ones are scarce or inexistent particularly in human tumors.

Methods: This study aimed to evaluate the electrical conductivity in human liver tissue, comparing normal versus tumor tissues and in-vivo versus ex-vivo conditions within a frequency range of 3 to 1000 kHz. Twenty patients were selected, and with informed consent, the electrical conductivity of their liver was measured during surgery and post-dissection.

Results: Our results showed higher values for the tumor compared to the normal, both under in-vivo and ex-vivo conditions. At 3.08 kHz, in-vivo tumor tissue showed a value of 0.44±0.12 S/m, while ex-vivo tumor tissue was of 0.31 ±0.04 S/m. In contrast, ex-vivo normal tissue showed the lowest value (0.11±0.04 S/m) followed by the in-vivo normal tissue (0.16±0.05 S/m). The electric properties also demonstrated a promising potential for distinguishing between different types of tissues including metastasis, cholangiocarcinoma (CCA), hepatocellular carcinoma (HCC), hepatic cirrhosis, and normal liver, in both in-vivo and ex-vivo conditions. At 3.08 kHz, electrical conductivity for cholangiocarcinoma, HCC and metastasis (in-vivo) were 0.35±0.00049, 0.49±0.25, and 0.45±0.12 S/m, respectively.

Conclusions: These findings could potentially improve diagnostics of liver diseases by means of electrical measurements and treatment techniques involving electric fields particularly IRE applications. Future research should focus on expanding the sample size to refine categorization and comparison processes across diverse human liver tissues.

S20 - PhD Students as important bricks in the wall of funded projects and basic research

Tuesday morning Track A

Sep 17, 10:40 - 12:10

OR-62

Gene electrotransfer of plasmid encoding Interleukin-12: off-target effects in murine cancer cells in vitro

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During cancer gene therapy, therapeutic genes are inserted into cells and recognized by cell defence mechanisms as exogenous nucleic acids and consequently as pathogen-associated molecular patterns (PAMPs). Exogenous nucleic acids are then recognized by endosomal and cytosolic nucleic acid-specific pattern recognition receptors (PRRs). Activation of PRRs induces a specific immune response that may lead to antitumor effects. One of the gene therapies that has already been proven to be safe and effective, having good local tumor control and an abscopal effect in the treatment of melanoma skin metastases, is gene electrotransfer (GET) of plasmid DNA coding for cytokine interleukin-12 (IL-12). Plasmid DNA introduced into cells by GET could activate different cytosolic sensors, therefore the aim of our study was to evaluate the effect of GET of plasmid DNA coding for IL-12 on PRRs. Cells (B16-F10 melanoma and CT26 colon carcinoma cells) in suspension ($25x10^6$ cells/ml) were prepared for GET using different plasmids: plasmid DNA encoding murine IL-12 (1 mg/ml, pmIL12), noncoding backbone plasmid DNA (1 mg/ml, pScramble), and a plasmid coding for a green fluorescent protein (1 mg /ml pEGFP). Cells were exposed to electric pulses through parallel electrodes with a 2-mm gap. A clinically used pulse protocol was used for GET; 8 times 1300 V/cm pulses of 100-microsecond duration at a frequency of 5 kHz were applied with CLINIPORATOR®. Transfection efficiency was determined 2 days after the electrotransfer of pEGFP using fluorescence microscopy. Cell viability was determined 3 days after treatment using the PrestoBlue™ Cell Viability Reagent. The expression of 15 different PRRs (Dai, Ifi16, Ifi204, Ddx60, Dhx9, Dhx36, Aim2, Cgas, Sting, Ddx41, Lrrfip1, Ku70, Mda5, Lgp2, Rig-I) was determined using qRT–PCR 4, 24 and 48 hours after treatment. Activation of cytosolic sensors leads to cascade of events resulting in cytokine production, thus we also determined expression of 23 different mouse cytokines at 24, 48 and 72 hours after GET in culture media of exposed cells. Transfection efficacy was higher in B16-F10 cell line compared to CT26 cells. Cell viability was significantly reduced after pmIL-12 and pScramble GET in both cell lines. GET of plasmid DNA led to upregulation of both DNA and RNA binding PRRs with higher basal levels of expression in CT26 cell line. Intriguingly, basal levels of these PRRs were inherently higher in CT26 cells compared to B16F10 cell line, similarly as to the concentration of cytokines observed to be more elevated in the CT26 cell line relative to the B16F10 cell line. Moving forward, future research will explore the comprehensive modulation of these sensors and cytokines following mRNA GET, offering insights into the broader landscape of GET off-target effects.

OR-63

Randomised Controlled Clinical Trial Investigating the Effect of Reduced Bleomycin in Electrochemotherapy Treatment on Patients with Cutaneous Malignancies

Marie Tolstrup, Julie Gehl
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Introduction: Skin tumours across cancer types have effectively been treated with electrochemotherapy (ECT) for over two decades. This treatment involves administering a standard dose of chemotherapy (15.000 IU/m2 bleomycin) intravenously, followed by briefly applying electricity to the skin tumours. The cell membranes of malignant cells will briefly become permeable, facilitating easier entry of chemotherapy into the cells and subsequent destruction. Literature has described studies where the chemotherapy dosage has been reduced during the execution of ECT for skin tumours, due to facts such as advanced age and reduced renal function. Despite the reduced dose of chemotherapy, positive results have been consistently achieved.

We aim to investigate whether halving the chemotherapy dosage during ECT for skin tumours is non-inferior to the standard ECT treatment.

Methods: We plan to conduct a double-blinded randomized clinical trial, aiming to include 55 participants with any kind of biopsy-verified cutaneous tumour. We anticipate that the patients will have approximately 110 cutaneous tumours in total. Participants will be randomized in a 1:1 ratio to receive either full or half dose of chemotherapy. The primary endpoint will be the overall tumor response after three months assessed according to the modified Response Evaluation Criteria in Solid Tumours (RECIST). Follow-up will continue for up to a year. Additionally, we will collect biological samples to measure bleomycin distribution in normal tissue, tumours and blood during the execution of ECT. Furthermore, a biopsy counting percent of tumour cells in tissue will be compared to bleomycin concentration in a biopsy for the same tumour. The collected data will be uploaded to the database of the International Network for Sharing Practices of Electrochemotherapy registry (InspECT).

Additionally, we aim to conduct qualitative interviews with 16 of the patients, both before the treatment and after three months, to understand their experiences with cutaneous tumours and ECT.

Discussion: As we make advancements in treating various cancers and prolonging patient survival, we are likely to see an increasing incidence of cutaneous malignancies. If halving the dose of bleomycin proves to be noninferior to the standard dosage, it could create opportunities for treating elderly and fragile patients as well as those with reduced kidney function. Additionally more repeated treatments can be performed if necessary.

The patients participating in this double-blinded, randomized controlled trial are those who would receive ECT treatment regardless, meaning there is no additional risk associated with the treatment. This setup closely resembles real-life scenarios, allowing us to apply the conclusions to everyday patient care.

Implications of this study will potentially be relevant not only to treatment of cutaneous metastases but also to treatment of deep seated tumours with use of ECT.

OR-64

Validation of in situ electroporation performed within a single cell microwave biosensor

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Université de Toulouse, France

Context: ElectroPoration (EP) is widely used in clinical practice within ElectroChemoTherapy (ECT) treatments, that consist in the combination of pulsed electric fields' application and anti-cancer agents' administration. The entry of the anti-cancer agents into cells is facilitated as the membranes are transiently permeabilized, thereby increasing drugs' cytotoxicity.

If the efficacy of ECT has been demonstrated in the treatment of solid tumors, in ~20% of cases, relapse occurs. Being able to predict treatment efficacy as early as possible constitutes a main challenge. Conducting studies at the single-cell level is essential for a better understanding of the dynamics of the EP phenomenon and enables the study of inter-cellular differences. Among the different existing techniques for electrical characterization of cells, Microwave Dielectric Spectroscopy (MDS) is a promising approach to obtain the dielectric properties of cells directly in their culture medium, in a non-invasive way. MDS can be performed at the single-cell level [1], and it has been effective in detecting cellular states following the application of EP and ECT treatments [2].

In the current study, we aim to perform in-situ EP with conventional microwave probes, in a biosensor initially designed for performing MDS analysis. Such probes are used here to apply DC voltages.

Methods: We conduct this study on human cancer cells (THP-1), which we pre-label with Calcein AM. After trapping a single-cell in the biosensor, we deliver electric pulses through microwave probes, placed on either side of the central strip of a coplanar waveguide, which has a 5 µm-capacitive gap at its center.

The applied electric pulses are similar to those commonly used for ECT treatments (8 pulses of 100 μs width, at a frequency of 1 Hz). We

calibrate the electric field by studying the impact on permeabilization at different voltages, ranging from 0 to 15 V.
Electropulsation buffer containing propidium iodide is used while applying the electric pulses. We assess the state of cellular permeabilization by studying the entry of propidium iodide and leakage of Calcein AM.
Results: We validate in-situ EP using an existing biosensor originally designed for single-cell MDS analysis. This study allows us to 1) define the permeabilization threshold for the studied cell line; 2) validate the use of microwave probes to apply DC voltages and perform EP; 3) pave the way for further MDS analysis of the EP phenomenon performed in-situ.

References:
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OR-65

Empowered Cellular and Subcellular Modeling for Microdosimetric Investigation of PEF Exposure

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Electromagnetic (EM) fields play pivotal roles in biomedicine, facilitating gene and drug delivery, cellular manipulation, and differentiation [1]. For instance, microsecond pulsed electric fields (μsPEFs) induce cell electroporation, enhancing membrane permeability for applications like electrochemotherapy and gene transfer [2]. In this context, microdosimetry emerges as crucial tool for comprehending the interaction between biological matter and electric fields, offering insights at the microscopic level. The European RiseUp project [3] exemplifies innovative approaches, aiming to regenerate neuronal function post-spinal cord injury (SCI) using an innovative, wireless, rechargeable implantable device combined with Stem Cells (MSCs and iNSCs). Microdosimetric analyses on realistic cell models are needed for accurately evaluating induced electrical quantities [4].

To this regard, a novel semi-automatic procedure for ad hoc reconstructing 3D cell models and their internal organelles from high-resolution confocal microscopy images was fine-tuned. The 3D reconstruction process was composed of four main steps: (i) image acquisition of cells, (ii) image preprocessing to improve the quality of the image, (iii) segmentation and 3D reconstruction of numerical cell, using 3D Slicer v. 4.11 software, and (iv) model optimization in Blender v3.0 software. The resulting advanced cell models were then combined with already available 3D mesenchymal stem cell (MSCs) models [4] in order to obtain a numerical cell sample formed by a mixture of iNSCs and MSCs. To assess the local electrical quantities induced by μs PEF on a cells sample, the planar device developed in RiseUP Project was considered and reconstruct in Comsol Multiphysics v. 6.0.

Results reveal complex distributions of induced electric fields and transmembrane potentials (TMP) within cell structures. The proximity of cells influences field redistribution, affecting exposure and electroporation. TMP distributions on plasma and internal membranes exhibit varying arrangements, correlated with cell and organelle sizes. The study emphasizes the importance of realistic cell and organelle shapes in microdosimetric analyses, highlighting the need for a comprehensive understanding of electroporation phenomena at the cellular and subcellular level. This study paves the way for numerically assisted experimental applications of μsPEF for the controlled manipulation of cells and subcellular organelles, enhancing the efficacy and safety of emerging therapies.

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OR-66

Effects of Intense Electric field on TRPV4 ion channel: a Molecular Dynamic study

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Pulsed electric fields are increasingly used in medicine to stimulate cells, enhancing membrane permeability via electroporation to deliver therapeutic molecules into the cell [1]. One event that contributes to the increase in membrane permeability is the formation of pores in the membrane lipid bilayer [2]. Electrophysiological measurements suggest that even membrane proteins as voltage-gated ion channels are affected by the application of the E field, although the molecular mechanisms by which the electric field could affects these molecules remain unidentified [1]. To fill this gap, molecular dynamic (MD) simulations are used to unravel the molecular events that take place in different ionic channels when exposed to an intense electric field [3].

Thus, the aim of this study is to investigate, by using a MD approach, the effects of high-intensity E-field on non-selective calcium channels, as the transient receptor potential (TRP) channels, which besides sharing a high degree of similarity with the voltage-gated ones, are found in numerous tissues and cell types [4]. These channels are responsible for various sensory like heat, cold, pain, stress and vision and can be activated by a variety of stimuli. The presence of the TRP in different tissues, the interaction with physiological pathways and distinctive structure makes them promising targets for treating numerous diseases [5].

In this study we will examine not only alterations in the protein's configuration and functioning but also its interaction with the surrounding environment. This investigation provides valuable insights into potential therapeutic applications through 100 nanoseconds MD simulations [2]. The human(h)TRPV4 model was implemented using MODELLER [6] starting from a model derived from the open source PDB [7]. The simulation box is represented by the hTRPV4 embedded into a lipid bilayer membrane (POPC) and hydrated by 178096 water molecules. The overall dimension of the simulation box is 18x18x21 nm^3. The study was carried out considering E-field intensity in line with ones usually adopted in literature [3]. Each MD simulation was performed in the NPT ensemble, with temperature and pressure kept at 310 K and 1 bar, respectively. Preliminary results reveal different modification of the hTRPV4 according to the applied E-field.

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OR-67

Anti-tumor efficacy of new high-frequency electrical protocols on in vitro three-dimension colorectal cancer model

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For more than 20 years, electrochemotherapy has been increasingly used in the treatment of can-

cer, as it increases the cellular uptake of hydrophilic antitumor drugs (bleomycin, cisplatin) and has already proven its effectiveness on tumors in human medicine and in veterinary practice. However, this treatment requires local or even general anesthesia, as the electrical pulses can be painful and cause muscle contractions. Several studies have shown that the application of high-frequency pulses (above 5000 Hz) is much less uncomfortable for the patient than the 1 Hz protocol used in clinical practice, despite a slight increase in temperature. The use of bipolar pulses can also reduce muscle contractions, although the amplitude of the field and the number of pulses must be increased to achieve the same effectiveness.

In order to reduce the pain associated with the contractions, but also to maintain the efficacy of the treatment, we have developed new protocols that use a high-frequency generator associated with a new set of multipolar electrodes. Cell death measurements were done on a colon cancer cell line (HCT-116) cultured in both 2D and 3D (spheroids) with different molecules (cisplatin, bleomycin, calcium and carboplatin). Using our new optimized protocols on cell suspension, the viability rate were around 10 % at 48h after treatment and less than 5 % at 10 days after treatment whatever the cytotoxic molecules. The results on 3D spheroid models show a significant decrease in spheroid size, suggesting growth impairment after a single treatment . These results were similar to the ESOPE (European standard operating procedures for electrochemotherapy) protocol currently used in the clinic. In addition, the mechanisms of cell death induced by these high-frequency protocols were investigated, suggesting apoptosis death that does not appear to occur via the mitochondrial degradation pathway. Ongoing clinical trials on cats and horses at the Veterinary School in Toulouse have shown that 80% of patients respond completely to treatment without noticeable pain. Therefore, our painless high-frequency electroporation protocols appear very promising for the widespreading of electrochemotherapy as an effective cancer treatment.

P1 - Electroporation and immune response

Tuesday morning Track B

Sep 17, 10:40 - 12:10

OR-68

Immunological changes in murine tumor cell lines following electrochemotherapy in vitro

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Electrochemotherapy (ECT) initiates an immune response within treated tumors, yet the impact of various cytostatics and the temporal dynamics of alterations across diverse tumor models remain uncertain. Commonly observed types of cell death following ECT include apoptosis, necrosis, and immunogenic cell death (ICD), which effectively induces adaptive immune responses against neo-antigens released by dying or dead cells. This inflammatory reaction is facilitated by damage-associated molecular patterns (DAMPs) released from cells, acting as danger signals. Key DAMPs capable of activating ICD include surface exposure of calreticulin (CRT), ATP release, and high mobility group box 1 (HMGB1) release. Additionally, immunologically significant changes in tumor cells, such as defects in antigen presentation (e.g., MHC I, MHC II), or alterations in immune-relevant cell markers like PD-L1 influence the immune response. This study aimed to assess alterations in DAMP [removed]CRT, HMGB1 and ATP) and immune relevant cell markers (MHC I, MHC II, PD-L1) following ECT in vitro. Three murine cell lines forming immunologically distinct tumor models in mice (B16F10 melanoma, 4T1 mammary carcinoma and CT26 colorectal carcinoma) were subjected to ECT using three different cytostatic drugs at IC30, IC50 and IC70 concentrations: cisplatin (CDDP), oxaliplatin (OXA), and bleomycin (BLM). Changes in expression were determined at 4, 24, and 48 hours post-treatment. Our results demonstrated that ECT with all three tested cytostatic drugs induced ICD-associated DAMPs, but the induced DAMP signa-

ture was cell line and drug concentration specific. Moreover, we showed that ECT with CDDP, OXA or BLM can modify the expression of MHC I, MHC II and PD-L1 cell surface markers that are important in boosting the immunogenicity of the therapy. Similarly, as for DAMPs, the potential of ECT to change their expression was cell line and cytostatic drug concentration specific. Our results thus put the ECT with clinically relevant cytostatic drugs CDDP, OXA and BLM on the map of ICD inducing therapies and provide the necessary mechanistic insights needed to harness the potential of ICD to elicit a systemic anti-tumor immune response, especially when combined with immunotherapies.

OR-69

Electroporation of 3D-cultured breast cancer cells elicits T lymphocyte-mediated killing

Mariangela De Robertis[1], Ramona Marino[2], Elisabetta Sieni[3], Mario Cioce[2], Andrea Marra[2], Vincenzo Maria Perriello[4], Nico Martarelli[4], Annj Zamuner[5], Sonia Perrelli[1], Monica Dettin[5], Maria Teresa Conconi[5], Vito Michele Fazio[2], Flavio Keller[2], Emanuela Signori[6]

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Cancer cells destruction induced by electrochemotherapy (ECT) can trigger a local and systemic antitumor immune response as a result of releasing tumor antigens from electroporated cancer cells. 3D scaffolds composed of hyaluronic acid and ionic-complementary self-assembling peptides can enable extracellular matrix organization, thus mimicking the complexity of the tumor microenvironment. They therefore represent a valid system to study the anticancer effect of Electroporation (EP) protocols.

We propose that the use of EP alone on cancer cells embedded in 3D scaffolds may trigger high antigens exposure, inducing T lymphocytes migration and activation that could lead to cancer cells elimination.

We co-cultured HCC1954 breast carcinoma cells with Jurkat T cells in 3D scaffolds, applying two different EP conditions (600 V/cm and 1000 V/cm) commonly used in ECT protocols, to modulate T lymphocytes activation. Our results revealed that EP of co-cultures of HCC1954 cells with resting T cells significantly influenced the number and size of cancer cell-associated 3D structures (spheroids). T lymphocytes infiltration and cancer cells death were associated to the reduction in 3D cancer cell spheroids. The addition of PHA-M activated T cells significantly enhanced this overall effect. Following these results, we co-cultured HCC1954 cells with human T lymphocytes to evoke a more clinically relevant condition. Using flow cytometry, we confirmed the activation of T cells and their cytotoxic activity, mediated by the ability of EP to induce the release of IL-2, INF-gamma and TNF-alfa at the mRNA level.

Our study demonstrates that EP alone can exert anticancer effects by increasing tumor cell killing by activated T lymphocytes. We speculate that this is facilitated by EP-mediated increase in antigen exposure on tumor cells.

OR-70

Electroporation alters the proteomic output in human ex vivo GI cancer explant models: boosting systemic anti-tumour immunity and polarizing immune cell populations

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Electroporation-based antitumor therapies, calcium electroporation (CaEP) and irreversible electroporation (IRE), have been shown to be very effective on directly treated tumours by altering the tumour microenvironment (TME) and triggering a systemic immune response. We investigated the proteomic secretome following reversible electroporation (rEP), CaEP and IRE in upper and lower

Gastrointestinal (GI) cancer explants. We analysed the proteomic secretome to further understand the immunological response to EP and investigated the effect of electroporated tumour conditioned media (TCM) on immune cell polarization.
Hypothesis: rEP and IRE are reported to trigger an abscopal effect, resulting in a systemic anti tumour immune response. We hypothesise that different EP treatments will differentially affect the proteome released from GI cancer explants and polarize immune cells in the TME enhancing an anti-tumour immunity.
Methodology: Following patient consent, ex-vivo tumour and matched normal tissue explants from GI cancer patients were exposed to rEP with/without 5.0mM CaCl2 and IRE using the ePORE electroporator (Mirai Medical, Galway). Treated explants were cultured at 37°C for 24 hours. The supernatants, termed tumour or normal conditioned media, TCM and NCM respectively, were analysed via MSD 54plex ELISA to assess secreted factors on angiogenic, chemokine, cytokine, inflammatory, TH17 or vascular injury panels. The effects of TCM and NCM on dendritic cell and macrophage polarisation was assessed by flow cytometry.
Results: MSD profiling of TCM and NCM from EP treated explants, showed a varied proteome that was dependent on the treatment delivered and tumour origin. In gastric cancer myeloid cell attractants such as IL-8 and MCP-4 were elevated on EP treatment and not in colorectal cancer (CRC). Normal tissue was less responsive to all treatments than tumour tissue. Inflammatory markers such as IL-1α (p=0.03), TNF-α (p=0.022) and IL-10 (p=0.048) and macrophage released chemokines MIP-1α (p=0.048) MIP1-β (p=0.018) and MIP-3α (p=0.0043) that attract lymphocytes were altered with CaEP. DCs and macrophages were differentially affected by EP treatment at different GI sites, this needs to be considered when designing treatment regimens. In vitro conditioned media from tumour explants following EP treatment lower M1 macrophage marker expression in CRC but differentially affects in upper GI cancers. In vitro conditioned media from CRC tumour explants following EP treatment alter DC marker expression, highlighting the immunomodulatory potential of EP treatments.
Significance: Alterations in the immunomodulatory secretome of tumour tissue after EP could affect immune cell function, alter the TME and illicit an abscopal response. Further interrogation is required to fully elucidate the effects of EP at different sites and could facilitate tailored regimens based on tumour site whilst enhancing a systemic immune response to clear distal metastatic tumours.

OR-71

Enhanced antitumor efficacy of bleomycin electrochemotherapy combined with anti-PD-1 in mouse tumor models

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Electrochemotherapy (ECT) has emerged as an effective treatment modality for various tumor types in both preclinical and clinical settings within human and veterinary oncology. Recent studies have unveiled its potential to activate innate immunity by triggering a signaling pathway through damage-associated molecular patterns (DAMPs). This activation leads to specific antitumor immunity and subsequent immunogenic cell death. The observed immunogenic responses resulting from ECT using bleomycin (BLM) suggest a potential synergy with immunotherapy, a pivotal component of current treatment modalities, particularly PD-1 inhibitors, known for their significant effectiveness in various cancer types. Therefore, our study aimed to evaluate the antitumor efficacy of combining ECT with BLM treatment alongside anti-PD-1 in mouse tumor models of various histological types. In vitro sensitivity of colorectal MC-38 and CT26 carcinoma, mammary 4T1 carcinoma, B16F10 melanoma, and WEHI 164 fibrosarcoma to ECT with BLM was assessed using the Presto Blue viability assay. Subsequently, the antitumor effect of combined ECT with BLM and anti-PD-1 was investigated in subcutaneous colorectal MC-38 carcinoma, B16F10 melanoma grown in C57Bl/6 mice, and colorectal CT26 carcinoma, mammary 4T1

carcinoma, and WEHI-164 fibrosarcoma grown in Balb/c mice through tumor growth delay assay.
Our data revealed that WEHI-164 cells displayed heightened susceptibility to ECT with BLM compared to the other cell lines examined. Specifically, a significant lower dose of BLM was required to achieve a 50% reduction in cell viability (IC50) of WEHI-164 cells following electroporation, whereas B16F10 and 4T1 cells demonstrated comparable sensitivity to ECT with BLM in vitro. Correspondingly, in vivo experiments demonstrated a complete remission rate of 100% in fibrosarcoma, a 50% in mammary carcinoma, a 13% remission rate in colorectal carcinoma tumors and no remission in melanoma tumors. Adjuvant immunotherapy was used in tumor models that demonstrated lower sensitivity to ECT with BLM. This led to complete remission in the 4T1 mammary model and a remission rate of 78% in colorectal carcinoma MC-38, while in melanoma, it reached up to 14%. Monotherapies, on the other hand, displayed limited efficacy, with only up to an 8-day delay in tumor growth compared to controls in all tumor models.
Overall, our study underscores the enhanced antitumor effectiveness of combining ECT with immunotherapy, particularly in less responsive tumor types, such as colorectal carcinoma, and to some extent, melanoma. These results underscore the potential of ECT to modify the tumor microenvironment synergistically with immunotherapy in cancer treatment. Nevertheless, further investigations are warranted to elucidate the underlying mechanisms of this combined therapeutic approach.

OR-72

Immunotherapy in combination with electrochemotherapy (Immune-ECT) in head and neck cancer

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Introduction: The combination of immunotherapy and electrochemotherapy with Bleomycin (Immune-ECT) is successfully used for the treatment of metastatic melanoma. The association of these two procedures is based on the “abscopal” effect of ECT that is the systemic effect induced by local treatment. Locally, cellular necrosis determines the release and exposure of tumor antigens, triggering an inflammatory response with consequent activation of the systemic immune response. This process allows the antineoplastic effect of immunotherapy to be supported and amplified.
Based on these assumptions and the evidence of the effectiveness of the treatment in other tumors, we decided to apply this protocol for locally advanced or recurrent tumors of head and neck.
Materials & Methods: Since 2016, 11 patients (4 M and 7 F, average age 74 years, range 43-88 y.o.) with locally advanced or recurrent cancer underwent Immune-ECT with palliative aim. Nine patients were already submitted to surgery and/or chemo-radiotherapy; 7 patients had squamous cell carcinoma, 3 melanoma and 1 Merkel cell carcinoma. Immunotherapy was done before ECT in 2 patients, after ECT in 4 patients, concomitant with ECT in 5 cases. Tumor response was evaluated clinically and radiologically within 3 months.
Results: No major or minor complications were observed. Complete clinical and radiological resolution of the carcinoma was achieved in 3 patients with a mean follow-up of 33 months. Three patients are alive with persistent/progressive disease. The remaining 5 patients had disease progression and died after an average of 22 months.
Conclusions: Immune-ECT is successfully used in clinical practice for metastatic melanoma. The safety and efficacy of the procedure could lead to consider Immune-ECT a valid alternative in the treatment options of locally advanced or recurrent tumors of the head and neck. Further studies are necessary to understand why some tumors respond to Immune-ECT protocols and other do not. Clinical trials comparing immunotherapy alone versus Immune-ECT are needed. Further studies are necessary to standardize this new therapeutic procedure.

P5 - Electroporation and cellular processes and pathways

Tuesday morning Track C
Sep 17, 10:40 - 12:10

OR-73

Optimizing Electroporation: Efficiency and Cell Viability in the Simultaneous Transfer of Bovine Serum Albumin, Propidium Iodide and Nucleic Acids

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The delivery of exogenous proteins into cells via electroporation has been used for biological inquiries, such as CRISPR-Cas9 gene editing and intracellular signaling detection. However, there currently exists no theoretical model for the entry of proteins via electrotransfer. Their context-dependent behavior could be observed by revealing its potential synergies or interferences with various other migrating molecules, including small molecules and nucleic acids, under electroporation. Since this approach may increase membrane damage and/or endocytosis during their transfer across the membrane, the concerns about cell viability should also be adressed. The aim of this study is to determine the effects of protein transfer on the efficiency of simultaneously electroporating it alongside propidium iodide and plasmid DNA into mammalian cells and vice versa, using varying pulse numbers to optimize multi-molecule delivery while maintaining cell viability. CHO cells were exposed to 1, 9, or 17 high-voltage (HV) pulses of 60 µs at 1000 V/cm and 1 Hz to facilitate simultaneous and separate entry of bovine serum albumin (BSA) Alexa Fluor 488 conjugate and propidium iodide (PI). After 30 min., the percentage of viable BSA+ and PI+ CHO cells was measured by flow cytometry, analyzing 10000 events in the P1 region (FSC-A/FSC-H) using FL1 and FL2 channels on an AccuriTM C6 cytometer. Another experimental points were tested for the uptake of BSA and mCardinal separately and together, using the pulse number that resulted in the highest uptake for BSA and PI. Cell viability was assessed 24 hours post-electroporation using the MTT-PMS assay. Concurrently, the percentages of viable BSA+ and RFP+ cells were determined by flow cytometry (FL1 and FL4). The corresponding control groups were treated with BSA for baseline uptake regardless of whether BSA was co-delivered with PI or pDNA; they were not exposed to electric pulses. Additionally, fluorescent microscopy will be used to confirm the presence of fluorescence suggesting pRFP expression and the uptake of BSA and PI within cells, providing a visual evaluation of the electrotransfer efficacy. The preliminary results have shown that with BSA-Alexa Fluor 488 rising to 12.92% at 17 HV pulses, affirming the method's efficacy, co-electroporation of BSA and PI slightly reduced PI uptake from 96.12% to 93.4% ($p<0.05$). The presence of BSA modulated pRFP uptake post-electroporation, with a decrease in RFP-positive cells by 28.38%, while BSA uptake increased by 20%. Furthermore, MTT-PMS assay revealed that pRFP and BSA co-electroporation decreased cell viability to 23.1% compared to 60% for pRFP alone and 48% for BSA alone ($p<0.05$). These findings might indicate the complex dynamic between BSA, PI, and pRFP where these cargoes could affect each one's uptake and overall cell viability.

OR-74

Mitochondrial Depolarization and ATP Loss During High Frequency Nanosecond Electroporation

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It is predicted that ultra-short electric field pulses (nanosecond) can selectively permeabilize intracellular structures (e.g., mitochondria) without major effects on the outer cell plasma membrane. Such a phenomenon would have high applicability

in cancer treatment context and could be employed for modulation of cell death type or immunogenic response.

In this study, we compare the effects of 100 µs x 8 pulses (ESOPE - European Standard Operating Procedures on Electrochemotherapy) and bursts of 100 ns pulses for modulation of the mitochondria membrane potential. We characterize the efficacies of various protocols to trigger cell plasma permeabilization, depolarize mitochondria, evaluate the extent of ATP depletion and generation of reactive oxygen species (ROS). Finally, we employ the most prominent protocols in the context of Ca2+ electrochemotherapy in vitro.

We provide experimental proof than 100 ns pulses (7.5–12.5 kV/cm, n = 100–1000, 1 MHz) can be used for modulation of mitochondrial potential, however, the permeabilization of the outer membrane is still a pre-requisite for depolarization. Similar to 100 µs x 8 pulses, the higher is the permeabilization rate the higher is the mitochondrial depolarization. Nevertheless, 100 ns pulses result in lesser ROS generation when compared to ESOPE, even when the energy input is several-fold higher than for the microsecond procedure. It was also shown, that 100 ns pulses can be successfully used for Ca2+ electrochemotherapy ensuring excellent cytotoxic efficacy, while it is concluded, that even shorter pulses (i.e., sub-100 ns) are required to observe the phenomenon of selective mitochondrial depolarization.

OR-75

Generation of hypochlorous acid by high-voltage pulses and its influence on the cell plasma membrane

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Pulses of strong electric field utilized for cell electropermeabilization, also cause electrolysis reactions at the electrode–solution interfaces. Tissues and solutions usually contain high amounts of chloride ions. As a result of electrolysis, Cl2 gas can be formed at the anode. Chloride ions react with the water molecules and form hypochlorous acid (HOCl), which is a powerful oxidant – it can react with a wide variety of biomolecules including DNA, RNA, cholesterol, and proteins. For practical applications of electroporation, e.g, when using electroporation to extract DNA, RNA or proteins (e-biopsy), it is important to avoid any contamination of samples.

The aim of this work was to study the formation of HOCl acid as a result of electrolysis during high-voltage pulses, as well as its influence on the viability of cells and the barrier function of the cell plasma membrane. The formation of hypochlorous acid was evaluated with fluorescent indicator of hypochlorite 3'-p-Aminophenyl fluorescein (APF) along with the scavengers of various reactive oxygen species (ROS). The viability of Chinese hamster ovary (CHO) cells was determined by a colony-forming assay. The size of the pores created in human erythrocytes was estimated by studying the protective action of xylitol (152 Da), mannitol (182 Da), and sucrose (342 Da) against colloid-osmotic lysis.

It has been obtained, that during high–voltage electric pulses, ROS are generated. In cell–free media, micro–millisecond pulsed electric field increased fluorescence of hypochlorite indicator APF proportionally to the pulse number and amplitude. APF fluorescence was reduced by both vitamin C and mannitol. Also, it has been shown that ROS formation was more intensive in the case of stainless–steel electrodes comparing to the aluminum ones. The results of this work can be useful for optimizing the electroporation technology used in biotechnology, medicine, and food industry.

The influence of hypochlorous acid on the viability of Chinese hamster ovary (CHO) cells in vitro was evaluated. HOCl caused the reduction of CHO viability. Less than 50 % of CHO cells survived, when the concentration of hypochlorous acid in the cell growth medium was 0.8 mM.

The influence of HOCl on the plasma membrane of human erythrocytes was also studied. HOCl increased the permeability of the cell plasma membrane to ions and small molecules, what caused

haemolysis of erythrocytes. The estimated radius of permeable structures, which appeared in the plasma membrane of erythrocytes, was about 0.3–0.5 nm. This is close to the size of the pores generated by the exposure of cells with pulses of strong electric field.
Conclusion: hypochlorous acid can be formed due to electrolysis during high-voltage pulses. It can increase permeability of the cell plasma membrane to ions and small molecules, which can cause the reduction of the cell viability.

OR-76

Application of Pulsed Electric Fields to Gating Blood-Brain Barrier for Drug Delivery

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The blood-brain barrier (BBB) serves as a protective layer separating blood circulation from neural tissue. It is crucial for preserving the delicate extracellular environment within the neuronal parenchyma. Neuroinflammatory events can disrupt the BBB by affecting adherens junctions (AJs) and tight junctions (TJs). The BBB presents a significant obstacle to drug delivery to the central nervous system (CNS). It is composed of a continuous layer of specialized endothelial cells connected by tight junctions, along with pericytes, a no-fenestrated basal lamina, and astrocytic foot processes. VE-cadherin, a key component of the vascular system, is particularly essential for the formation of AJs and the overall structure of the BBB. This intricate barrier regulates and restricts the entry of therapeutics into the CNS. Various innovative approaches have been investigated to improve the transport of therapeutics across the BBB, each offering distinct benefits and drawbacks. We hypothesized that the application of pulsed electric fields could open the BBB for drug delivery.
Human microvascular endothelial cells were grown as monolayers to 90% confluence on 8-well plates. They were pulsed with 15-50 electrical pulses (EP) of 100 μsec duration, frequency of 1 Hz, and applied an electric field of 200-1000 V. Four hours later the cells were fixed, permeabilized, and immunostained for visualization of VE-Cadherin. Control groups included unpulsed cells, as well as cells pulsed with 15 pulses of 200 V (100 μsec duration, frequency of 1 Hz); both control groups exhibited physiologically normal expression of VE-cadherin. Endothelial cells, pulsed with 15 EP at 500 and 1000 V (100 μsec duration, frequency of 1 Hz) demonstrated a reduction in VE-Cadherin immunostaining, indicative of endothelial barrier disruption. Additionally, 50 EP at 500 V completely disrupted the endothelial barrier. This data suggests that microsecond EP affects VE-Cadherin expression. Additional studies are needed to understand the mechanisms behind these observations.

OR-77

Is irreversible electroporation immunologically superior to thermal ablation or cryoablation? A closer look at antigen presentation, T cell activation and synergy with immune checkpoint blockades

Qi Shao
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Minimally invasive tumor focal ablation technique (commonly known as focal therapy, FT), including irreversible electroporation (IRE), thermal therapy (Heat) and cryosurgery (Cryo) possesses immunomodulatory properties and can synergize with immunotherapy to engage the immune system for systemic and long-term disease control. In particular, the combination treatment of FT and immune checkpoint inhibitors (ICIs) holds great promise in eliminating established tumors and preventing tumor recurrence, as demonstrated by encouraging results in preclinical studies (since 2004) and clinical trials (since 2011) across a variety of cancer types. Although comprehensive reviews have compared the clinical performances among distinct tumor ablation approaches, the immunological effects specific to FT modalities have not been fully assessed or compared, the underlying mechanisms are not fully understood.
Utilizing the in vitro assessment platform with the OVA model, we found that T cell proliferation and DC activation following IRE treatment of B16-OVA cells were significantly more robust than with Heat

or Cryo treatments, even though Cryo resulted in the highest release of protein, antigen, ATP and HMGB1, followed by IRE and Heat. IRE treated B16 cell lysates induced the most significant improvement in DC activation and T cell proliferation compared to Heat and Cryo treatments when the same quantity of antigen (naive OVA) was present. In a murine colorectal cancer model (MC-38), the therapeutic efficacy of anti-PD-1 in combination with FT is evaluated, utilizing well-characterized miniature probes. IRE exhibits the most favorable syngenetic effect with anti-PD-1 immunotherapy than Heat or Cryo, leading to greatest primary tumor growth delay, longest tumor-free survival, and highest protection against secondary tumor challenge. Furthermore, the co-administration of IRE and anti-PD-1 significantly fosters the infiltration of CD8+ T cells into the tumor coupled with a remarkable stem-like progenitor phenotype.

Our in vitro study suggests that IRE is a promising approach to induce immunogenic cell death, activate APCs, enhance antigen presentation and T cell activation, and stimulate anti-tumor immune response. IRE, emerged as the most immunogenic treatment, is largely attributed to its immunologically favorable “antigenicity” and “adjuvanticity”.

Our in vivo study demonstrates that IRE stands as a promising modality that can potentiate the antitumor efficacy when the tumor is poorly responding to the ICI monotherapy. In addition, IRE has a superior capacity to activate immunity and enhance the immunogenicity of MC-38 tumors, in comparison to cryoablation and thermal ablation at the given settings.

It is important to acknowledge that the immunological outcomes of each ablation modality can vary depending on the energy field distribution, the targeted tissue and the extent of energy-tissue interaction. These variations are heavily influenced by the specifics of cell death, tissue damage, inflammation induction, and subsequent wound healing processes.

OR-78

Involvement of mitochondria in the selective response to microsecond pulsed electric fields on both healthy and cancer stem cells in the brain

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In the last few years, pulsed electric fields have emerged as promising clinical tools for tumor treatments. This study highlights the distinct impact of a specific pulsed electric field protocol named PEF-5 (0.3 MV/m, 40 µs, 5 pulses) on astrocytes (NHA), medulloblastoma (D283) and glioblastoma (U87 NS) cancer stem-like cells (CSCs). We pursued this goal performing ultrastructural analyses corroborated by molecular/omics approaches to understand the susceptibility or resistance mechanisms triggered by PEF-5 exposure in the different cell types. Scanning and Transmission Electron Microscopies analysis highlighted membrane filopodium-like protrusions disappearance on the surface of all pulsed cells accompanied by a rapid cell swelling. Moreover, morphological alterations in the cytoskeleton and internal compartments, including organelles, were observed after the exposure. Intracellular ATP evaluation and mitochondria membrane depolarization results confirmed that mitochondrial organelles were the elective target of PEF-5. Overall, our results suggested that the mitochondrial perturbation was proportionally correlated with CD133 content on the cell surface of analyzed cells. Where a high CD133 content was present we observed its decrease due to the protein localization on the lost membrane protrusions. This decrease contributed to mitochondria dysfunction and ATP depletion which further conferred to cytoskeleton alterations. The medulloblastoma CSCs showed a significant decrease of CD133-positive cell proportion and severe damages on

mitochondria leading to massive cell death, glioblastoma CSCs maintained the principal vital processes, suggesting that the PEF-5 perturbation was transient and reversible and finally NHA activated multivesicular bodies formation as a protective mechanism.

Altogether, these findings suggest the possibility to use PEF-based technology for developing potential therapeutic strategies to target selectively mitochondria of brain CSCs, in correlation with CD133 content, preserving healthy cells.

P13 - General applications of electroporation for food processing

Tuesday morning Track D
Sep 17, 10:40 - 12:10

OR-79

Nondestructive extraction of functional molecules in yeast using 100 kV/cm class electrical pulses

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Proteins, amino acids, and lipids contained in yeasts are widely used for foods, feeds, cosmetics, and pharmaceuticals because yeasts are inexpensive and mass-producible nature. Also, gene transfer enables us to make yeasts produce any designed proteins. Thermal or chemical methods are used to extract the yeast components for the mass production, whereas non-thermal and physical methods are preferred to extract functional molecules without destroying them. Although pulsed electric fields (PEFs) are known to permeabilize the yeast cell membrane, the thick cell wall remains unchanged. The wall interrupts the intracellular large molecules to go outside. We have tried to destroy the yeast cell wall using ultra-high PEFs more than 100 kV/cm and to extract the intracellular molecules such as enzymes without destroying them. The PEF treatment cell consisting of stainless-steel parallel plate electrodes with a 2 mm gap was installed in a pressurized, temperature-controlled flow system. Phosphate-buffered saline solution including Saccharomyces cerevisiae was constantly flown with 100 mL/h for the treatment. Pulsed voltages of 400 ns duration and up to 30 kV were supplied between electrodes at 1 Hz using a spark-gap driven Blumlein pulse formation network. Inlet temperature of the solution was adjusted so that the outlet temperature did not exceed 50□. To analyze yeast morphology, cell membrane permeability was observed using propidium iodide and cell wall morphology using concanavalin A-FITC in addition to brightfield imaging. Also, the cell surface was observed using an electron microscopy. To analyze the supernatant after centrifugation of the treated solution, protein concentration was quantified using the BCA method whereas the qualitative analysis was conducted using Native-PAGE. Furthermore, functional activity of invertase, a hydrolytic enzyme, was examined using an enzyme function assay kit. PEF up to 150 kV/cm was continuously applied under 0.3 MPa. Cell morphology clearly showed that the cell wall of the yeast subjected to 100 kV/cm PEF was damaged. The amount of protein increased with an electric field above 70 kV/cm. Our experiment shows the ultra-high PEF is capable of destroying the yeast cell wall to extract intracellular functional molecules.

OR-80

Pulsed Electric Field Treatment for Preservation of Chlorella Suspensions

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Pulsed electric field (PEF) processing has emerged as an alternative to thermal pasteurization for the shelf-life extension of heat-sensitive liquids at industrial scale. It offers the advantage of minimal alteration in physicochemical characteristics and functional properties.

In this study, a pilot-scale continuous PEF processing (T < 55 °C) was applied for the preservation of microalgae Chlorella vulgaris (Cv) suspensions (pH = 6.5). Cv suspensions have been proposed as minimally processed, nutritious

and functional ingredient for manufacturing meat analogues via high moisture extrusion. Various PEF conditions (electric field strength E = 16, 19, 22, 25 and 28 kV/cm and pulse repetition rate = 100, 120 and 140 Hz, with a pulse width of 20 μs and an inlet product temperature T of 30 °C, n = 3) were tested on Cv inoculated with potential food spoilage microorganisms (Pseudomonas guariconensis, Enterobacter soli and Lactococcus lactis). The aim was to evaluate the PEF induced microbial reduction and monitor the microbial outgrowth during a 10-day cold storage period (10 °C). Additionally, selective media were used to differentiate between injured and intact cells, and image processing techniques were employed to study the impact of PEF on colony size during growth.

Maximum inactivation of 4.1 ± 0.2, 3.7 ± 0.1 and 3.6 ± 0.2 logs was achieved (28 kV/cm and 120 Hz) for the isolates mentioned above, respectively. Under these conditions, the critical electric field strengths E above which inactivation was observed, ranged from 22.6 to 24.6 kV/cm. The significant increase in injured cells compared to intact cells (approximately 100x) suggests considerable potential for further inactivation. The observed inactivation resulted from a synergy between thermal and electric effects. A second PEF cycle demonstrated similar inactivation efficiency, indicating its potential to enhance shelf-life further.

These findings are of industrial relevance and offer valuable insights into innovative technologies that not only ensure the quality and longevity of plant based food ingredients but also contribute to minimizing the risks associated with traditional thermal processing, thereby promoting healthier and more sustainable food options.

OR-81

Correlation of PEF induced biological autochemiluminescence with yeast cell electroporation

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Pulsed electric fields (PEFs) show promise in biotechnology and food industries. However, real-time monitoring of PEF treatment efficiency, especially at industrial and continuous production scales, is challenging. To address this, we developed a simple setup for real-time detection of yeast biological autochemiluminescence (BAL) during pulsing. We exposed Saccharomyces cerevisiae to 8 pulses of 100 µs width with electric field strengths of 2-7 kV/cm. Our method's sensitivity in detecting yeast electroporation was compared to established methods such as impedance measurements, propidium iodide uptake, cell growth assay, and fluorescence microscopy. Results demonstrate instantaneous monitoring of yeast electroporation during pulsing, making it ideal for industrial applications. The setup's simplicity allows integration into continuous liquid flow systems. We also established quantitative BAL indicators via statistical analysis.

Acknowledgments: The authors thank the Czech Science Foundation project no. 20-06873X for the support.

OR-82

Germination and stress tolerance of oats treated with pulsed electric field at different phases of seedling growth

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This study explores the impact of pulsed electric field (PEF) application on oat seedling growth and stress tolerance. PEF treatment (99 monopolar, rectangular pulses lasting 10 µs each, with a frequency of 13 Hz and a nominal electric field strength of 2250 V/cm) was applied at two growth stages: (i) when the seedlings had 0.2 cm roots emerging from the kernel, and (ii) when they

had a 0.4 cm shoot emerging from the kernel. Post-treatment, the seedlings were hydroponically grown for 8 days. To induce stress, the hydroponic medium was augmented with PEG (15%) to induce drought stress and NaCl (150 mM) to induce salinity stress. Results demonstrate that applying PEF improved the growth of the root and shoot of oat seedlings. This effect was more pronounced when applied to more developed seedlings. When PEF was applied during the later stage of germination, seedlings exposed to salinity stress showed enhanced shoot growth compared to the control. Under the studied conditions, the application of PEF had no impact on the growth of seedlings under drought stress.

OR-83

Effects of different combinations of pulsed electric field and pH shifting treatment on the aggregation structure and functional properties of soybean protein isolates

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Foshan University, China

Soybean protein Isolate (SPI), a kind of a protein product made from defatted soybean meal, has been widely used in food, materials and cosmetics industries for its high nutritional value and functional properties. However, when SPI has been extracted and produced, the treatments such as alkali dissolution and acid deposition, heat sterilisation and freeze/spray drying would cause protein aggregation and generate protein aggregates, which results in decreasing solubility of SPI and blocking its functional properties, thus restricting its application in the food industry. Therefore, we attempted to modify SPI using pulsed electric field (PEF) and pH shifting techniques to reduce the degree of aggregation of SPI and improve its functional properties. The effects of three different combined treatment methods of PEF and pH shifting (PEF before pH shifting, PEF after pH shifting, PEF combined with pH shifting) on the aggregation structure and functional characteristics were investigated. The results show that PEF combined with pH shifting was the best method for depolymerization of SPI aggregates. SPI treated with 10 kV/cm combined with pH 11 shifting has the highest solubility (90.23%), the smallest turbidity (0.072), the largest absolute zeta-potential value (44.4mV) and the smallest particle size (63.5nm). The combination of PEF with pH shifting treatment can induce the secondary and tertiary structure of SPI to unfold the folded part, resulting in the transformation of SPI from the spherical packing state to a molten sphere state with lower levels of α-helix and β-sheet and higher levels of random coiling. The SPI modified by different methods had better emulsifying, foaming and binding properties with small molecules. The improvement effect of the three modification methods on SPI functional characteristics was ranked as follows: PEF combined with pH shifting > pH shifting followed by PEF > PEF followed by pH shifting. Compared with the untreated group, the emulsification activity (EAI), emulsification stability (ESI), foaming activity (FA) and binding constant to lutein of SPI after PEF combined with pH shifting were increased by 119.24%, 39.33%, 59.03% and 245.81%, respectively. The processing conditions of this method are mild, which is conducive to improving the functional properties of SPI without impairing the sensory properties and biological activity of the protein. It is compatible with the processing concepts of “green processing” and “minimal processing” of food, and has the potential to become the mainstream technology in the modification of plant proteins in the future.

OR-84

Analysis of temperature dependent dielectric properties of bacteria for effective PEF pasteurization

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Pulsed electric field (PEF) is the most promising low-temperature pasteurization method for protein-rich liquid foods, such as milk, liquid egg, etc. Target liquids are often preheated for the PEF applic-

ation since bactericidal effect of PEF is known to be dependent on the temperature at which PEF is applied. On the other hand, higher preheating temperatures compel us to lessen the electrical input of PEF for preserving the ingredients. In this paper, we discuss the optimal preheating temperature for effective PEF pasteurization based on the temperature dependence of bacterial membrane properties measured using an impedance spectroscopy. The impedance analyzer (PSM1735-IAI, Newtons4th) was used to measure the capacitance and conductance of bacterial suspension of Enterobacter hormaechei (1010 CFU/ml) in a 2 mm gap electroporation cuvette. The capacitance and the conductance reflect state of the bacterial membrane and the leakage of intracellular ionic substances, respectively. The temperature of the bacterial suspension was monitored using a fluorescent fiber thermometer (FL-2400, Anritsu) and precisely controlled by our homemade system. When the temperature of the bacterial suspension was slowly increased from 30□, the capacitance was decreased linearly with increasing temperature up to 43□. The decrease rate of the capacitance was increased between 43□ and 55 □, which implies to be a phase transition of the membrane. On the other hand, the conductance was increased with increasing the temperature, and the increase rate was increased between 43 □ and 55 □. This indicates the leakage of the intracellular ionic substances. The survival rate of the bacteria to a single 2 μs-long 20 kV/cm PEF at the temperature between 35□ and 50□ was investigated. The temperature dependence of the bactericidal effect was correlated with the temperature dependence of the capacitance and the conductance determined by impedance analysis.

PFA Industry Panel

PFA Industry Panel Track
Sep 17, 13:20 - 14:10

OR-085

Pulsed Field Ablation – gaps in knowledge and future directions of development

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Pulsed Field Ablation is a new ablation modality based on irreversible electroporation. Since first proposed (and soon abandoned in 1980s due to major risks and side effects) it has gained traction following the development of irreversible electroporation in 2000s. PFA is now approved for atrial fibrillation treatment in Europe and by the FDA. The drive propelling the rapid uptake by cardiac electrophysiologists is its superior safety and speed with respect to cryo- and radiofrequency ablation and its comparable efficacy as evidenced by available clinical studies. It seems that we have “tamed the lion” in the last two decades.

Preclinical and clinical results indeed provide expected results with respect to safety by almost complete absence of “standard” complications of cryo- and RF-ablations: phrenic nerves, pulmonary veins, and esophagus damage and most rare but feared atrio-esophageal fistula. Somewhat unexpectedly, other complications have been reported: most notably cardiovascular spasm and hemolysis, but also silent cerebral emboli and phrenic nerve palsy have been reported. Can we expect more and others when numbers of treated patients will increase, and new PFA systems will be introduced into the market?

Somewhat disappointing was relatively low efficacy, i.e. comparable to cryo- and RFA (but still long-term follow-up results are missing). It needs to be emphasized that any new technology requires optimization (also from a procedural standpoint) It also seems fair to note that results obtained by PFA first generation devices are unfair to be compared to those obtained by RFA systems that benefited of decades of perfecting them. Much so also for cryo-ablation.

How can we address the new safety and/or therapy related concerns such as hemolysis, coronary spasm, cough, neuromuscular stimulation? Can we improve the efficacy? Is there a magic waveform that balances off safety concerns while it im-

proves efficacy? Is there a way to compare different PFA systems? How will regulators enable new and improved waveforms and devices to get on the market for the benefit of the patients? How can improved understanding of electroporation and basic research help address these questions.

S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)

Tuesday afternoon Track A
Sep 17, 14:20 - 15:20

OR-86

Initial single centre experience with pulsed field ablation for treatment of cardiac arrhythmias

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Background: Pulsed field ablation (PFA) is an emerging ablation modality for treatment of cardiac arrhythmias, based on irreversible electroporation.
Methods: We investigated procedural parameters and clinical outcomes from our PFA registry. CentauriTM (Galvanize Therapeutics, Inc., USA) PFA system was used. It is connected instead of a radiofrequency generator and thus easily adopted in routine clinical procedures, which were performed in deep sedation between February 21st, 2023 and March 5th, 2024. Follow up included scheduled outpatient clinic visits and ECG recordings guided by symptomatic arrhythmia episodes.
Results: We included 43 consecutive patients where at least one PFA application was delivered. Mainly persistent (53%) and paroxysmal (33%) atrial fibrillation (AF) were treated with pulmonary vein isolation lesion set (81%) as recommended by the manufacturer. For persistent AF isolation of left atrial (LA) posterior wall (47%) was added. Patients were male (65%), mean age 60,6 years, overweight (29,3 BMI). Totally, 92 ± 60 (mean ± SD) PFA applications were delivered per patient, with skin-skin procedural time 171 ± 64 (mean ± SD) min. Acute procedural efficacy was 81% for the whole cohort and follow up procedural efficacy was 60% and 42% for paroxysmal and persistent AF respectively. We observed 3 PFA related complications.
Discussion: As PFA has proven higher safety profile, which is believed to be due to non-thermal nature, LA posterior wall isolation was done as per our clinical experience and expert consensus, nevertheless one patient had postprocedural esophagitis, that resolved 2 weeks later. Follow up analysis showed, that for this patient higher 25A dose (intended for anterior aspect of LA) was used in LA posterior wall. One patient had acute pericarditis. Coronary spasm presenting with acute ST elevation was noticed in one procedure, while ablating at the ostium of the left inferior pulmonary vein.
Additionally, we used PFA in 4 patients after failed radiofrequency ablation of ventricular tachycardia. Despite highest dose (25A) and multiple applications delivered in the same location, only transient effect was achieved in all cases. This could be because CentauriTM system was developed for ablation of thin atrial wall, thus reaching deeper midmyocardial tachycardia isthmuses only with reversible electroporation field.
Conclusion: Based on small and heterogeneous patient cohort clinical efficacy comparison to the established clinical practice is difficult. Point-by-point PFA seems moderately effective for treatment of AF and ineffective for intramural ventricular substrates. We believe that the biggest advantage of the PFA system presented here is its possibility of toggling between different energy sources especially in the vicinity of neighbouring tissues (oesophagus) during extensive ablation of the LA posterior wall.

OR-87

Intraoperative Assessment of Irreversible Lesion Formation During PFA

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Background: Currently, there are no methods to assess and confirm irreversible lesion formation in real-time during Pulsed Field Ablation (PFA). Loss of local electrograms is a poor guide to confirm irreversible lesion formation.
Objective: To evaluate the feasibility of assessing irreversible lesion formation in vivo (in swine) using High Frequency Dielectric Sensing (HFDS) using a focal PFA catheter with its ablation electrode designed as an antenna-sensor at MHz-GHz frequencies in real-time during PFA.
Methods: A 9F focal ablation catheter with its ablation electrode redesigned as an onmidirectional antenna-sensor electrode was used in vivo (swine) to perform PFA. High Frequency Electrical Properties (HFEPs), namely, resonant frequencies (Fr), of the antenna sensor-electrode were simultaneously monitored in 150MHz-to-2.5GHz frequency sweep measurement. Changes to HFEPs, namely Fr, were monitored to confirm electrode-tissue contact, e-field deposition in tissue and durable lesion formation. PFA was carried out by applying trains of biphasic pulses, in a monopolar application, at different voltages ranging from 250 V to 1800V, 2 μs pulse widths 1000 pulses per application. Ablation locations were cataloged using Ensite mapping system and lesions were assessed by gross histology using TTC staining 12-14 hr post ablation.
Results: A total of 29 lesions were assessed in 2 animals (farm pigs, RA and RV lesions). Distinctly different HFEPs i.e. Fr, are observed when electrode is in blood, when contacting myocardium, and during PFA pulse application. Baseline Fr in blood 450±15 MHz which increased to 500±15 MHz confirming stable electrode-tissue contact ($p<0.001$). Following stable electrode-tissue contact confirmation, PEF pulses were applied and Fr increase in the range of 545MHz-to-630 MHz was observed (for voltages >1400V). An increase in Fr i.e. ΔFr>30 MHz correlated with irreversible lesion formation on histology. PFA in blood resulted in Δ Fr <10MHz. 21 out of 29 intracardiac lesions which were confirmed in real-time by HFDS during PFA were confirmed by histology and were transmural or >2.5 mm deep. 6 lesions where ΔFr was <10 did not result in a discernable lesion, whereas 2 lesions where ΔFr <30 resulted in superficial lesion ~1 mm deep.
Conclusion: HFDS can potentially assess procedure parameters and confirm irreversible lesion formation in real-time during PFA. This method does not rely on local electrogram detection and can be used to assess completeness of ablation during PFA.

OR-88

Lesion Durability Prediction based on Real-Time Impedance Analysis Algorithms: Validation with First-in-Human Clinical Data from the RESET-AF Trial

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Pulsed field ablation (PFA) is a non-thermal ablative strategy that achieves cell death in cardiac tissue by irreversible electroporation. PFA is becoming widely adopted as a technique for the treatment of atrial fibrillation (AF). A catheter is placed in patient's left atrium (LA) and navigated to each of the four pulmonary veins (PVs). Pulsed-field energy is applied to achieve isolation of all PVs from the body of the LA. Determining the quality and uniformity of the ablation catheter contact with the tissue is essential for the success of PFA procedures. Poor electrode-tissue contact is detrimental to achieving circumferential and transmural PV lesions. Additionally, patient movement may also lead to catheter displacement which can lead to lower efficacy. Therefore, it is important to detect such catheter movements, as well as poor contact, and to alert users accordingly. These alerts may result in stopping treatment or in repositioning the catheter. Data for this abstract was obtained from the RESET-AF clinical trial. RESET-AF is a prospective, nonrandomized, premarket first-in-human (FIH) study.
The aim of this study was to evaluate a PFA system that utilized real-time impedance measurements to predict treatment efficacy based on contact uniformity and movement detection algorithms. We used impedance data from 18 patients of the RESET-AF. A single PFA application was delivered to each PV followed by a second 'insurance' applic-

ation without repositioning the catheter. PV isolation was confirmed by 3D-mapping and exit block. Additional applications were only allowed if acute PV isolation was not achieved. All patients underwent mandatory invasive remapping at 3-months. The remapping data evaluated the lesion durability and were used to define the prediction algorithm accuracy.

Acute isolation was achieved in 100% of the PVs (n=72) using 2.1±0.4 applications/PV. PV isolation durability at 3 months was confirmed in 91.7% of the PVs and in 77.8% of the patients. For the isolated veins, the contact uniformity algorithm, in conjunction with the average electrode impedance value, were able to predict durable lesions with a positive predicted value of 94.7%. The movement detection algorithm was able to detect all movement instances of single electrodes, as confirmed by fluoroscopy or 3D navigation.

In this FIH clinical trial, PV isolation was achieved safely and effectively in patients with AF using a novel, single-shot PFA system without repositioning the catheter. Real-time impedance algorithms were shown to add an additional layer of efficacy as means to predict lesion outcomes.

OR-89

Investigation of bubble formation in intracardiac pulsed field ablation

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Introduction: Pulsed field ablation is a treatment method for cardiac ablation based on electroporation. Electroporation requires charge transfer between the electrodes and the cell suspension or tissue, which leads to redox reactions at the electrodes, which also produce gasses. The energy input is so intensive that it leads to considerable heating and can reach the boiling point of water.

There are three possible mechanisms of bubble formation and growth: a.) release of gas due to lower gas solubility at higher temperatures of the medium (we call this degassing), b.) release of gas as water vapor (boiling) and c.) release of gas as a product of hydrolysis. We performed an in vitro study with pulsed fields in saline using several different treatment protocols and a modified RF catheter for bipolar delivery and high-speed camera imaging. Moreover, numerical simulations to investigate thermal effects near the electrodes and predict boiling.

Materials and methods: We used monophasic (100 μs) and biphasic protocols and with varying duty factor to deliver pulses to 0.45% NaCl solution. Pulses were delivered bipolar via a modified 5 mm tip catheter (ConductR, Medtronic, Minneapolis, MN, USA) between the tip and the ring electrodes for intracardiac electrogram recording. The voltage and current during the pulses were measured, and the area between the tip and the most distal ring electrode was imaged at 10 kfps using a Phantom v1212 high-speed camera (Vision Research, Wayne, NJ, USA). Numerical modeling was performed with COMSOL Multiphyiscs (COMSOL AB, Stockholm, Sweden). An axisymmetric 2D model of the catheter was created. The model takes into account the heating due to pulse delivery, the increase in conductivity of the saline solution due to heating and the phase transition between the liquid and gaseous phase of the water, i.e. boiling.

Results: High-speed video recordings show that bubbles form in an even layer on both electrodes (anode and cathode) during a monophasic 100 μs pulse protocol. These bubbles are of electrochemical origin. Above a certain voltage threshold, an additional effect is observed near the leading edges of the ring electrodes: rapid appearance and disappearance of bubbles, which is consistent with the boiling process. In biphasic protocols, the electrochemical bubbles are not present, but boiling also occurs at sufficiently high voltages. The numerical model was also used to determine the maximum volume of degassed air due to the reduced solubility of nitrogen and oxygen at elevated water temperatures. The volume of degassed air was always less than the volume of steam.

Conclusions: The choice of treatment protocol for energy delivery and electrode design critically af-

fect the generation of bubbles near the electrodes. Careful electrode design and numerical modelling can aid in preventing bubbles. The results obtained in saline may differ from results in blood.

S08 - Public health risks and pulsed electric fields in the food industry

Tuesday afternoon Track B
Sep 17, 14:20 - 15:20

OR-90

A multivariate study on continuous-mode pulsed electric field treatment of E. coli in water

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Pulsed electric field (PEF) technology in the liquid food processing sector can be operated in batch- or continuous-mode. For industrial integration it is necessary to move from a research-focused batch-mode treatment to the more industrially feasible continuous-mode. Although it is more complex to integrate a PEF system in continuous-mode, it is more advantageous as the transient temperature can be controlled because of the liquid flow. Whereas, in batch-mode heating of the liquid food is more prevalent and should be closely monitored. In this work the role of the voltage, pulse number, and initial bacterial concentration as predictors of bacterial reduction of Escherichia coli JM109 were investigated. A closed-loop continuous-flow system was designed with a peristaltic pump, reservoir containing 100mL of the inoculated water, and custom-made cuvette.
E. coli, at three concentrations (5, 7, and 8 log CFU.mL-1), was inoculated in water and treated in continuous-mode PEF at two voltage levels. The pulse protocol was applied in four ‘steps’, each step of 60k pulses was applied with a 5-minute resting time between steps. The resting time was used to allow the liquid to cool down but also to extract a sample to investigate the role of pulse number. The sample media used in this treatment was water. The peak temperature of the pure water samples only increased by a few degrees as the sample’s electrical impedance is very large. However, when a small amount of salt was added the peak temperature was below 37 °C which will not influence/inactivate E. coli independently.
The bacterial reduction increased linearly for most of the treatments applied with some showing saturation as the pulse number (or specific energy input) is increased. The log reduction ranged between 1.1 to 6.6 across all PEF treatments. All three variables (voltage, pulse number, and initial bacterial concentration) were found to be independently statistically significant predictors of bacterial reduction, however no interaction between these variables were identified to also be statistically significant.

OR-91

Inactivation of zoonotic parasites by PEF, beyond single-cell electroporation

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Parasites are concerning food-borne pathogens. Some of them are currently not being routinely controlled in food, probably because their burden on public health is underestimated. Parasitic incidences could be avoided if preventive technologies were applied during food processing. Effective inactivation treatments are currently based on heat or freezing, but their side effects collide head-on with current consumer trends and new culinary habits.
Recent research supports the potential application of Pulsed Electric Field (PEF) technology in the control of food-borne parasites, to reduce the viability and infectivity of parasite transmission stages without affecting food quality.
The feasibility of PEF as an alternative to traditional freezing processes for the inactivation of Anisakis in fish has been demonstrated. The viability of Anisakis larvae was highly dependent on field strength and specific energy, while pulse width only exerted a notable influence at the lowest field

strengths tested (1 kV/cm). Central composite design helped to define a PEF treatment of 3 kV/cm and 50 kJ/kg as the one capable of inactivating almost 100% of Anisakis larvae in pieces of hake, while affecting the investigated quality parameters (moisture, water holding capacity, and cooking loss) to a lesser extent than freezing and thawing.

The inactivation of larvae of the nematode Trichinella, causing parasitic infections after the consumption of uncooked meat or meat products, also has been studied. Meat inspection is costly and tends to progressively be derogated in pig holdings applying controlled housing conditions in certain EU regions. PEF has been evaluated in the inactivation of Trichinella sp., including excysted larvae isolated by artificial digestion and encapsulated larvae found in meat from naturally infected wild boars. The excysted larvae were inactivated ten minutes after an intermediate PEF treatment (1 kV/cm, 0.41 kJ/kg). Treating excysted larvae with the mildest PEF treatment (0.5 kV/cm, 0.05 kJ/kg) combined with a 3% NaCl incubation resulted in synergistic inactivation. The application of 3 kV/cm (20 kJ/kg) to wild boar meat resulted in the inactivation of over 90% of encapsulated Trichinella larvae. The viability of Trichinella in meat was inversely correlated to the field strength applied (1-6 kV/cm) for equal energy input (20 kJ/kg).

The development of new PEF equipment is advancing at a rapid pace, allowing for food treatment at a scale that would have been unimaginable some years ago. A review of more basic-science studies carried out on buffer media would contribute to progress in addressing the underlying drawbacks that remain to be solved. Thoroughly different fields (parasitology, physics, food engineering, water sanitation, etc.) should converge to achieve the industrial implementation of PEF for the inactivation of food-borne parasites.

OR-92

Rapid Recovery of Bacterial Membrane Following Exposure to Pulsed Electric Fields

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PEF is a potential non-thermal sterilization technology, which has good application prospects for liquid food sterilization. he principle of PEF sterilization is to apply a high potential difference across the cell membrane, disrupting its structure (formation of pores) and thereby sterilizing the cell– a phenomenon called electroporation. However, there are two states for these pores: recoverable and non-recoverable, depending on their size. Recoverable small pores can quickly heal in a suitable environment (rich in nutrients, suitable temperature) within a few minutes, which may weaken the bactericidal effect. However, there are two states for these pores: recoverable and non-recoverable, depending on their size. Recoverable small pores can quickly heal in a suitable environment (rich in nutrients, suitable temperature) within a few minutes, which may weaken the bactericidal effect. The recovery process is anticipated to progress in seconds or less from the viewpoint of the thermal motion of lipids in the membrane, however, there are few studies reporting the recovery process within minutes following PEF treatment.

The purpose of this study was to utilize fast dielectric spectroscopy to investigate the time scale of recovery of injured bacteria from electric field exposure at different field strengths. The advantages of dielectric spectroscopy are that it can measure phenomena within a few seconds after PEF application and that it eliminates arbitrariness because the measured values are averages of all bacteria contained in the target solution. Enterobacter hormaechei (Gram-negative bacteria) suspended in buffer solution containing calcium ions were irradiated with a single PEF 2 μs, 5-20 kV/cm) and measured using an impedance analyzer (PSM1735-IAI, Newtons4th) at 1 MHz, a frequency that is less affected by the electric double

layer.
Dielectric measurements revealed that exposure to PEF with field strengths of 10 kV/cm or higher led to a significant decrease in capacitance and an increase in conductance of the bacterial solution. The decrease in capacitance suggests an increase in the permeability of the bacterial membrane, while the increase in conductance indicates permeability and leakage of intracellular ionic material into the solution. At 12 kV/cm, slightly above the critical field strength, the microscopic membrane dielectric constant decreased once and then recovered to the value before PEF exposure. This provides evidence of recovery of the electro-permeable membrane. However, for E > 15 kV/cm, no recovery was detected. These results indicate that the cell membrane recovers at different speeds depending on the electric field intensity. Additionally, the length of the gap time after the application of PEF will be one of the important parameters to consider in industrial applications of PEF combinatorial sterilization.

OR-93

Limitations of PEF for Food Pasteurization: role of membrane resealing in the microbial inactivation kinetics

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The capacity of Pulsed Electric Fields to inactivate vegetative forms of pathogenic microorganisms has led to its extensive study as a non-thermal technology for pasteurizing liquid food. Efforts have been made to define PEF processing parameters to ensure food safety. However, some studies revealed limitations in PEF treatments that could hinder its implementation at an industrial scale. In particular, the kinetics of PEF-inactivation often exhibit a tail, which may require extremely energetic protocols for cold-pasteurization. The present study explored the responses of various microorganisms subjected to electric fields to comprehend the reason behind the increment of microbial resistant to PEF during processing.
Salmonella Typhimurium, Bacillus pumillus and Saccharomyces cerevisiae were evaluated as Gram-negative and Gram-positive bacteria and yeast strains respectively. PEF-treatments (12-30 kV/cm) were applied in static conditions at low frequencies (1 Hz), resulting in a negligible temperature increase (< 5 °C). The study compared the lethality of PEF-treatments applied in two trains of pulses (n/2 + n/2) separated by a period of time ranging from 1 minute to 24 hours with the same treatment applied in a single train (n). Microbial viability and cytoplasmic membrane permeability were monitored over experimental time by the plate count method and propidium iodide staining protocols.
When a single train of pulses was applied, all the strains exhibited a rapid inactivation in the first moments but then the inactivation barely increased by augmenting the number of pules (tailing). However, for the three microorganisms, this tendency was mitigated when pulses were applied in two separate trains, being more evident with a longer resting time in between. Therefore, the tail of the survival curves was a consequence of a transient increment of the resistance of a proportion of the population to the external electric field. Membrane permeabilization studies indicated that, during the time between trains, a certain percentage of cells progressively recovered their membrane integrity. Therefore, it seems that an intact cytoplasmic membrane is required to be electroporated by PEF. This observation might be related to the formation of reversible local defects that increases the conductivity of the cytoplasmic membrane and prevents the increment of the induced transmembrane voltage (ITV) during subsequent pulses. After a resting period of time, the reversibly electroporated cells can recover their membrane integrity and become sensitive to subsequent PEF treatments.
In conclusion, splitting the delivered pulse train with a delay period between them may contribute to reducing the energy requirements of PEF protocols to achieve the required inactivation levels to guarantee food safety and stability.

S09 - Treatment of spinal cord injury: novel strategies and updates from the RISEUP project

Tuesday afternoon Track C
Sep 17, 14:20 - 15:20

OR-94

Sensorimotor contributions to human cognition and emotion: clinical neuroscience clues for optimizing engineering approaches to functional restoration in people with spinal cord lesions

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The massive body-brain disconnection consequent to spinal cord injuries implies a loss of sensory and motor bodily functions below the lesion but no direct influence on higher-order brain regions. Based on this, dichotomic views of mind-body interactions posit that basic perceptual and motor functions are separated from higher order ones that in turn are exclusively based on the manipulation of abstract, amodal symbolic systems. Telling, however, Embodied Cognition Theories postulate that creating and maintaining cognition and emotion may be shaped by somatosensory and motor representations. In this vein, somatosensory and motor bodily representations are not purely instrumental, ancillary components of mental functions but are fundamentally important for their optimal functioning. Here, we review clinical neuroscience studies suggesting not only that space and action representations but also higher order functions like fluid mental abilities (e.g. attention, executive functions) and emotional reactivity and regulation are impaired in people with SCI. While the relevance of factors like completeness, severity and level of the lesion needs to be clarified, the existing data hint at the crucial role of bodily functions in modulating cognition and emotion. Thus, taking into account the bi-rectional link between bodily and higher-order mental functions is of key importance for any engineering-based attempt to restore functions in people affected by spinal cord injury.

OR-95

Mechanisms of spinal cord regeneration

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Understanding why axons fail to regenerate has been one of the longest standing problems in contemporary neuroscience. For over eighty years, it was believed that the primary obstacle encountered by regenerating axons was the formation of an astrocyte border, which many believed acted as a physical and chemical barrier. My research overturned this longstanding and incorrect dogma, and demonstrated that the formation of an astrocytic border aids, rather than prevents, axon regeneration (Anderson et al, Nature, 2016). Following this discovery, I went on to expose three fundamental biological requirements to achieve axon regeneration across severe SCI: i) upregulation of dormant developmental growth programs in neurons above the injury, ii) expression of axon growth permissive molecules within the core of the injury, and iii) chemoattraction to guide axons to relevant targets below the injury. This strategy, based on gene therapies, propelled severed axons through and past anatomically complete SCI in mice, but they failed to reestablish movements (Anderson et al, Nature, 2018). My team and I recently discovered additional mechanisms that are required for such axon regeneration to mediate the recovery of neurological function: one must reestablish the projections of specific neuronal subpopulations to their natural target region (Squair et al, Science, 2023). When all these mechanisms are targeted with the appropriate spatial and temporal sequence, paralysis can be reversed despite the complete interruption of nerve fibers in the spinal cord. Finally, this understanding also informed experiments that exposed the efficacy of targeting individual requirements to improve neurological func-

tions following incomplete spinal cord damage, or when spontaneous capacities decline due to age (Skinnider, Gautier, et al, Nature, in press).

OR-96

Towards neuronal reconnection after a spinal cord injury using graphene-based nanocomposites – The NeuroStimSpinal project

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University of Aveiro, Portugal

NeuroStimSpinal (NSS) was a collaborative project funded through the H2020 FET Open program (No. 829060). The mission of NSS was to contribute to the development of a treatment for spinal cord injuries (SCIs). SCIs result in para- and tetraplegia due to the disruption of descending motor and ascending sensory neurons on a partial or complete basis. The objective was to produce an innovative, stimulus-responsive scaffold capable of stimulating neural tissue repair following SCI. The scaffold was composed of graphene-based materials (GBM) and decellularized human adipose tissue (adECM) and was intended to be implanted at the site of a traumatic injury. By combining the scaffold with an electrical stimulation device, it was aimed to promote neuron outgrowth and reconnection.

A series of innovative scaffolds based on adECM and GBM, showing promising applications for neural tissue regeneration, were developed. The scaffolds encompassed 3D foams, nanofibrous scaffolds, hydrogels, and bioinks. The NSS project advanced the understanding of cellular interactions with 3D adECM/rGO foams. The unique biochemistry of adECM allows neural stem cells to adhere and grow. It is important to note that high levels of rGO directly control cell fate by turning NE-4C cells and embryonic neural progenitor cells into neurons. Furthermore, increasing rGO modulates primary astrocyte fate by boosting the expression of reactivity markers, while unaltering the expression of scar-forming ones.

To explore and optimise the electrostimulation parameters delivered to the scaffold during the in vitro cell cultures, a new device, “A multi-well graphene-multielectrode array device for in vitro 3D electrical stimulation and its fabrication method,” was developed, and an international patent application was published (WO 2023/209676 AI).

The adECM and adECM-rGO scaffolds were shown to be safe in vivo studies, with no systemic reactions or toxicity seen after implantation. The results of histological studies showed that the scaffolds allowed cells to successfully invade and integrate into the host tissue. This supports the idea that they could be used for neural tissue engineering. The research further delved into the implantation of scaffolds in SCI models, noting the significant tissue integration and limited fibrous encapsulation, especially in adECM-rGO foams. Moreover, the macrophage-mediated uptake of rGO suggests a favourable biodegradation profile for these materials. Following these results, the hemisection in rats at the 10th thoracic vertebrae SCI implantation without electrical stimulation (ES) was performed on 46 animals divided into different groups. The experiment combining the scaffold with the ES device was conducted on a limited number of animals; however, it allowed us to demonstrate the proof of concept.

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OR-97

Neuroprotective effect of Pulsed Electromagnetic Fields after Acute Ischemic Stroke

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Acute cerebral ischemia is characterized by several pathological processes evolving during time, which contribute to the final tissue damage. Secondary processes, such as prolonged inflammatory response, impaired mitochondrial function and oxidative stress, are responsible for the progression of brain injury to the peri-infarct area, called “penumbra.” Adenosine has been shown to play a crucial role in regulating the inflammatory

cascade following brain ischemia.

Pulsed electromagnetic fields (PEMFs) act as modulators of adenosine receptors, increasing the functionality of the endogenous adenosine. In particular, PEMF exposure induces a significant upregulation of A2A and A3 adenosine receptors in different neuronal cell types.

Several lines of evidence suggest that PEMF exposure might play a neuroprotective role after ischemic damage.

PEMFs counteract hypoxia-induced apoptosis and ROS production in neuronal-like cells and exert a strong anti-inflammatory effect on microglial cells. Data from stroke animal models showed that PEMFs exposure is able to reduce the size of the infarct area and decrease the levels of pro-inflammatory mediators. In clinical studies, PEMFs stimulation proved to be safe and well tolerated.

A randomized, placebo-controlled, double-blind study aimed at evaluating whether PEMFs exposure is able to promote recovery in acute ischemic stroke patients (NCT02767778, I-NIC study) has been recently completed. The MRI evaluation performed at 45 days after stroke showed PEMF treatment induces a significant reduction of brain ischemia accompanied by a large clinical recovery compared to controls.

Altogether, our data demonstrate the efficacy of PEMFs against several mechanisms underlying ischemic damage and suggest that PEMF treatment should be considered to reduce the neuronal damage occurring in the “penumbra”, offering to clinicians the opportunity to extend the time for intervention in patients suffering from ischemic stroke after the narrow time window for reperfusion treatments.

P11 - Electroporation modeling and mechanisms

Tuesday afternoon Track D
Sep 17, 14:20 - 15:20

OR-98

Multi-stages pulse modulation strategy (MSPM) enhances electroporation-mediated intracellular delivery by regulating the distribution and accumulation of drugs

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Electroporation-mediated intracellular drug delivery provides an efficient and universal method in biological and biomedical applications [1] such as cellular monitoring [2] or cell therapy [3]. Reversible membrane permeabilization is preferred in this process which is crucial for the reconstruction of cellular homeostasis and cell survival [4]. Pulse modulation matters as it plays various roles at different stages of membrane pore formation, development, and resealing [5]. Here, we propose a multi-stages pulse modulation (MSPM) strategy that uses the microsecond high-voltage pulses (HSPs) and the millisecond low-voltage pulse (LLP) in a progressive manner to realize effective perforation and enhanced delivery efficiency, respectively. This methodology is effective for the delivery of both small charged molecules and nucleic acids.

For small charged molecules, MSPM leverages conventional HSPs (eight pulses, 1 Hz, 100 μs, 1000 V/cm) for electrochemotherapy and LLP (100 ms, 75 V/cm) with individually optimized field strength for HeLa cells. Various time sequences of pulses are tested with LLP before or/and after HSPs with the same overall electric dose (LLP-HSPs, LLP-HSPs-LLP, HSPs-LLP). Of which, HSPs-LLP achieves the best balance between cell viability and delivery efficiency at selected parameters. To better understand this process, a numerical model integrating molecule transport into traditional electroporation theoretical framework is built by introducing the dynamic pore conductivity to simulate the delivery process. Since pore evolution process is highly dynamic, there is a necessity to couple molecule transmembrane transfer with the fluctuation in pore current [6]. The modified model aligns well with the experimental results, where propidium iodide (PI) is utilized as a fluorescent indicator to track the delivery process. MSPM can augment rapid entry and distri-

bution of PI during pulse implement and ultimately increase intracellular accumulation by about 30% compared to using HSPs alone, which indicates the involvement of diffusion and enhanced electrophoresis. Besides, we evaluate the short-term and long-term disturbances of MSPM alone on cells by transcriptome analysis, flow cytometry and MTT assay, which shows tolerable influences on cellular activities upon parameter optimization. Further experiments confirm that MSPM effectively enhances the delivery of small charged chemotherapy drugs (bleomycin and cisplatin), with approximately 10% of decrease in IC50 for 24 h compared to using HSPs alone. Though at reduced bleomycin concentration, MSPM still increases the proportion of apoptotic cells and effectively triggers apoptosis-related pathways within 12 hours.

Additionally, since nucleic acids-related drug delivery has gained broad popularity in cancer therapy, we also investigate the effectiveness of MSPM in delivering siRNA. MSPM outperforms traditional RNAiMAX Lipofection for in-vitro siRNA delivery with higher viability (69.89% vs 48.51%) though knock-down efficiency (56.58% vs 55.10%) is similar at further optimized parameters for MDA-MB-231-Luciferase cells (fifty pulses, 10 Hz, 20 μs, 750 V/cm for HSPs and 100 ms, 75 V/cm for LLP). Besides, we find it also shows promise for in-vivo siRNA delivery with minimal side effects.

In summary, MSPM provides a feasible solution for safe, efficient and rapid intracellular delivery, further improving the delivery efficiency of charged chemotherapy drugs and reducing the dose demand by time-scaled regulation of molecular entry, distribution and intracellular accumulation compared to using HSPs alone. It also shows significant potential for nucleic acid drug delivery, making it a valuable tool in biomedical research and therapy.

OR-99

Multi-scalar microscopic molecular dynamics, coarse-grained and macroscopic study of voltage-gated protein interactions and complex lipid pore formation during cellular electropermeabilization

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Increasing experimental evidence shows that application of pulsed electric fields on cells during electropermeabilization affects voltage-gated ion channels, produces cell membrane peroxidation and alters the action potential. However, few studies have been conducted on these effects, with challenges remaining due to the wide range of temporal and spatial scales involved in experiments and applications. In this regard, we undertake a multi-scalar approach combining molecular dynamics, coarse-grained simulations, continuum models and artificial intelligence to simulate and analyze the formation of complex pores and increased permeability of human Na+, K+ and Ca2+ ion-channels during electropermeabilization. We explore the effect of different electrical pulse parameters on the electroconformational changes of individual channels, protein-protein and lipid-protein interactions. The results from this work provide a better understanding of pore formation and membrane protein function modulation by external electric fields, with potential biomedical applications targeting excitable and non-excitable cells, such as gene therapy, DNA vaccination, cardiac ablation, and electrochemotherapy for cancer treatment.

OR-100

Comparison of sharpness and electrical field distribution of different electrode needles for electrochemotherapy

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Introduction: Electrochemotherapy (ECT) is an accepted treatment used for human and veterinary cutaneous primary and secondary tumors of varied histologies. Several aspects of ECT remain to be elucidated.

The aim of this work is to develop a new needle for electrochemotherapy electrodes that resists mechanical wear providing adequate sharpness and electric field distribution throughout the treatment.

Materials and Methods Three types of needles were evaluated; conical, beveled, and triangular shaped. Electric field simulations were performed using COMSOL Multiphysics () to address the best orientation of the different shapes in the conformation of a six-needle electrode. To evaluate the difference in the insertion force required to pass through the tissue with the different types of needles, several insertions were performed on an ex vivo tissue model. The insertion force was estimated using a scale, and three different operators performed several applications in the tissue. The average weights were calculated, as well as the maximum and minimum values. To evaluate the durability of the sharpness, the insertion force was measured in over 300 insertions. Curves for the strength required were performed on each type of needle. The new electrodes developed were validated in veterinary patients with spontaneous tumors.

Results: Triangular shaped electrodes required less force for the insertion in the tissue followed by beveled and conical needles respectively. The electric field distribution was more homogeneous in conical shaped needles, followed by triangular and beveled shaped electrodes respectively. Great inhomogeneities are seen close to the tip of all the tested electrodes. Conical shaped electrodes lose sharpness before 50 insertions. Beveled needles show adequate insertion force, but great electric inhomogeneities in the surroundings of the tip, and greater tolerance to sharpness wear, resisting almost 150 insertions. Triangular needles provide adequate sharpness for more than 200 insertions, with an acceptable homogeneous electric field in the proximity of the tip, provided the flat faces of the needles are facing each other.

Conclusions: An electrode using triangular needles could provide an adequate electric field distribution and optimal sharpness after several applications for the treatment of cutaneous and subcutaneous lesions with electrochemotherapy.

OR-101

Cell electropermeabilization with subnanosecond pulsed electric fields

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The ability of short pulsed electric fields (PEFs) to generate cell electropermeabilization has been extensively studied for decades. This knowledge has led to the development of a wide scope of applications in various fields, ranging from the biomedical field to the food processing industry. To optimize these applications and to develop new ones, it is important to investigate all the mechanisms involved in this phenomenon, taking advantage of the progress in the generation of transient voltage pulses of ultrashort durations. The ultrashort pulses might display distinct properties on biological membranes and might cope with some technical limitations associated with the exposure to longer pulses. If the action of µs-PEFs and ns-PEFs on the cell membranes have been already well characterized, studies investigating the ability of subnanosecond-PEFs to electropermeabilize cells are still relatively scarce, mainly as a consequence of the scarcity of subnanosecond pulsed power exposure systems itself [1]. The present work focuses on the effects of ca. 910 ps duration PEFs on the level of electropermeabilization of E. coli DH5α to YO-PRO-1, a cell-impermeant DNA binding dye. The influences of several parameters have been investigated, such as the effect of the number of pulses applied, the pulse repetition frequency, the

electric field amplitude or the temperature of the sample during the exposure to the pulses. While the effects of some parameters are in line with those observed with pulses of longer duration (for example, the electropermeabilization as a function of the number of pulses applied), the effects of other parameters surprisingly differ. This has led to the elaboration of new mechanistic hypotheses, consistent with the fact that events commonly leading to electroporation with pulses of longer duration do not have the time to occur within hundreds of picoseconds. Importantly, the electric field amplitudes reported here to generate an efficient electropermeabilization of E. coli to YO-PRO-1 are considerably lower than those typically used in studies involving pulses of subnanosecond duration [2,3]. The achievement of efficient electroporation of E. coli using subnanosecond pulses of relatively low electric field amplitude is a step forward in the perspective of developing novel technologies for PEF delivery.

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S23 - Electroporation-based treatments in veterinary medicine

Tuesday late afternoon Track A
Sep 17, 16:50 - 18:20

OR-102

Electro-Chemo-Immuno Therapy: activating local and systemic immunity

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Introduction: Electrochemotherapy (ECT) is an approved and efficacious treatment for local control of cutaneous and subcutaneous tumor nodules. Nonetheless, ECT results in no impact on inaccessible, distant metastases, thus resulting in no significant effects on patients' overall survival. Preliminary evidences suggest that ECT can activate the Immune System. However, this issue has never been investigated in details. Based on a similar technology, we have developed a genetic vaccination platform based on the use of muscle DNA electrogenetransfer (DNA-EGT). Erb-eVax is a vaccine targeting the oncogene HER2/neu, overexpressed in breast cancer and canine osteosarcoma. Here, we hypothesize that standard ECT can induce a low, but detectable immune response against Tumor Associated Antigens (TAA) and that ECT and DNA-EGT can achieve synergic effects.
Methods: In this study, we have utilized a clinically validated device for Veterinary electroporation called Vet-ePorator, based on Cliniporator technology currently utilized and approved in Europe for ECT applications and adapted to EGT. Mice challenged with a breast cancer cell line overexpressing HER2 were treated with ECT, DNA-EGT or their combination. Dogs with metastatic breast cancer or Osteosarcoma are currently being treated with ECT and/or Erb-eVax.
Results: ECT induced a detectable cell-mediated immune response against HER2/neu and transient control of tumor growth. DNA-EGT was able to delay tumor growth over time whereas the combination ECT/DNA-EGT resulted in complete tumor rejection. ECT reduced tumor masses and Erb-eVax induced cell mediated and antibody immune responses against HER2/neu in treated dogs.
Conclusions: Our findings provide new data on tumor immunobiology and most importantly improve the efficacy of ECT and may open up novel immunotherapeutic approaches, thus having a significant impact on patient's survival and quality of life.

OR-103

Electrochemotherapy for bilateral limbal squamous cell carcinoma in a horse

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Objective: To describe the procedure and outcome of electrochemotherapy (ECT) with bleomycin as a first-line treatment for bilateral squamous cell carcinoma in the eye of a horse.
Animal Studied: A client-owned 5-year old Haflinger gelding with limbal-conjunctival squamous cell carcinoma.
Procedures: During general and local anaesthesia, injection of bleomycin in the ocular tumour was followed by electroporation, applied with a 15 mm needle electrode perpendicular to the ocular surface.
Results: Treatment with ECT resulted in therapeutic electric pulses, and complete tumour response with no recurrence during follow up. Tumour toxicity as a result of treatment was mild, with no adverse effect to normal tissue.
Conclusions: In this case of bilateral ocular tumours, staged ECT with bleomycin was shown to be a safe and effective treatment with complete tumour remission. The result suggests ECT as a competitive treatment in both human and veterinary ocular tumours, with further research recommended.

OR-104

Predictive factors in electrochemotherapy with or without IL-12 gene electrotransfer in dogs and cats

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Electrochemotherapy (ECT) is a relatively new method of tumour treatment in both human and veterinary oncology. In veterinary oncology, ECT is used to treat various histological tumour types, most commonly perianal tumours, soft tissue sarcomas (STS) and mast cell tumours (MCT) in dogs and squamous cell carcinomas (SCC) in cats. Although the success of therapy varies depending on the type of tumour, considerable differences can be observed even within the same histological tumour types. A good knowledge of biomarkers, such as histologic tumour type, is an indispensable tool for tailoring treatment regimens and predicting local and systemic response to treatment. Therefore, it is important to identify other types of biomarkers that could serve as predictive factors for treatment success in order to contribute to the increasing recognition of ECT for tumour treatment in veterinary oncology.
Previous studies have shown that the expected objective response (OR) depends on the type of tumour: epithelial tumours (perianal adenomas and adenocarcinomas) respond in 100%, round cell tumours (MCT) in 85%, mesenchymal tumours (STS) in 75% and oral tumours (oral melanoma (OM), oral SCC) in 40%. The OR in ECT as adjuvant therapy for unclean margins can be expected to be 90% for both STS and MCT. The OR also decreases with advancing clinical stage.
In MCT, the combined treatment of ECT and gene therapy with cytokine interleukin 12 (IL-12 GET) resulted in better efficacy due to systemic support of the antitumor immune response. Similar to studies using only ECT, the response was worse in tumours > 2 cm3. In addition, the poorer response is also associated with a lower proliferation index (Ki67) and increased PD-1 and PDL-1 expression in tumour tissue prior to treatment. In contrast, the response was better in tumours with a higher infiltration of CD3+ cells. Serum/plasma levels of IL-12 and IFNγ, ferritin and nucleosomes were also not related to clinical response. In contrast, we have shown that elevated LDH levels may be helpful in predicting treatment response.
The results of our large study on MCT in dogs suggest that the combination of ECT and IL-12 GET is successful, but additional biomarkers are needed for a more accurate disease prognosis.

OR-105

Comparison of intratumoral or peritumoral IL-12 gene electrotransfer in combination with electrochemotherapy for the treatment of spontaneous mast cell tumors in dogs

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The use of electrochemotherapy (ECT) and interleukin-12 (IL-12) gene electrotransfer (GET) for the treatment of various histological types of spontaneous tumors in dogs has been described in several clinical studies. Although the results of these studies show that the treatment is successful and safe, the routes of administration of IL-12 GET have been either intratumoral (i.t.) or peritumoral (peri.t.). Therefore, the aim of this study was to compare the two routes of administration of IL-12 GET in combination with ECT and their contribution to the enhanced response to ECT. Seventy-seven dogs diagnosed with spontaneous mast cell tumors (MCT) were divided into three groups: one group was treated with a combination of ECT + GET peri.t. (29 dogs), the second with the combination of ECT + GET i.t. (30 dogs) and the third with ECT alone (18 dogs). In addition, immunohistochemical analysis of tumor samples before treatment and flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) before and after treatment were performed to determine any immunological effects of treatment. The results showed significantly improved local tumor control in the ECT + GET i.t. group ($p < 0.050$) compared to the ECT + GET peri.t. and ECT alone groups. In addition, a remarkable prolongation of the disease-free interval (DFI) and progression-free survival (PFS) was observed in the ECT + GET i.t. group compared to the other two groups ($p < 0.050$). The results in terms of local tumor response, disease-free interval (DFI) and progression-free survival (PFS) were consistent with the immunological assessments, as evidenced by the increased presence of anti-tumor immune cells in the circulation after treatment in the ECT + GET i.t. group. This observation suggests that a systemic immune response was induced. Moreover, no adverse severe side effects were observed. Finally, due to the more pronounced local response after ECT + GET i.t., we suggest that the treatment response assessment should be performed at least two months after treatment, which is in line with the iRECIST criteria.

OR-106

A Veterinary Electrotransfer System that employs Heat and Impedance – Progress Toward Commercialization

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Pulsed electric fields have broad potential for molecular delivery applications for applications such as gene therapy, protein therapies, vaccination, and chemotherapy. Countless studies have examined different electrode configurations as well as wide ranges of electrical parameters such as pulse width, voltage applied, number of pulses, and period all generally focused on maximizing/optimizing delivery or tissue response. More recently, localized moderate heating (to ~43°C) during the delivery of plasmid DNA using pulses electric fields has been shown to increase expression of a delivered foreign gene. Furthermore, tissue impedance measured during electrical treatment has been used in a feedback manner to adjust electrical treatment in real-time to customize the electroporation process during delivery. Based upon lab prototype devices and results in small animal models, it was clear that there could be many applications in human and veterinary clinics. As a first step, a commercial device for providing electrochemotherapy to veterinary tumors was developed, designed, and produced in conjunction with LifePulse™, Inc. The device consisted of a handle that had a replaceable array of needle electrodes. After placement of the array into a tumor, the tumor was heated using warmed air. Temperature was maintained using a FLIR camera that was also contained in the handle. Pulses were applied using a custom pulse generator, and real-time impedance measurements were used to guide pulsation. The entire system was automatic and controlled using a graphical user interface and software on a laptop. Ten of these first commercial devices have been circulated among about 20 veterinary oncology practices across the United States during the past two years. A multitude of different tumor types have been treated mostly in dogs and horses. De-

tails of the system operation and features will be provided along with features of the final commercial designs. 4 case studies will also be presented. These will be from the treatment of an equine sarcoid, equine melanoma, canine soft-tissue sarcoma, and canine nasal squamous cell carcinoma.

OR-107

Electrical characterization of VX2 tumor in rabbit model for electroporation purposes

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Within the diverse fields of application of electroporation, oncological treatments have a great interest. Although several treatments such as tumor ablation by irreversible electroporation or electrochemotherapy do already have clinical application, there is still a need to improve the safety and control of these therapies. To carry out this research it is necessary to develop tools such as simulation and experimental models, which are complex due to the great variability of biological systems. Specifically, within this branch it is necessary to study and model biological tissues in order to adequately estimate the distribution of the electric field, the feasibility and efficiency of the treatments, and the possibility of thermal side effects.

The aim of this work is to present the electrical characterization of an animal tumor model widely used in this field. The rabbit VX2 is a large animal model of cancer used for studying and developing oncological treatments and consists of one implantation in the hepatic lobe of a rabbit of VX2 carcinoma, that is an anaplastic squamous cell carcinoma derived from a virus-induced papilloma in rabbits. For this purpose, an impedance measurement system has been designed to carry out in vivo and ex vivo measurements in real time. With this system and the acquired measurements, FEA models have been developed to accurately estimate the electrical properties of the tissue.

The experimental process consisted of the implantation of 9 animals divided into 3 groups to characterize the model at different time points of development. To perform the impedance measurements with the developed system, tumors with a diameter between 0.5 and 2 cm were considered, so the time points studied were: 14, 17, and 21 days. It is remarkable that, this last time point already presented a significant internal volume of necrosis. The implementation procedure consisted of a laparotomy implanting 1 mm3 portions of tumor in a small incision in a left hepatic lobe. The measurements were taken in vivo and 10 minutes after the sacrifice of the animals.

In conclusion, an impedance measurement system has been developed to carry out an electrical characterization of a very interesting tumor model for the study of electroporation. The data have been statistically analyzed to obtain representative conclusions and models. The final version of this paper will include experimental results and future insights demonstrating the feasibility of this proposal.

S07 - Potential applications of PEFs technology in vegetable and fruit processing

Tuesday late afternoon Track B

Sep 17, 16:50 - 18:20

OR-108

How does PEF impact membrane integrity and the volatile profile of leek?

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In the past few decades, pulsed electric fields (PEF) technology emerged as an innovative non-thermal processing technique for several types of food. At low electric field strengths (< 4 kV/cm), PEF has the potential to induce irreversible cell membrane permeabilization in plant-based matrices, which is currently mainly been investigated in the context of enhanced drying and enhancement of extraction and cutting effectiveness. However, PEF at low electrical field strength can also impact enzyme-substrate interactions as a

result of membrane disintegration (i.e., electroporation), thereby imparting their volatile profile, especially in matrices where enzyme and its corresponding substrate are present in different cells or different cell compartments, physically separated by membranes. There are only limited studies that focus on the potential of PEF in this context. Due to the inhomogeneous microstructural characteristics of many vegetables (e.g., different cell types and cell sizes), it is hypothesized that the spatial orientation of the product in the treatment chamber might influence the PEF-induced effect. The latter aspect has not been fully elucidated, but is interesting to consider as quality characteristics of similar food products could be affected to different extents if orientation in the treatment chamber is modified.
In this research, PEF treatments at low electric field strengths were applied to leeks (Allium ampeloprasum var. porrum), a vegetable that is characterized by prominent compartmentalized enzymes and substrates which can determine the volatile profile of the vegetable. As a first research question, the effect of varying the total specific energy input of PEF treatments was investigated on the membrane integrity and volatile profile. A second research question aimed to investigate the effect of the spatial orientation of leeks during PEF treatment, relative to the applied electric field, on both the vegetable's membrane integrity and volatile profile. To assess the membrane integrity, measurements of tissue and medium conductivity were performed. The volatile profile was analyzed using an untargeted headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) approach. This work showed that PEF at low field strengths is able to induce membrane disintegration in leeks. A substantial effect of applying different total specific energy inputs between 1.50 and 22.50 kJ/kg at a constant low electric field strength of 1.01 kV/cm on the resulting cell membrane disintegration and volatile profiles of leeks was observed. Furthermore, this study demonstrated that the spatial orientation of leeks during PEF treatment has an influential role in inducing membrane electroporation and shaping the volatile profile.

OR-109

Practical application using Pulse Electric Field (PEF) approach in milking the roots from aeroponic system

Sylwester Ślusarczyk, Kajetan Grzelka, Joanna Jaśpińska, Adam Matkowski

Wroclaw Medical University, Poland

The aim of this work was to develop and optimize PEF treatment for the reversible electroporation from the roots and rhizome of 3 model plants Iris domestica (syn. Belamcanda chinensis L. DC. Iridaceae) Scutellaria baicalensis L and Cicerbita alpina (L.), all cultivated in aeroponic system. Electroporation can be successfully used in extraction of compounds from plant tissue what is very well known.[1,2] but is it possible such extraction for living plants?
In our work we expand on these findings and further optimize the treatment parameters. 3 months old aeroponic cultivar of model plants were electroporated using different pulsed electric field strength from 0,2 to 7.5 kV/cm with constant 50 or 100 μs duration of pulse and repetition N 50, 100 and 200 pulses. We successful milking the roots (extracted) of secondary metabolites after PEF in different solvents system. We developed special chamber for this purpose. Treated plants survived this process and it can be regularly repeated on the same individual plants as a continuation of milking the roots. The most promising variant was when we applied: E = 0.5 kV/cm N = 100 f = 1 Hz t = 100 us (A) and E = 0.5 kV/cm N = 200 f = 10 Hz t = 50 us (B) For extraction following solvents were used: choline chloride : glucose (1:2) + 30% water; choline chloride : ethylene glycol (1:2); choline chloride : fructose (1:2) + 30% water; choline chloride : saccharose (1:2) + 40% water and tap water. After PEF plants were cultivated in aeroponic systems and observed throughout the next 4 months, compare to control. For HPLC-MS analysis all collected sample (directly after PEF and after week) were first purified on SPE system to 10 mg/ml concentration. Main disadvantage of this approach is frequent infection of aeroponic cultivars with typical pests and frequent fungal and bacterial overfeeding, which must be combated by

biological protection and keeping sterility regime.

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OR-110

Seaweed processing with pulsed electric fields: from batch to continuous process development for functional ingredients production

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Marine macroalgae Ulva sp. and Gracilaria sp. can reach a high protein content and a high composition of essential amino acids. We have developed a novel device and investigated the parameters for a continuous Pulsed Electric Field (PEF) process, coupled with enzyme treatment, to facilitate the aqueous fractionation of those seaweeds. The process demonstrated 8.79±0.58 (% w/w) Ulva protein extraction yield. Furthermore, the aqueous fractionation of Ulva protein exhibited a substantial 41.45% essential amino acid content and a branched amino acids composition of 17.58%. Additionally, it displayed an in vitro relative digestibility of 87.4±1.36% compared to soy protein, along with a water holding capacity of 7.15 ± 0.17 g water/g sample and an oil holding capacity of 1.76 ± 0.11 g oil/g sample. For Gracilaria sp., the water extracts from the PEF-treated biomass showed higher protein yield (37.82 ± 7.96 to 47.56 ± 10.54 mg/g initial dry weight (DW) of biomass, depending on PEF conditions) compared to extracts without PEF (33.82 ± 6.01 mg/g initial DW of biomass). PEF followed by aqueous extraction removed 69.11% of the ash from the Gracilaria sp. biomass. The in vitro digestibility of the Gracilaria sp. proteins in the water-soluble extract from the PEF process was 70% compared to that of casein. These findings suggest that employing a continuous PEF process could position marine macroalgae as an important potential source for novel proteins.

OR-111

Optimizing valuable compound recovery from food side streams and microbial Biosynthesis through PEF-Induced Extraction and Stress Strategies

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Aim: In the last years several studies have demonstrated the feasibility of pulsed electric fields (PEF) as a good pre-treatment technology able to enhance the bioactive compounds extraction. Within the EU founded project SHEALTHY it was investigated to what extend the extraction yield of essential oils as well as polyphenols could be increased with PEF in the range of 1-5 kJ/kg in comparison to an untreated sample.
Methods: Based on the literature, higher electric field strength are necessary to extract valuable compounds from side streams, especially from orange peels. The HVP 5 PEF (30 kV) systems with a 2 cm treatment cell was used to aplly an electric field strength up to 10 kV/cm. The experimental parameter setup for orange peels was based on literature, where different electric field strength for the extraction of polyphenols from different side streams were applied. For the PEF-treatment of olive leaves, parameters from a study conducted by UGR were used. The knowledge of the possible effective process conditions was translated into a Design of Experiments (DoE). After the treatment the samples were dried and send to the analytic partner for analyses.
Results: For orange peels the electric field strength was between 1.0 – 2 kV/cm. Based on the DoE the optimal treatment conditions for the increase of extraction in comparison to the control 50 % (Flavon-

oids), 26 % (Polyphenols), 32 %(Hesperidin), 68 % (Narirutin) was 1.5 kV/cm, 30 pulses and a pulse width of 30 us. In comparison to ultrasound optimal conditions for TPC 22 % more was extracted. The optimal treatment conditions for olive leaves were 0.8 kV/cm, 110 Hz, 11 s of treatment time and a pulse width of 15 us. This lead to an increase of TPC by 9% and of DPPH by 22 % in comparison to the control. Ultrasound and PEF extraction had similar results.

Conclusion: PEF extraction shows high potential for the extraction of valuable compounds from side streams in comparison to untreated as well as other green extraction technologies like ultrasound. The validation of the optimal treatment conditions as well as the scale up are ongoing.

OR-112

Increasing the yield of juice and bioactive compounds extracted from blueberries using pulsed electric field

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Pulsed electric fields (PEF) is a widely used processing technique in the food and beverage industries for extraction, safety and/or quality of liquids, semi-solid, and solid foods. One of the main disadvantages of PEFs is that the generators used to create them are dependent voltage sources. The output pulse characteristics such as the voltage and pulse width are highly dependent on the sample's electrical properties. In this work we have developed a method to treat high-value solid food products without voltage dependence on the sample. Blueberries were chosen as a target high-value food product to show the proof-of-concept which can be applied to any other solid food.

The method uses pure water to set the electrical impedance of the sample which is not influenced by the target food product (in this case blueberries). Pure water has a large impedance (low conductivity), whereas the blueberry itself cannot influence the electrical impedance until the PEF excitation has begun. In biochemistry buffers are used to fix the pH at a near constant value; similarly pure water can be used to reduce volatility in the transient temperature during PEF excitation. This method can be used to treat any solid food product placed between electrodes surrounded by pure water.

The pH, sugar content, total solids, colour, total phenolic content, anthocyanin content, and DPPH antioxidant activity were investigated after pulsed electric stimuli applied to blueberries. No significant differences between PEF and non-PEF samples were identified for pH, sugar content, nor total solids. The colour of the 'pure water' used for PEF treatment changed significantly as the specific energy input increased.

The phenolic content of both the blueberry juice and skin was significantly different to the control (no PEF). The anthocyanin content in the blueberry skin was found to be significantly different to the control. The DPPH antioxidant activity was statistically significant in the blueberry skin, however not in the extracted juice.

PEF through the electroporation and electropermeabilization processes have shown to significantly facilitate and support the extraction process from blueberries. The method used in this work can be further used in future work targeting any solid foods.

S09 - Treatment of spinal cord injury: novel strategies and updates from the RISEUP project

Tuesday late afternoon Track C

Sep 17, 16:50 - 18:20

OR-113

Boosting the development of Electro Pulsed Bio-hybrid implantable devices through advanced modelling in vitro and in vivo

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Spinal cord injury (SCI) is one of the most debilitating conditions existing [1]. Researchers are working worldwide on innovative treatments to restore the lost functions [2]. Particularly, the project “Regeneration of injured spinal cord by electro pulsed bio-hybrid approach” (RISEUP, FET Open of Horizon Europe Program 2020) aims at developing an innovative Electro Pulsed Bio-hybrid (EPB) device for spinal cord regeneration based on stem cells transplantation and electric stimulation via μs-Pulsed Electric Fields (μsPEF) and direct current (DC) stimulation.

In this context 3D advanced computational modelling represents a fundamental step to potentiate the engineering work of design and production of the device able to generate field necessary to induce the neuroregenerative processes on cells.

Herein, we present the workflow to attain the 3D realistic model of the EPB working in its intended environments of applications, i.e., in vitro and in vivo. The computational approach to this full characterization of the EPB starts from modelling the initial device and subsequently adding details that increase complexity and level of realistic representation.

As a first step, bare electrodes are simulated to define and optimize their configuration, so obtaining the electrified part of the EPB. Secondly, a reliable and fast reconstruction procedure to obtain realistic 3D models of the microfibril lanes is carried out, starting from microscopy images, so reproducing the bio-hybrid scaffold, in tight contact with the electrodes.

Once that the EPB is numerically characterized, its behavior during in vitro experiments must be reproduced. An additional degree of detail is added, by simulating the presence of the stem cells over the microfibril lanes, with highly realistic cells models, obtained from high-resolution confocal microscopy images: 3D-microdosimetry simulations allow to identify conditions able to guarantee threshold levels needed to attain the desired effect. Finally, these optimal conditions identified in vitro must be ensured in the in vivo environment, where the concept of neuronal regeneration should be proved. Hence, an accurate 3D model of a spinal cord (SC) injured rat is needed. The semi-specific approach is applied [3], by manipulating licensed detailed rat computational phantoms to host the model of a SC lesion obtained from microscopy images. The EPB model is placed over the lesion, as done during rat surgery: dosimetric results assess whether the optimal stimulation conditions can be achieved in the lesion environment, and allow the design of additional stimulation devices when needed.

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OR-114

Materials solutions for an electrostimulable device for use in spinal cord injury model in rat

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The use of natural and synthetic biomaterials forming hydrogels or scaffolds has served as a successful interface for physical, biological, and chemical support to endogenous and transplanted cells. This is a very promising field with great potential to overcome the low efficacy of cell transplantation in spinal cord injury (SCI). In this work, an Electro-Pulsed Biohybrid (EPB) device is developed as part of the FET-OPEN project “Regeneration of injured spinal cord by electro pulsed bio-hybrid approach” (RISEUP, n.964562). Due to the characteristics of the implant for SCI in rat model, the device had to meet a series of specific properties. Biocompatible and biodegradable materials (polylactic acid (PLA) and hyaluronic acid (HA)) were selected with the aim of achieving a slow degradation rate that would provide integrity to the device throughout the entire regeneration process of the SCI. The device had to be able to deliver cells to the injured area using the smallest possible volume of material. Therefore, the scaffold had to have a high specific surface area and, at the same time, present a high flex-

ibility. This was achieved by using polylactic acid microfibers of 10 microns in diameter arranged in a monolayer, forming a microfiber lane. This assembly had to be subjected to electrical stimulation. For this purpose, a PLA membrane was designed to place the electrodes. Since the conductivity levels required for the electrodes were very high, it was necessary to use metal electrodes instead of electroconductive polymers, and gold electrode tracks were incorporated on the PLA supportive membrane. Tissue adhesions after surgery can compromise the effectiveness of cell transplantation. In order to minimize this danger, a hydrogel coating of HA was produced on the face of the device opposite to that of the electrodes. HA is a natural hydrogel that prevents these processes. With all these premises, a device was designed and manufactured, consisting in an electrified supportive membrane (PLA) with a monolayer of microfibers (PLA) on one face and a hydrogel coating (HA) on the other. Two adapted designs of the device were developed taking account of the circumstances of in vitro experiments and of in vivo surgery in a rat SCI model.

OR-115

Electromanipulation of calcium oscillations in Mesenchymal Stem Cells, a control of cell fate?

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Mesenchymal stem cells (MSCs) are adult multipotent stem cells which can differentiate into various connective tissue cell types such as osteoblasts, adipocytes… 1 In addition, under specific conditions, their differentiation abilities can extend to other specialized cell types, such as muscle or neuron-like cells 2. Not only due to this promising multipotency, but also to interesting secretory activities 3 as well as rescue functions towards damaged cells in their environment 4, these cells have attracted increasing interest in the context of regenerative therapies in last decades. In another respect, calcium (Ca2+) is an important second messenger in cells. Ca2+ signaling often occurs in the form of oscillatory patterns, referred to as Ca2+ oscillations, whose frequency and/or amplitude embeds information that is subsequently decoded by effector proteins whose activity is Ca2+-sensitive 5. Spontaneous Ca2+ oscillations have been observed in MSCs, the frequency of which varies over the course of differentiation or proliferation events. The question subsequently arises to wonder whether taking the control over Ca2+ oscillations could be used as a mean to influence the cell fate. As a first step, we focused on assessing the changes in Ca2+ oscillation patterns along proliferation or differentiation events by developing time-lapse microscopy with fluorescent Ca2+ probes and Fast Fourier Transform analysis, allowing the analysis of Ca2+ oscillations in hundreds of cells in a single experiment. In a second time, we took the control of Ca2+ oscillations using microsecond pulsed electric fields generating a slight permeabilization of the cell membrane to Ca2+. This limited and controlled permeabilization to Ca2+ subsequently triggers Ca2+ oscillations similar to natural ones (in shape, amplitude and duration) due to the Ca2+-induced-Ca2+-release response 6. The evolution of Ca2+ oscillation patterns in proliferation and in various differentiations led to the hypothesis of a link between Ca2+ oscillations and cell cycle progression in MSCs. In this context, the effect of the frequency of Ca2+ oscillations on specific signaling pathways associated with the cell cycle progression and the evolution of the Ca2+ oscillation patterns within a cell cycle are currently under investigation. This work carries both fundamental and applicated aspects: to assess the role and the importance of the Ca2+ oscillations in various cellular processes in MSCs, and to develop a suitable device to control them on the long-term, in the perspective of therapeutic applications.

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OR-116

Effects of microsecond electrical pulses on the inflammatory response

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Spinal cord injury (SCI) is a neurological and pathological state caused by direct damage of the spinal cord or of the tissue and vertebrae surrounding it, characterized by permanent or temporary changes in the motor, sensory, and autonomic function.

Two different phases characterize SCI. The primary injury occurs immediately after the traumatic insult which damages or destroys the tissue and is due to the hemostatic response and acute cell death. This first phase is followed by the progressive secondary injury cascade, characterized by ischemia, proapoptotic signaling, and peripheral inflammatory cell infiltration. Over the subsequent hours, the strong inflammatory response, mediated by cell activation and release of proinflammatory cytokines, makes the SCI environment hostile to any kind of therapeutic treatment. Indeed, so far, several therapeutic strategies for SCI have been studied, but an effective protocol for spinal cord regeneration is still missing.

RISEUP project, funded by the European community in the H2020 FET-OPEN program (grant agreement N° 964562), provides for the development of an innovative system for the regeneration of SCI based on the transplantation and the microsecond electric pulses (μsPEFs) stimulation of mesenchymal (MSCs) and induced neuronal (iNSCs) stem cells, through a biocompatible, biodegradable, and electrified support.

The hypothesis is to modulate through μsPEFs the intracellular calcium fluxes resulting in the control of proliferation and differentiation of these cells; iNSCs could differentiate in mature neurons to regenerate the lesioned area, and MSCs in neuronal-like cells to release neurotrophic factors.

To better understand the effect of this device on the pro-inflammatory environment of SCI, the two μsPEFs protocols (proliferation and differentiation), identified in the context of the project, have been tested on a human cell line of monocytes (THP-1), macrophages (obtained by treating monocytes with PMA) and microglia (HMC3).

Here we present the first results obtained by applying these stimulation protocols to the cells mentioned above in terms of proliferation, gene expression, and inflammatory response.

The purpose of these analyses is to assess the effect of the final device on the pro-inflammatory environment of SCI, to avoid any side effects, hoping for the induction of a beneficial anti-inflammatory effect.

OR-117

Emerging Approaches to Neural Tissue Regeneration: Electrical Stimulation of Stem Cells

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Spinal cord injury (SCI) treatment remains a significant challenge due to inability to regenerate lost neurons and restore functional connections after an SCI. The complex cascade of events that

follow an SCI, including a non-permissive environment and limited capacity for axonal regrowth, often leads to a degenerative chronic stage [1]. This complexity necessitates multifaceted strategies for functional restoration, including cell substitution, neuroprotection and axonal growth promotion.

Mesenchymal Stem Cells (MSC) have the potential to generate multiple differentiated progenies and provide a suitable microenvironment for tissue regeneration [1]. MSC are present in several tissues of the human body, making them a readily available source of stem cells for therapeutic use. Also, it is reported that MSC transplantation into SCI provides immunoregulatory effects. However, the transplantation process is challenged by a number of issues such as a low survival rate and the lack of neural differentiation and maturation. Therefore, in order to improve cell-based therapies it is important to develop complementary treatments for example electrical stimulation particularly if it may avoid using exogenous chemical compounds.

Our work explores a new combinatory therapy for SCI treatment that employs high voltage microsecond electric pulses (μsPEF) stimulations and low amplitude direct currents in conjunction with stem cell transplantation on a biocompatible device. The purpose of using μsPEF stimulation is to generate cell electropermeabilisation to calcium (Ca2+), a simple molecule which performs a multitude of functions within cells [2]. It serves as universal second messenger in many biological process [3]. Minor changes in Ca2+ concentration, known as Ca2+ oscillations, are crucial for cellular differentiation and function. Thus, the control of Ca2+ oscillations could potentially optimize the process of cell transplantation.

This objective is thus the development of electrical protocols that can induce differentiation or proliferation. We started using a 2D model and we are now performing experiments in a more realistic 3D system. Indeed, MSC exhibit spontaneous Ca2+ oscillations, which vary if MSC are in differentiation or proliferation processes [4]. This study is the continuation of our previous work [5], exploring the changes that might be triggered by μsPEF stimulation, which could promote cellular proliferation or differentiation events.

In conclusion, this work aims at defining the electrical stimulation protocols that are able to induce specific changes in the Ca2+ oscillations of MSC which may promote proliferation or neural differentiation. This could potentially lead to significant advancements in the treatment of SCI, paving the way for more effective recovery strategies.

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OR-118

Regeneration of Injured Spinal Cord by applying subdural electro pulsed stimulation and stem cell bio-hybrid approach

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Spinal cord injury (SCI) is a dramatic event that leads to an irreversible disabling condition with loss of motor and sensory functions (1). The main symptoms are the temporary or permanent loss of sensations, muscle strength, bowel and bladder function. SCI condition affects around 300.000 people each year with different incidences in the different countries and the severity of the SCI mainly depends on two aspects: i) the anatomical structure involved and ii) the site of the lesion (closer the trauma is to the cervical vertebrae and greater will be the area af-

fected by the consequences of the trauma), (1). SCI could be subdivided in two pathophysiological phases: the primary injury, induced directly by the trauma, leading to neural cell death and axonal network damage and the delayed secondary injury characterized mainly by further dead of neurons and oligodendrocytes, increased astrocytes and immune cell activation and free radical formation (2,3). Among all, the loss of oligodendrocytes and the consequent progressive demyelination result in axonal dysfunction, degeneration and loss of sensory and motor functions (4). Although many progresses have been done to minimize the spinal cord damage and optimize the functionality of spared connections, nowadays there is no effective therapy for the treatment of SCI (5). Multifaceted strategies are considered the unique solution for functional restoration by including cell substitution, neuroprotection and axonal growth promotion. RISEUP project (https://www.riseup-project.eu/) proposes to achieve neuronal functional regeneration after SCI by an unprecedented and unique bio-hybrid-compatible electro-activated and potentially be wireless rechargeable implantable technology. RISEUP introduces high voltage microsecond electric pulses (micro pulses) stimulations and low amplitude direct currents on a combination of stem cells (induced neural stem cells and multipotent stromal cells), whose transplantation is facilitated by an innovative scaffold biomaterial. The RISEUP concept is that micro pulses, being able to impose and control cytosolic Calcium oscillations (6), will facilitate cell maturation, survival and neurotrophic factors secretion. Because Calcium signaling is essential for neuronal activity, endogenous neuronal re-connections will also be favored. RISEUP goal, even if ambitious, is concrete due to the multidisciplinary partners' competences, initiating from TRLI a radically new line of technology (electro-activated, remotely controlled, biocompatible, biodegradable cell-containing implants for the repair of neuronal lesions) establishing its proof-of-principle (TRL3). The long-term vision of RISEUP is the radical change in SCI treatment modality to assure the cure delivery without any machinery connection, dramatically improving patients' quality of life.

1 Alizadeh, Dyck et al. 2019
2 Waxman 1989
3 Anjum, Yazid et al. 2020
4 Alizadeh and Karimi-Abdolrezaee 2016
5 Varma, Das et al. 2013
6 Hanna, Andre et al. 2017

P7 - Electroporation for clinical use

Tuesday late afternoon Track D

Sep 17, 16:50 - 18:20

OR-119

Optimization of Bipolar Microsecond Electric Pulses for DNA Vaccine Delivery

Robert H. Williamson[1], Matthew Dewitt[2], Driss Elhanafi[1], David Zaharoff[1], Michael Sano[1]

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Gene-related therapeutics such as DNA vaccines are currently hindered by poor cellular uptake. Reversible electroporation (RE) has been used to significantly improve DNA delivery, but traditional monopolar protocols induce severe muscle stimulation and pain in vivo. Novel bipolar microsecond and sub-microsecond pulses have been shown to alleviate this stimulation when used for irreversible electroporation (IRE); however, these pulses introduce several parameters which have yet to be explored in the context of RE. In this study, the parameters of waveform, delivery rate, and dose were explored to begin optimizing bipolar microsecond pulses for clinical use as a DNA delivery vehicle.

To accomplish this, cells were embedded in 3D collagen tissue mimics and allowed to achieve native morphologies. A coaxial ring-and-pin electrode was used to deliver treatments with varied waveforms, delivery rates, and doses while exploring a wide range of electric field intensities. Samples were maintained at 37°C to replicate physiological conditions and active temperature control was used during treatments to ensure peak temperatures did not exceed 42°C, eliminating thermal effects as a confounding factor. RE thresholds were assessed

via addition of propidium iodide prior to pulse delivery. IRE thresholds were assessed 24 hours post treatment in matched experiments using PI and Calcein AM to indicate live and dead cells, respectively.

A combination of finite element models and a MATLAB algorithm were then used to determine the electric field intensities associated with RE and IRE outcomes with optimal parameter sets being those which maximized the difference between these thresholds. DNA transfection with a plasmid encoding for green fluorescent protein was then evaluated via microscopy in a subset of best-performing groups and compared to traditional electroporation protocols.

Of the parameters tested, dose had the most significant impact on RE and IRE thresholds, with higher doses reducing the magnitude of these thresholds, but reducing the safety margin between reversible and irreversible effects. The rate of energy delivery also influenced these thresholds, with higher initial rates resulting in higher RE and IRE thresholds and a larger safety margin between thresholds. Accordingly, when an optimal parameter set was used in a final transfection validation study, we achieved a 7730% increase in gene expression over traditional monopolar pulse protocols.

These results indicate that RE offers a promising avenue for DNA delivery and that bipolar microsecond pulses offer significant improvements over current technology, which warrants more research to further explore the effects of these and other bipolar pulse parameters on RE outcomes.

OR-120

Keloid treatment with Electrochemotherapy

Sebastian D. Michinski[1], Ana Dimitri[3], Ana Campastri[2], Antonella Cilio[2], Raquel Lertora[3], Felipe H. Horacio Maglietti[2]

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Background: Keloids are characterized by the growth of a firm, extensive scar beyond the edges of the original wound. These occur due to injuries deep enough to affect the reticular layer of the dermis (burns, insect bites, surgeries, tattoos and piercings) and are the result of abnormal healing responses, producing excess fibrinogen and collagen (hyperactivation of keloid fibroblasts). Keloid scars are often disfiguring, profoundly impair quality of life, and cause immense physical and mental discomfort in affected individuals, especially those who have symptomatic (pruritic, painful) and/or hyperpigmented scars. Surgery, corticosteroids, and even intralesional bleomycin, among many others, are the treatments proposed for this pathology. Despite this, many of these scars do not improve or if they do, they recur in the vast majority of cases. It is proposed that electrochemotherapy (ECT) could be effective in treating of recurrent keloids, even when other treatments have failed. Here we present the preliminary results of 4 patients (5 keloids).

Materials and Methods: Four patients with five keloids were treated with one session of ECT, three patients with one keloid (two on the chest and one on the back) and one patient with two keloids on the ear. For the ECT procedures, Oncopore electroporator (Biotex SRL, Buenos Aires, Argentina) and a 6-needle electrode were used. Bleomycin (Bleocris 15, LKM) was applied intralesionally. The concentration of the solution used was 1,000IU/cm3. The volume used was calculated by using the formula V = ab2 π /6, in each case. Patient follow-up was performed in a weekly basis for the first month, and monthly for the following 2 months. The lesions were measures on each follow-up visit.

Results: Three months after treatment, the overall average reduction in the volume of the keloids was 73% (range, 41.8 to 100%). In all cases the symptoms were reduced (itching, pain), but the hyperpigmentation remains. Treatment was well tolerated by patients, and no serious adverse events were observed.

Discussion and Conclusion: There is little experience in using ECT to treat keloids, but the results have been encouraging (1,2). Here we present only the preliminary results of 4 patients (5 keloids). We conclude that ECT is safe and could be effective in recurrent keloids, even when other

treatments have failed.

1- Electrochemotherapy treatment of a recalcitrant earlobe keloid scar with chronic lymphocytic leukaemia infiltration. https://doi.org/10.1016/j.bjps.2010.05.008
2- Treatment of Keloids and Hypertrophic Scars with Bleomycin and Electroporation. DOI: 10.1097/PRS.0b013e3182a053c8

OR-121

Development of a specific gel for skin cancer electrochemotherapy.

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Introduction: Electrochemotherapy (ECT) is an accepted treatment used for cutaneous primary and secondary tumors of varied histologies. Several aspects of ECT remain to be elucidated, for example how to mitigate the side effects of electrolysis during treatment. Electrolysis byproducts; acid at the anode and base at the cathode, can cause needle deterioration, tissue damage, and skin irritation.

Aim: To study the magnitude of pH changes produced by the electrolysis and to develop a gel that neutralizes these changes.

Materials and Methods: We used agar-agar gels with a conductivity similar to tissues, adjusted by the addition of 0.5% sodium chloride. Tissue conductivity was calculated using current data obtained from ECT treatments previously performed in veterinary and human medicine. pH fronts were stained using methyl red for the acidic front and phenolphthalein for the basic front. The formation of the fronts were captured by means of a high-speed camera (SAMSUNG 108 MP, 920 FPS at a resolution of 1280x720). The images were processed and analyzed with the NIH ImageJ software implemented in an image processing station for quantifying pH front extension.

We added sodium bicarbonate (as a buffer) at increasing concentrations in the agar-agar gels, until the application of the pulses did not induce the formation of pH fronts or they were quickly neutralized.The final gel was prepared with carbopol 940 + established buffer concentration (sodium bicarbonate) + an adjusted conductivity by addition of 0.5% sodium chloride

Results: Pulse delivery induces extreme pH changes, which deteriorate the surface of the needles demonstrated by the emerging of irregular fronts in heavily used needles. This irregular electrical conduction leads to irregular electric fields, which lowers treatment efficiency. The gel developed effectively neutralizes extreme pH changes, preventing electrode damage.

Conclusion: The specific gel developed for electroporation reduces electrode wear increasing treatment efficiency. In addition, it may ameliorate skin side effects provoked by extreme pH changes.

OR-122

The Synergy of Conductive Nanoparticles with Nanosecond and Microsecond Pulse Bursts for Bleomycin-based Electrochemotherapy

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[3]Wroclaw Medical University, Poland

The application of nanotechnology and nanoparticles in the area of drug and gene delivery is heavily exploited. Recently, it was shown that conductive nanoparticles can be used for electric field amplification in a close proximity with the cell membrane, introducing the capability to improve available electrochemotherapy methods and solve problems associated with field non-homogeneity. However, the effects of conductive nanoparticles were not yet characterized in the context of nanosecond pulse bursts or in the context of drug delivery, i.e., electrochemotherapy. Therefore, in this work, we characterize the potential use of gold nanoparticles (AuNPs: 13 nm) in combination with

microsecond: 0.6–1.5 kV/cm × 100 μs × 8 (1 Hz) and nanosecond: 6 kV/cm × 300–700 ns × 100 (1, 10, 100 kHz and 1 MHz) electric field pulses. We show that in the nano-pulse range, the pulse repetition frequency significantly affects the synergy between AuNPs and electroporation. Basically, the synergistic effects (improved permeabilization and electrotransfer) are profound only with increased burst frequency and are non-existent with low-frequency bursts. Additionally, AuNPs not only reduce the permeabilization thresholds but also affect pore resealing, i.e., irreversible electroporation is triggered in case the field is not adjusted.

Finally, we have tested the most prominent protocols (microsecond and nanosecond) in the context of bleomycin-based electrochemotherapy (4T1 cell line). It was shown that a saturated cytotoxic response with AuNPs can be triggered at significantly lower electric fields, which are considered sub-threshold and/or not applicable for electrochemotherapy otherwise. At the same time, the nanoparticles themselves are non-toxic for the cells either separately or in combination with bleomycin, indicating the methodology's prospective applicability in the context of electrochemotherapy.

Acknowledgments: The research was supported by the Research Council of Lithuania, Grant Nr. S-PD-24-5.

OR-123

Development of novel genetic vaccine platforms: from the idea to GMP production

Luigi Aurisicchio

Takis, Italy

The COVID-19 pandemic demonstrated the urgent need to develop versatile vaccination platforms that could be quickly implemented for infectious diseases. It is of utmost importance to provide a vaccine in a time-critical manner for such diseases, as the frequency of such epidemics and pandemics as we see them today, will be heavily increasing due to rise in global travel, global warming, increase in population density, penetration into previously uninhabited areas and animal trade. Nucleic acid vaccines, such as those based on mRNA are endowed of these features.

To address the urgent need to find solutions to the SARS-CoV-2 Pandemic, Takis has developed COVID-eVax, a vaccine approach based on genetic engineering and DNA electroporation as part of the X-eVax platform, previously developed. The project started in 2020 and consisted of the molecular design of the vaccine, the development of the reagents and tests necessary to test its effectiveness and the experiments in animal models. Subsequently, GMP-grade material (Good Manufacturing Practices) was produced, all regulatory studies were conducted (toxicology, biodistribution, immune response) and finally a phase 1 study in humans, which ended in December 2021, achieving all the objectives set and providing the basis for evaluations in other applications.

DNA vaccines advantages are: (1) simple and quick production of DNA encoding the antigens by PCR or synthetic methods (potential game-changers for new variants especially vaccine resistant strains), (2) easy large-scale production, (3) safety compared to inactivated virus vaccines, and (4) higher thermostability (minimal cold-chain requirements), which is an issue with some vaccines. The DNA-based platforms offer great flexibility in manipulating the encoded vaccine antigen and have a great potential for accelerated development. Recently, the first DNA vaccine against SARS-CoV-2 (ZyCov-D) has been registered in India for human use; moreover, DNA vaccines have been extensively tested in multiple clinical trials in the oncology field and are commonly used in veterinary medicine. These vaccines (as opposed to mRNA-based vaccines) are stable, do not require cold-chain supply, and can easily be produced in large amounts in bacteria. All these advantages make this platform technology an attractive tool, as it overcomes several shortcomings of alternative approaches (e.g., complex production processes, stability issues, purchase price).

In this presentation, opportunities and challenges of DNA-based vaccines and Takis biotech experience will be discussed.

OR-124

Low-Dose Electrochemotherapy Enhances DNA Damage and Overcome Resistance through Synergistic Drug Delivery

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[3]Mie University, Japan

Introduction: A lethal dose of bleomycin (BLM) is delivered into cells during electrochemotherapy (ECT) to induce acute cell death. Given BLM promotes double-strand breaks (DSBs) in DNA, we investigated whether the dose could be titrated to achieve DNA damage equivalent to external beam radiation therapy. Subsequently, we tested our strategy of low-dose ECT (LD-ECT) to treat radiation sensitive glioblastoma (GBM), studying synergy with standard of therapeutics; temozolomide (TMZ) and olaprib (OLA), and illuminating novel avenues for enhanced therapeutic efficacy in GBM treatment.

Methodology: TMZ-sensitive (U87) and -insensitive (T98G) GMB cell lines were screened for optimal reversible electroporation parameters, and BLM dosing was titrated to maximize DNA damage as measured by ɣ-H2AX analyzed by flow cytometry. Synergy with TMZ and OLA was tested at different dose ratios (0:3, 1:1, 1:2, 1:3) with LD-ECT, and outcomes evaluated with clonogenic assay for survival and proliferation. Radiation therapy at 2, 5 and 10Gy served as positive control. In Murine subcutaneous tumor model, TMZ or OLA was delivered for 10 consecutive days after LD-ECT, then discontinued. Tumor volume and survival were recorded. Further evaluations conducted in an orthotopic GBM nude mice model, U87 cells injected into left frontal cortex and treated with TMZ or TMZ + LD-ECT. Mice were monitored with bioluminescence imaging, and histological evaluation was performed at euthanasia. Results: In vitro studies revealed that delivery of nanomolar concentration (0.625µg/mL) of BLM (ECT) increased DNA damage >85% in T98G (****p<0.0001) and >65% in U87 (**p<0.01) compared to free drug BLM without compromising viability significantly. However, when combined with DNA repair pathway-targeting chemotherapeutic drugs (TMZ or OLA), viability significantly decreased from 95% (TMZ free drug) to < 40% (ECT 1:1 TMZ, ****p < 0.0001) in T98G and further reduction <15% viability (ECT 1:2 TMZ **p<0.005) U87 cell lines. Similarly, viability decreased from 90% (OLA free drug) to <50% (ECT 1:1 OLA, ****p<0.0001) in both cell lines. Clonogenic assay showed comparable cell toxicity to high dose irradiation (10Gy) with no significant difference compared to ECT+TMZ or ECT+OLA. In vivo results demonstrated complete tumor eradication in subcutaneous mouse model (**p<0.01) when compared to untreated control at week 4, with doubled survival from 4 weeks to 8 weeks untreated and ECT+TMZ or ECT+OLA treatment respectively. Intracranial GBM model showed reduction of bioluminescence as early as 1-week post treatment, supported by histological data which showed no signs of tumor cells at 8-week sacrifice. Conclusion: Non-ablative LD-ECT mimics high-dose radiation, enhancing standard-of-care therapeutics' efficacy and overcoming resistance, with potential superior healthy brain sparing.

S04 - Advanced applications of PEF for food quality enhancement, food component modification, and structural alterations

Wednesday morning Track A

Sep 18, 10:40 - 12:10

OR-236

Introduction to Advanced Applications of PEF for Food Quality Enhancement, Food Component Modification, and Structural Alterations

Jessica Genovese

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Pulsed electric field (PEF), also known as electroporation, is a biophysical process in which an external electric field is applied to conductive materials, such as cells and tissues, increasing cell membrane permeability and inducing physicochemical

modifications of the cell membrane. The widely accepted mechanism underlying electroporation is the formation of hydrophilic pores in the cell's phospholipid bilayer. This process also causes mechanical, osmotic, viscoelastic, and hydrodynamic instabilities in biological materials.

PEF treatment has been found to induce the production of highly reactive oxygen species (ROS), such as superoxide anion and hydroxyl radical, partly generated by electrochemical reactions at the electrode-electrolyte interface, but are primarily the result of the cell's response to oxidative stress (only present when the electroporation is reversible). Chemical reactions at the electrode interface, due to the passage of the electric current through an aqueous solution, generate hydroxide ions at the cathode and protons at the anode, resulting in localized pH alterations. These changes can influence the charge, reactivity, and stability of various biomacromolecules, including polysaccharides, proteins, nucleic acids, and lipids.

In summary, the effects of PEF treatment are exerted through a complex interplay between electrophysical and electrochemical mechanisms. Electrophysical mechanisms include the induction of transmembrane voltage (TMV), electrophoresis, rotation of molecule dipoles, potential heating, and the generation of mechanical waves, directly altering cellular structures and functions. Electrochemical mechanisms, such as pH changes, water electrolysis, metal cations release from electrodes, and the generation of ROS, further impact cellular responses and can induce alterations in the microstructure and macromolecular interactions of biomacromolecules.

Overall, the diverse applications of PEF treatment have the potential to revolutionize various aspects of food production and preservation, waste management, and food ingredient modifications.

Acknowledgement: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for proposals No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".

OR-125

PEF for more sustainable, nutritious biomass and macromolecules for food applications with a case study on microalgae

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Combining emerging processing technologies with new raw materials and the inclusion of sustainability assessment, covering nutritional, social, and economic aspects, could form a holistic approach for developing sustainable food systems. New materials like microalgae offer alternatives to creating more sustainable, nutritious, protein-rich foods. Technologies including pulsed electric fields (PEF) can facilitate their adoption by enhancing the efficiency of associated value chains. This presentation highlights PEF applications for sustainable, nutritious food development, using microalgae as a case study.

Upstream, nanosecond PEF (nsPEF) (100 ns pulses, 10 kV cm−1) maximizes the efficiency of single-cell bioprocesses up to 17.53 ± 10.46 percent across a range of diverse organism domains, including Arthrospira platensis (3×256±67 J kgsus−1) and heterotrophic Chlorella vulgaris (3×227±60 J kgsus−1).

Downstream, combining microsecond PEF (μsPEF) with an incubation step can modify the cell wall structure, potentially increasing nutrient bioaccessibility. Applying 10×5 μs pulses at 20 kV cm−1 (31.8 kJ kgsus−1) enhances C. vulgaris lipid bioaccessibility 3-fold while preserving oxidative stability, therefore enhancing raw material nutritional value. Microalgae protein extraction via μsPEF combined with incubation (80.7 ± 1.9 kJ kgsus-1) is more energy-efficient compared to conventional methods like high-pressure homogenization. Additionally, PEF facilitates protein separation by microfiltration by preserving the cell structure, reducing fouling and improving flux rates. PEF yields small soluble proteins with specific functional properties for food applications. This talk showcases the effect of PEF on the

quality and functional properties of microalgae and extracted biocompound fractions, highlighting the potential of advanced PEF applications for novel food raw material components modifications and structural alterations.

PEF could emerge as high-impact technology, addressing challenges in single-cell-based value chains by enhancing upstream efficiency while improving the downstream recovery of biocompounds. PEF supports the provision of nutritious foods by facilitating the adoption of new raw materials and, thus, contributes to establishing more sustainable food systems.

OR-126

The influence of a pulsed electric field on the osmotic dehydration process and selected physical properties of orange fruits dehydrated in unconventional solutions

Agnieszka Ciurzyńska, Katarzyna Rybak, Dorota Witrowa-Rajchert, Katarzyna Pobiega, Sabina Galus, Małgorzata Nowacka

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Aim: The aim of the work was to investigate the effect of pre-treatment with a pulsed electric field (PEF) before osmotic dehydration (OD) treatment on the properties of osmodehydrated orange fruit.

Method: The samples were treated using a pulsed electric field with specific energy input of 1.5 and 6.5 kJ/kg, and then subjected to the osmotic dehydration using two solutions: 50% sucrose solution and rosehip juice solution with the addition of sucrose. The process was carried out at a temperature of 30°C for 3 hours, with the evaluation of fruits after 0.5, 1, 2, and 3 hours. The ratio of the mass of the solution to the mass of the raw material was 1:4. The osmotic kinetics based on changes in total mass, water mass, and solids mass were evaluated. Additionally, the chosen properties of the orange fruit were analyzed (color, dry substance content and water activity, structure and texture).

Results: The results showed that PEF treatment had the greatest effect on tissue diffusivity during short osmotic dehydration. In the case of dehydration for 0.5 and 1 h, treatment with a pulsed electric field of 1.5 kJ/kg resulted in the highest increase in extract, increase in dry matter content and loss of water. In most cases, the influence of the type of osmotic solution on the kinetics of the process was insignificant. The highest dry matter content was observed in fruits dehydrated in a rosehip juice solution with the addition of sucrose, and the lowest water activity was observed in fruits dehydrated using a sucrose solution. The texture parameters of oranges depended on the type of osmotic solution used and the duration of the dehydration process, while the use of a pulsed electric field before the osmotic dehydration process did not significantly change the texture of dehydrated orange fruits, but the structure did change. The use of PEF pre-treatment and dehydration in a 50% sucrose solution and a 50% rosehip solution with the addition of sucrose increased brightness, color saturation, and the share of yellow in the osmodehydrated oranges. The visual assessment showed a darkening of the tissue of fruit dehydrated in an unconventional rosehip solution with the addition of sucrose compared to samples dehydrated in a sucrose solution. PEF significantly increased the ΔE coefficient, which indicates a visible difference in color between osmotically dehydrated and PEF-treated tissue and fresh tissue.

Acknowledgment: This project received funding from transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofunds, under the Joint SUSFOOD2/CORE Organic Call 2019 (MILDSUSFRUIT), as well as the National Centre for Research and Development (POLAND, decision DWM/SF-CO/31/2021).

OR-127

The manifold manifestations of electroporation effects on plant tissue and how their quantification depends on the method of analysis

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The use of Pulsed Electric Fields (PEF) has been successfully implemented in the potato industry, but its application in the vegetable industry remains limited due to the lack of knowledge on the behavior of various plant tissue types in subsequent processing steps. The exposure to PEF reduces the needed blanching time as the electroporation increases the rate of heat and mass transfer in tissue, allowing lethal temperatures for browning enzymes like peroxidase to be reached more quickly in PEF treated plant tissues. However, this effect has not been consistently confirmed across all tissue types and in some cases an increased enzyme activity was measured. Our work aimed to investigate the non-uniform effects of PEF treatment due to the structural diversity of plant tissues commonly used in industrial applications. These include mostly homogeneous tissues (red beet, sweet potato), tissues with lignified parts (broccoli), and tissues with varying characteristics (inhomogeneities) within the same raw material (parsnip, carrot). The applied PEF treatment ranges from a field strength of 0.4 to 3 kV/cm with specific energy inputs up to 6.5 kJ/kg. Electrical impedance measurements were conducted before and immediately after the PEF treatment as well as after a holding time of 10 minutes to monitor effects of water redistribution. Texture analysis was performed using compression and cutting force measurements to determine the responsiveness of PEF treated tissue to different force applications by comparing thresholds. The results show that the more complex the tissue structure, the less homogeneous the PEF treatment. This can be attributed to factors such as air pockets, low-conductivity structures (e.g. lignin), and variations in water content and distribution. Due to these, the orientation of the tissue in the electric field also plays a crucial role when treating inhomogeneous materials. Varying temporal changes in the average impedance ratio (after-to-before the treatment) were observed for different treatment protocols and need to be considered for industrial processes when holding times are part of the production line. Based on our findings, appropriate PEF assessments and treatment conditions can be defined for different groups of plant raw materials, ensuring a gentler and more efficient production process.

OR-128

Enhancing Chemical Reactions and Modification of Food Ingredients Using Pulsed Electric Fields: An Alternative Technique

Xin-An Zeng

Foshan University, China

Pulsed Electric Fields (PEF) represent a promising non-thermal technology in food processing, enhancing chemical reactions and modifying the physical properties of food ingredients without compromising their nutritional and sensory qualities. Our investigations explored the application of PEF across diverse food matrices, including flavonoids, starches, and soy protein isolates. We found that PEF effectively augments the antiglycation activity of Noni flavonoids by modulating flavone glycosides into aglycones. Specifically, PEF treatment resulted in a substantial increase in the quercetin proportion from 0.08 to 0.91 (molar ratio with rutin) after 4000 pulses. This elevation in quercetin content significantly enhanced the NF sample's antiglycation potential, demonstrating up to 2.98 times higher inhibitory effects on fructosamine, α-dicarbonyl compounds, and advanced glycation end-products (AGEs) in bovine serum albumin (BSA)-fructose model. The enhanced antiglycation potential suggests significant health benefits, particularly in preventing oxidative stress-related diseases by inhibiting the formation of AGEs. In the starch modification, PEF treatments were applied to corn and potato starches, achieving notable improvements in freeze-thaw stability and pasting properties. After five cycles, PEF-assisted esterification reduced the syneresis of starch by 29.5%, compared to 10.17% without PEF, and lowered the pasting temperature by 7.6–15.1°C at 2–6 kV/cm. This PEF-assisted method also reduced reaction time and improved efficiency by 6.1–39.1% over the control. These enhancements are critical for applications requiring robust performance during storage and processing, such as in frozen foods and ready-to-eat meals. Additionally, the functionality of commercial soy protein isolate (SPI) was substantially improved by targeted PEF treatments

and pH shifts. Structural unfolding and promotion of soluble aggregate formation were induced under moderate PEF intensity (10 kV/cm) and alkaline conditions (pH 11), resulting in a significant increase in the solubility of commercial SPI from 26.06% to 70.34%. This combination of treatments also greatly improved the emulsification and foaming properties of commercial SPI, crucial for expanding its use in a wider range of food products where textural integrity and stability are paramount. The outcomes of our studies not only demonstrate the efficacy of PEF in enhancing the functional properties of food components but also its potential to contribute to the innovation and sustainability of food manufacturing processes.

OR-129

Opportunities for implementing pulsed electric fields for the enhanced processing of plant-based foods

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The increasing demand in sustainable plant-based foodstuffs necessitates the development of energy efficient techniques as pretreatments, targeting to milder and improved processing, thus retaining quality. Pulsed Electric Fields (PEF) selectively increase plant cell permeability at low energy inputs through electroporation, offering significant benefits in mass transfer operations to and from plant tissues such as drying, osmotic dehydration, extraction, and juicing, compared to conventional processes. Enhanced mass transfer rates during such processes lead to significant quality preservation and textural modification. This work provides an overview of successful case studies of the implementation of PEF processing as a pretreatment to core food processes for a diverse range of plant tissues, with the overarching principle being the improvement of further processing outcomes.

Plant materials were treated with PEF at electric field strengths ranging from 0.5 up to 2.0 kV/cm with specific energy inputs up to 30 kJ/kg. The effects of the pretreatment were assessed based on outcomes such as extraction yield, moisture transfer, extraction of quality-enhancing compounds or removal of undesired compounds.

In terms of juicing, PEF was successfully implemented as a pretreatment in carrot and peach, which pose challenges during comminution and juicing. PEF also modified the textural properties of the ligneous tissues thereby facilitating cutting, destoning, and juicing, while saving energy compared to thermal treatments (up to 99 %). Juice extraction yield was increased by up to 17% for carrot and 5% for peach. Olive processing also exhibited promising results, increasing oil yields by up to 10%. The enhanced extractability also led to increased bioactive compound concentrations in the oil; 7% more total phenolic compounds and 57% tocopherols, leading to increased oxidative stability during storage. The treatment of fruits and vegetables such as kiwi, cherry tomato, spinach, and goji berry benefited from the increased tissue permeability during osmotic dehydration and drying, exhibiting increased dehydration rates. The effective moisture removal also led to significant shelf-life extension, adding up to 34 d to the shelf life of osmotically dehydrated spinach leaves. PEF processing of zucchini also led to the reduction of oil uptake during deep frying by up to 36%, underlining the potential of PEF for healthier food production. PEF processing of chickpeas improved their rehydration characteristics and led to significant reduction of undesirable oligosaccharides during soaking, up to 87%.

The presented results highlight the potential and efficiency of PEF pretreatment at improving the yield, quality and stability of plant-based foods with minimal energy consumption. The availability of industrial scale continuous equipment makes the application of the process a highly realistic prospect after economic and environmental factors have been considered.

OR-130

Comparison of nanosecond and microsecond PEF for physical property and substance mobilization in potato

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Pulsed electric fields (PEFs) are gaining attention in the food industry as a non-thermal food processing technology, for sterilization, extraction, drying and texture control of agricultural and fishery products. The key factors of PEF effect on agricultural crops are field intensity, pulse duration and number of pulses. Much of what is expected from PEF in food applications is to increase the permeability of cell membranes. Generally longer pulses are more effective in increasing cell membrane permeability than larger electric fields. For this reason, microsecond or millisecond PEFs are often used in much of food processing, as it allows treatment with small electric fields. Nanosecond PEF has rarely been used. It is easy to imagine that the morphology of cell membranes subjected to nanosecond PEF is different from that of microsecond PEF [1]. Relatively high field, nanosecond pulses are expected to form a number of small pores on the membrane, whereas the microsecond pulses produce a few large pores. The variation of the membrane morphology dependent on the pulse waveform can be unitized to control the substances mobilizing through the membrane. In this study, 100's nanosecond PEFs was compared to 10 microseconds PEFs for the dielectric properties of potatoes and for the substances in the soaked juice, and for the drying rate. The capacitance and conductance of potatoes were measured using the impedance analyzer (IM3570, Hioki) to discuss the cell membrane permeability in the tissue. The substances in the soaked juice will also be analyzed using the high-performance liquid chromatograph (EXTREMA, Jasco) to discuss the substances mobilizing through the membrane in the tissue. Furthermore, the drying rate of potatoes was measured to discuss the fluidity of water in the tissue.

OR-131

Effect of PEF on ginger roots: Improving juice extraction yield or product quality

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This study investigated the combined effect of Pulsed Electric Field (PEF) treatment and (mild) heat treatment on the juice extraction yield and juice quality of ginger roots. Treatments were compared to a thermal treatment (30 min 80°C) which is an industrial standard.

Fresh ginger roots were subjected to PEF treatment (E = 2 kV/cm, τ = 8 µs, w = 6 kJ/kg: 5/7 ginger-water ratio, w/w) combined with different heat treatments (30 min 80°C, 2 min 90°C, 10 min 80°C, 10 min 50°C). After PEF treatment, the ginger roots were processed, which includes an initial juicing step using an Angel juicer to separate pulp and juice. The separated pulp and juice were then recombined and heated. This mixture was processed again using an Angel juicer to further extract juice. Control samples without PEF treatment were also prepared for comparison. The juice yield was measured by weighing the extracted juice. Quality parameters such as dry matter, soluble and non-soluble solids, color, and the gingerol content were analyzed.

The results indicated that a combination of PEF with heat treatment (30 min 80°C) improves the extraction yield of juice with 3% compared to the control sample using only heat. Combinations of PEF and milder heat had the same extraction yield as the control. For both PEF treated and control samples, the most heat intensive treatment (30 min 80°C) tend to darken the juice and reduce the yellow intensity. The other combined treatments of PEF with milder heat treatments retain the natural color.

Combination of PEF with heat can lead to interesting alternatives for conventional thermal treatment. Either one can add PEF to the current existing thermal treatment to increase juice extraction yield, while keeping the same quality. Alternatively, one can combine PEF with a milder heat treatment, leading to the same extraction yield as is the current

standard practice, but with a better quality. Reduction of the heating temperature or time could also contribute to a reduction of energy use.

S17 - Voltage control of biological membrane pores

Wednesday morning Track B
Sep 18, 10:40 - 12:10

OR-132

Voltage sensitivity of electropores limits the membrane potential

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Charging cells beyond physiological limits increases membrane conductance in a nonlinear manner. This effect does not depend on voltage-gated ion channels. The additional conductance pathway created by membrane overcharging is commonly associated with electropore formation. It remains to be established whether electropores are perforations in the lipid bilayer or in membrane proteins or if they are complex multicomponent structures. It is likely that several types of electropores can be generated, and their yield depends on the electric field parameters.

Voltage-clamp measurements in diverse mammalian cells electroporated by nanosecond-range electric pulses established that small, nanometer-sized electropores (“nanopores”) are voltage sensitive and selective to cations. These pores are also inwardly rectifying, meaning that they conduct more current when the cell is hyperpolarized than when it is depolarized to the same potential.

The formation of voltage-sensitive electropores can also be observed with ms-range pulses. By combining a whole-cell patch clamp with total internal reflection fluorescence (TIRF) microscopy, we were able to observe the formation of electropores concurrently with monitoring their conductance. The opening of large, high-conductance (> 1 nS) pores, manifested as an abrupt increase in the whole-cell current and a bright focal Ca2+ entry fluorescence transient, was typically delayed by milliseconds after the membrane had already reached the critical voltage.

At command voltages below -200 mV, the opening of high-conductance electropores reduced the efficiency of membrane charging. As a result, the induced transmembrane potential (TMP) became progressively smaller than the applied command voltage. In the range between -240 and -300 mV, the induced TMP reached a ceiling and could not be hyperpolarized any further. Larger command voltage steps increased the electropore conductance in an adaptive manner so that the TMP remained unchanged or even depolarized. This effect was reproducibly demonstrated in different cell types using different intra- and extracellular solutions, and with voltage step, voltage ramp, and current ramp protocols. For example, the most hyperpolarized TMPs that could be reached with voltage ramps averaged -270 ± 6 mV in HEK cells, -284 ± 5 mV in CHO cells, and -243 ± 9 mV in hippocampal neurons. The cell plasma membrane becomes essentially transparent to the electric field in excess of what is needed to reach this limit, thus allowing it to reach intracellular structures. The adaptive increase in pore conductance was reversible and protected cells from reaching still higher TMP that could disintegrate the membrane. It remains to be studied whether faster charging, e.g., with high-intensity nanosecond electric pulses, could override the increase in adaptive pore conductance and drive cells to an even higher TMP.

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OR-135

Using the same electrode to electroporate a chromaffin cell and measure the resulting exocytosis of catecholamine

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Adrenal chromaffin cells release the catecholamines adrenaline and noradrenaline as part of the fight-or-flight response. These hormones are stored in vesicles within cells, and, upon an appropriate stimulus, a vesicle will fuse with the

cell membrane and release its contents as a discrete packet via “quantal” exocytosis. Since catecholamines are readily oxidized, quantal exocytosis can be detected by an electrochemical electrode located immediately adjacent to the cell as a spike of amperometric current for each vesicle released. Here we report using a microelectrode to both stimulate and measure the release of catecholamines from individual vesicles in individual cells. Stimulation occurs by either eliciting action potentials in the cell or by electropermeabilizing the cell to allow entry of stimulating substances into the cell. Gold electrodes were fabricated on glass substrates using photolithography and insulated with a thick photoresist with openings to define the working electrodes at the bottom of cell-sized microwells to trap single cells. A customized potentiostat was used to allow large transient currents to be applied to stimulate cells followed by low-noise current recordings to resolve picoamp current spikes resulting from quantal exocytosis. Trains of voltage pulses triggered release with spike characteristics similar to those found with more physiological stimuli, therefore release occurs from individual vesicles. Uptake of trypan blue into cells demonstrated that cell permeabilization occurred with strong stimuli. Surprisingly, quantal exocytosis was dependent on the chloride concentration in the bath solution, but not the concentration of calcium. Using the same electrode to stimulate and record release of catecholamines from an individual cell allows detailed information about exocytosis to be obtained and can be used to load membrane-impermeant substances into the cell such as DNA or fluorescent markers.

OR-136

Molecular mechanisms of vesicle priming, fusion pore formation and transmitter release by electrodiffusion

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Various cell types release neurotransmitters, hormones and many other compounds that are stored in secretory vesicles by exocytosis via the formation of a fusion pore traversing the vesicular membrane and the plasma membrane. This process of membrane fusion is mediated by the Soluble N-ethylmaleimide-Sensitive Factor Attachment Proteins REceptor (SNARE) protein complex, which in neurons and neuroendocrine cells is composed of the vesicular SNARE protein Synaptobrevin 2 and the plasma membrane proteins Syntaxin 1 and SNAP25 (Synaptosomal-Associated Protein of 25kDa). Before a vesicle can undergo fusion and release of its contents, it must dock at the plasma membrane and undergo a priming process that makes it ready for release. Experiments using a SNAP25 based FRET construct indicate the incorporation of SNAP25 into the SNARE complex, which appears to be the final step and it is this step, which defines the formation of the primed state [1]. This step requires the presence of the vesicular SNARE Synaptobrevin 2. The assembly of the SNARE complex is followed by its disassembly, reversing the FRET ratio increase. The changes in the SNARE complex leading to fusion are stimulated by activation of voltage gated Ca2+ channels, increasing the Ca2+ concentration near the fusion site, which is sensed primarily by the vesicular Ca2+ sensor Synaptotagmin. However, Ca2+-independent but voltage-dependent secretion (CiVDS) has also been reported in primary sensory neurons and in the sympathetic nervous system [2, 3]. Fusion and transmitter release begins with formation of a narrow fusion pore connecting the lumen of secretory vesicles to the extracellular space. Release of charged neurotransmitter molecules through a narrow fusion pore requires charge compensation and is therefore a process of electrodiffusion. The charge compensation for release of positively charged catecholamines from chromaffin cells is coupled to Na+ influx through the fusion pore and not to co-release of anions [4]. Anion substitution experiments and molecular dynamics simulations indicate that very narrow fusion pores are cation selective but more dilated fusion pores become anion permeable. The transition occurs around a fusion pore conductance of ~300 pS [5]. The cation selectivity of a narrow fusion pore accelerates the release of positively charged transmitters such as dopamine, noradrenaline, adrenaline, serotonin

and acetylcholine, while release of negatively charged transmitters such as ATP or glutamate may require a more dilated fusion pore.

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OR-137

Lipid protein interactions guide fusion pore opening and expansion during regulated exocytosis

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It has long been known that synatpotagmin-1 (Syt1) is the sensor that triggers fast, evoked release of neurotransmitter by the fusion of synaptic vesicles to the presynaptic membrane in response to calcium. It is also well established that the SNAREs syntaxin-1a, SNAP-25, and synaptobrevin-2/VAMP-2 form the core of the membrane fusion machinery that drives calcium-triggered exocytosis not only in neurons but also in secretory cells like insulin releasing beta-cells. We recently proposed a mechanism where the lipid bilayer is intimately involved in coupling calcium sensing to fusion. Using TIRF- (total internal reflection fluorescence) and sd-FLIC (site-directed fluorescence interference contrast) microscopy, we demonstrate that fusion of purified dense core vesicles and insulin granules with supported membranes containing syntaxin-1a and SNAP-25 is strongly linked to the flexibility of the juxtamembrane region between SNARE domain and transmembrane domain of syntaxin. As the juxtamembrane region becomes more rigid, force is exerted on the SNARE transmembrane domains to drive the merger of the two bilayers while the trans-SNARE complex completes folding into the cis-SNARE complex. The flexibility of syntaxin's juxtamembrane region can be modulated by the order of the lipid bilayer, and the order of the bilayer is changed by Ca2+ dependent binding of the two C2 domains of Syt1 to the membrane. The strong dependency of vesicle fusion efficiency on membrane order is further confirmed in live INS1 cell experiments where the plasma membrane is acutely enriched with lipids of defined acyl-chain saturation. In addition to fusion efficiencies and fusion kinetics, TIRF data from single vesicle fusion events contains information about how the fluorescent content is released. Of particular interest is, how fast the content is released. We present data that show how interactions between PIP2 and conserved arginine residues of Syt1's C2B domains regulate fusion pore expansion.

OR-134

Voltage-activation mechanisms of ion channels with different electrical polarities

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are essential for rhythmic activity in the heart and brain. Mutations in HCN channels are linked to heart arrhythmia and epilepsy. HCN channels belong to the family of voltage-gated K+ (Kv) channels. Hyperpolarization-activated HCN channels and the related depolarization-activated Kv10.1 (EAG) and Kv11.1 (hERG) channels have very similar tetrameric structures with six transmembrane segments (S1-S6) per subunit: S1-S4 form the voltage-sensing domain (VSD) and S5-S6 form the pore domain (PD). In both Kv and HCN channels, S4 is the positively charged voltage sensor and the C-terminal part of S6 forms the gate. However, why HCN channels are activated by hyperpolarization whereas Kv channels are activated by depolarization is not clear. Using voltage clamp fluorometry, FRET, cysteine accessibility, and

cysteine crosslinking, we have measured the movement of S4 and the gate in HCN channels to determine the mechanism of activation by hyperpolarization. Our main hypothesis is that small differences in free energy between the closed and open states, due to different interactions between S4 and the pore in different channels determines whether HCN channels or HCN-related channels open by hyperpolarizations or depolarizations.

OR-133

Visualizing membrane fusion and budding in live cells

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Membrane fusion and budding mediate fundamental processes like intracellular trafficking, exocytosis, and endocytosis. Fusion is thought to open a nanometer-range pore that may subsequently close or dilate irreversibly, whereas budding transforms flat membranes into vesicles. Here I will describe a model of membrane fusion and budding obtained from direct visualization of vesicle fusion and endocytosis in live chromaffin cells. Fusion involves hemi-to-full fusion, pore expansion, constriction and/or closure while fusing vesicles may shrink, enlarge, or receive another vesicle fusion; endocytosis follows exocytosis primarily by closing Ω-shaped profiles preformed through the flat-to-Λ-to-Ω-shape transition or formed via fusion. Calcium/SNARE-dependent fusion machinery, cytoskeleton-dependent membrane tension, osmotic pressure, calcium/dynamin-dependent fission machinery, and actin/dynamin-dependent force machinery work together to generate fusion and budding modes differing in pore status, vesicle size, speed and quantity, controls release probability, synchronization and content release rates/amounts, and underlies exo-endocytosis coupling to maintain membrane homeostasis.

S18 - Bridging the gap between experimental and modeling studies in PEF electroporation: a Young Professional's perspective

Wednesday morning Track C

Sep 18, 10:40 - 12:10

OR-138

Evaluating biological membrane response to PEF: A multiscale computational approach

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In recent decades, there has been a surge in the study and application of pulsed electric fields (PEFs) in biomedical research. Characterized by short durations (ranging from milliseconds to nanoseconds) and high intensities up to megavolts per meter (MV/m), PEFs are utilized in established techniques like electrochemotherapy, as well as emerging methods such as gene therapy and controlled drug delivery [1]. The investigation of the biophysical mechanisms underlying the interaction of electromagnetic fields (EMFs) has led to a consensus that molecular-level alterations can trigger subsequent responses as biological complexity increases, ultimately yielding macroscopic consequences for the organism [2]. The challenges associated with studying these mechanisms through traditional experimental approaches underscore the necessity of numerical investigations. Consequently, a multiscale computational approach is proposed here, and it could be the key to planning and monitoring EMFs-based biomedical applications.

Such a multiscale approach endeavors to link the outcome of discrete modeling conducted on a molecular level with the inputs needed to provide a continuum simulation on a microscopic scale level. In fact for example Molecular dynamics (MD) simulations have elucidated that electropermeabilization arises from phospholipid rearrangements and the formation of aqueous pores within the membrane [3-4]. However, MD simulations are typ-

ically performed on reduced models (with dimensions up to tens of nm) and timescales (on the order of hundreds of ns) to mitigate computational costs while maintaining atomistic precision. On the contrary, whole cell exposure to PEFs induces various cellular signaling pathways, primarily through the well-known process of electropermeabilization or electroporation of cellular membranes. These phenomena usually can be represented via continuum models with dimensions up to □m and timescales on the order of hundreds of □s.

Thus, besides molecular modeling of membranes, microdosimetric simulations have recently garnered significant attention [5]. These simulations estimate the electrical quantities induced at subcellular and cellular levels, solving the quasi-static EM problem and the pore evolution over time in a multiphysics manner. Recent advancements focus on employing advanced 3D cellular models reconstructed from microscope images. These models accurately capture the irregular shape and dimensions of intracellular compartments, providing spatial distribution of electrical quantities that can be correlated with physicochemical measurements obtained from experiments [6]. While microdosimetric investigations offer valuable insights, their accuracy remains confined to the microscopic level.

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OR-139

Effects of pulsed electric fields on collagen self-assembly and collagen secretion by dermal fibroblasts

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Electroporation is a physical method that is gaining momentum in cancer treatment, to eradicate cancerous cells, while sparing surrounding healthy tissues. Pulsed electric fields act on cell membranes and can induce the occurrence of cell membrane “pores”. Nevertheless, more knowledge is needed on electroporation effects on the surrounding healthy tissues and on the extracellular matrix (ECM). Adjacent tissues are essential for structure and nutrition and include supporting/connective tissues and blood vessels. Within them, the ECM contains the collagen, which is one of the most abundant bodily proteins. Omnipresent in our body, this protein also plays an important role in diseases such as fibrosis or cancer. Thus, the study of collagen is primordial in cancer research and treatment, because the ECM is involved in tumor development, dissemination, and metastasis progression. Furthermore, type I collagen is among the most frequently up-regulated collagens in solid cancers, yet its implication in disease progression is still unclear [1].

To determine if square wave electric pulses, such as the ones used for electroporation, can contribute to the remodeling of the ECM, we evaluate if and to what extent electroporation parameters might contribute to the alteration of the collagen structure. This is also of particular importance in the context of lower intensity electric fields, which can stimulate wound healing and could increase collagen secretion by fibroblasts [2].

To shed light on biological effects on collagen itself and on collagen building blocks (tropocollagen) upon pulsed electric field exposure, dermal sheets, produced with primary dermal fibroblasts, and tropocollagen monomers are exposed to square wave micro- and millisecond electric pulses at different electric field intensities. The collagen is analyzed with second harmonic generation microscopy and its amount and distribution is assessed. The obtained data will be subsequently used to model structural alterations with Coarse Grain Molecular Dynamics (CG-MD) simulations using the MARTINI model.

This work highlights the implication of pulsed electric fields in collagen organization within dermal sheets. Moreover, it also address the question if collagen formation from tropocollagen monomers can be impacted, or if collagen secretion can be amplified by fibroblasts stimulation with pulsed electric field.

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OR-140

Exploring Vibrational and Electromagnetic Properties of Protein Tubulin using Normal Mode Analysis and Molecular Dynamics Simulations

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Proteins, serving as nature's intricate nanomachines, orchestrate essential biological processes by communicating motions across their complex structures. Understanding the elusive mechanism underlying the connection of distant sites within proteins often necessitates computational methods due to the challenging nature of direct experimental spectroscopic detection of these motions. In this investigation, we utilize normal mode analysis to explore the dominant vibration modes of an all-atom model of tubulin protein and scrutinize their interaction with electromagnetic fields. Our primary aim is to discern the first few vibrational modes and their frequencies, which correlate with specific conformations of tubulin dimers implicated in the dynamic assembly and disassembly of microtubules.
Our inquiry focuses on a model of the tubulin-$\alpha\beta$ dimer complexed with two GTP molecules, subjected to molecular dynamics (MD) simulations. We extract multiple snapshots from these simulations to enhance sampling and employ normal mode analysis to ascertain the vibrational frequencies. Our findings demonstrate that these frequencies are situated in the sub-terahertz (subTHz) band, particularly within the range of approximately 40 to 140 GHz for our model. Introduction of water layers into the model modulates the frequencies, resulting in reduced variations among snapshots. Notably, we discern a distinct cluster of the first three modes within the subTHz frequency domain. Leveraging our normal mode ensemble analysis, we predict the electromagnetic absorption of tubulin's vibration modes, incorporating vibrational damping as a variable.
This investigation advances our understanding of the vibrational and electromagnetic properties of tubulin, establishing a groundwork for future explorations into manipulating protein dynamics through external electromagnetic fields.
Furthermore, we delve into the complex and dynamic post-translational modification of tubulin termed polyglutamylation. Multiple molecular dynamics simulations, incorporating various lengths and positions of polyglutamate chains on the C-terminal tails of tubulin-$\alpha\beta$ dimers, are currently in progress. Our objective is to elucidate the influence of electromagnetic fields on the structural characteristics of tubulin and how the presence of polyglutamylated side chains influences the structure and dynamics of tubulin dimers.

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OR-141

Nanosecond pulsed electric fields and gold nanoparticles for cancer treatment

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The considerable progress in pulsed power allowed the generation and delivery of high-intensity (MV/m) ultrashort pulsed electric field (usPEF) of hundreds nanosecond and picosecond paving the way toward novel targeted cancer therapies. The unique capability of usPEF to trigger regulated cell

death or initiate an adaptive immune response showed high efficacy in treating cancers in pilot studies in vivo and clinical trials. The main advantage of this technique is achieving highly localized tumor destruction without thermal ablation. While most of published studies focus on pulses ≥ 100 ns, our recent research reveals that, under specific exposure conditions, pulses from 2 to 10 ns (up to 100 kV/cm) can elicit cellular death and inhibit the growth of 3D multicellular spheroids (MCSs) derived from HCT-116 colorectal carcinoma cells. In parallel, theory suggests that PEF can be locally amplified by conducting nanoparticles (NP), such as gold nanoparticles (AuNPs). Spherical AuNPs could theoretically enhance the electric field up to 3-fold and elongated NP may theoretically locally enhance the electric field up to 100 times. While attractive, this effect has not been extensively studied experimentally and has never been studied in combination with usPEF. While some studies aimed to assess the combined effect of electric pulses and spherical AuNPs on individual cells, the combined effect of spherical or elongated AuNPs on 3D MCSs remains to be investigated. This work aims to combine usPEF with AuNPs to develop a novel tool that could improve cancer therapy. Preliminary results have been performed on two types of spheroids derived from colorectal cancer (HCT-116) and hepatocellular carcinoma cells (Hepa 1-6). MCSs with or without AuNPs, located outside MCSs in the pulsing buffer or embedded within cancerous cells, were exposed in HBSS (Hanks' Balanced Salt Solution) buffer to trains of 500 unipolar pulses (20 Hz) with field intensity of approximately 50, 75, and 100 kV/cm. These two NP localizations (at the external membrane or within the cells) have a twofold interest. Outside the cells, the NPs could serve as antennas amplifying the electric field. Inside the cells, NPs could serve as internal local agents, destabilizing cells homeostasis. Cellular viability, spheroids growth, and local NPs's effect depending on their peri- or intra-cellular position were evaluated. In parallel, we are exploring the possibility to monitor in real-time, non -invasively and on living cells, the activity of ion channels possibly implicated in cancer to unravel the molecular mechanism underlying the interaction between electric pulses and ion channels. To achieve this, we are implementing an innovative biophysical spectroscopic methodology based on bioluminescence energy transfer, i.e., BRET. Detailed results will be presented and discussed at the conference.

OR-142

Deciphering the behavior of multicellular 3D spheroids exposed to high-intensity pulsed electric fields by a mathematical modeling approach

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While irreversible electroporation (IRE) is efficient in most cases, with a complete ablation rate of about 75% of treated tumor nodules after an IRE procedure [1], it is known that in some patients ablation is not successful and the event may lead to accelerated tumor growth. To better understand the effects of pulsed electric fields on tumor response, we used multicellular tumor spheroids as an in vitro microtumor model. Multicellular tumor spheroids are an important tool in cancer research as they bridge the gap between 2D cell models and in vivo tumors by mimicking the characteristics of early avascular tumors. In this work, multicellular HCT-116-GFP spheroids were exposed to different electric field intensities and their volumes were monitored by fluorescence and bright field microscopy. To characterize their regrowth after pulse delivery with respect to pulse magnitude, we developed a two-step approach based on mathematical modeling and data assimilation.

First, we consider a reduced Gompertz model - a very classical model for tumor growth [2] - for volume evolution. This volume approach showed encouraging preliminary results. However, it did not allow to properly quantify the effects of electroporation and to account for tumor heterogeneity.

These two limitations motivated the introduction of a system of partial differential equations in a second part. Three densities of tumor cells were considered: the proliferative cells, the quiescent

cells and the cells whose functioning was altered by the effect of the electric field. To fit the biological data with our models, we considered a parameter estimation strategy based on a population approach that allows to compensate for sparse sampling times and measurement uncertainties by constraining the variability of the parameters in the population. With this approach, it was possible to determine the percentage of cells destroyed and the percentage of cells whose functioning was altered by the effect of electroporation for each value of the electric field.

Interestingly, our results indicate that the partially irreversible electrical pulses may lead to accelerated cell regrowth.

The advantage of our approach, is that soon after the electroporation, we can detect the trend of tumor growth. In practice, this model can thus allow to assess which tumors were inefficiently treated, and that the patient is on his or her way to relapse.

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OR-143

On the complementarity of modeling and experimentation in the study of biological effects of subnanosecond pulsed electric fields

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The complementarity between modeling and experimental studies is undeniable. Modeling studies can be very useful to prepare the experiments or to explain the results already obtained experimentally. In turn, experimental studies are the ultimate confirmation of modeling results. In the field of electroporation research, some research subtopics may particularly highlight this complementarity. For example, in the context of studying the effects of subnanosecond pulsed electric fields (subnsPEFs) on the electropermeabilization of cells, it was a prerequisite from the beginning to know precisely the electric field to which the cells would be exposed. Therefore, it was necessary to perform 3-D electromagnetic modeling of the electric field distribution generated by voltage pulses inside the system. This, together with experimental measurements of the output voltage of the generator and the voltage recorded closest to the sample by a Vdot probe system, allowed a correct interpretation of the experimental results. This part of the study was performed in the SIAME laboratory specialized in high power pulsed systems. For experimental convenience, the exposure system was designed to allow the use of commercial electroporation cuvettes in which cells were placed in suspension. We needed to ensure the homogeneity of the electric field within the cuvette, the value of the electric field achieved, as well as the shape of the waveform. In particular, we now hypothesize that both the value of the electric field reached and the rise and fall times of the impulse are of paramount importance in the electropermeabilization obtained with such transient PEFs. Experimentally, we have carried out a parametric study in our laboratory to evaluate the influence of the number of pulses applied, the pulse repetition frequency, the electric field amplitude, as well as the temperature of the sample during exposure to PEFs. In the process of interpreting the experimental results obtained and further investigating the mechanisms behind the observed electropermeabilization, we resorted to other types of modeling studies. These included mathematical modeling performed at the Institut de Mathématiques de Bordeaux as well as molecular dynamics modeling studies. With regard to our question, all these elements have been complementary pieces of a puzzle, allowing us to consolidate our mechanistic hypotheses. This also high-

lights how the collaboration within the electroporation research community has been incredibly useful in our research on sub-nsPEFs.

S16 - Electroporation in veterinary and translational medicine

Wednesday morning Track D
Sep 18, 10:40 - 12:10

OR-144

Chimeric DNA vaccination against the Chondroitin Sulfate Proteoglycan 4: a potential allied in combinatorial approaches for the treatment of melanoma and osteosarcoma

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Discovering effective therapies to combat tumor relapse and progression remains an unmet need due to resistance to conventional treatments. Rational combinatorial approaches to attack tumor cells from multiple sides are needed for durable clinical response. Combining standard therapies with cancer vaccines against tumor associated antigens, such as the chondroitin sulfate proteoglycan (CSPG)4, which is barely expressed in normal tissues, but overexpressed by different tumors, could be pivotal. We have characterized the relevant CSPG4 role in malignant melanoma (MM) and osteosarcoma (OSA), leading to investigations of the potential of anti-CSPG4 DNA vaccination for their treatment alone or in combinatorial approaches with the standard of care.

Since the CSPG4 is a non-mutated, poorly immunogenic, self-antigen, we generated a human (Hu)-dog (Do)-CSPG4 hybrid plasmid, to overcome host's unresponsiveness. We tested HuDo-CSPG4 plasmid intramuscular injection followed by in vivo electroporation (electrovaccination) in mice challenged with transplantable tumors and in dogs with surgically resected spontaneous CSPG4+ MM and OSA. HuDo-CSPG4 was immunogenic and endowed with an anti-tumor potential in MM and OSA mouse models. In canine MM and OSA patients the electrovaccination procedure was safe, immunogenic and with a clinical benefit, significantly increasing the survival of vaccinated dogs as compared to controls. Induced-cellular and antibody responses, encompassing multiple mechanisms of action, correlated with patients' survival.

As a step forward, we have initiated to evaluate the potential combination of CSPG4-targeting with front-line therapies for MM (BRAF inhibitors, BRAFi, Vemurafenib) and OSA (chemotherapy). Ongoing studies suggests that CSPG4 could be related to the emergence of BRAFi-resistance: preliminary data show that in MM cells, CSPG4 expression drops in the early response to BRAFi, to reemerge again in resistant cells. Moreover, preliminary bioinformatic analysis of public datasets from melanoma patients' biopsies collected before and after targeted-therapy, revealed similar trend with an early drop in CSGP4 mRNA followed by a raise in therapy-resistant metastases. Our in vitro results suggest that the combination between HuDo-CSPG4 antibodies induced by electrovaccination and BRAFi could have a synergistic effect against the malignant behavior of MM cells.

Moreover, our in vitro studies have shown that when treated with chemotherapeutic agents, an increase in CSPG4 expression in OSA surviving cells is observed, suggesting a possible involvement of CSPG4 in resistance development. In line, combining vaccine induced anti-CSPG4 antibodies with chemotherapy enhance its effects on human and canine OSA cells and spheres.

In conclusion, these results highlight the relevant CSPG4 role in MM and OSA and provide the bases to propose HuDo-CSPG4 electrovaccination to effectively break immune tolerance and treat CSPG4+ MM and OSA patients in rational-based combinatorial approaches, including BRAF targeted-therapy for MM, and chemotherapy for OSA, to finally improve patients' survival.

OR-145

Electrochemotherapy plus IL-2+IL-12 gene electrotransfer in spontaneous inoperable stage iii-iv canine oral malignant melanoma

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Introduction: Electrochemotherapy (ECT) and Gene electrotransfer (GET) are valuable tools in the treatment of cancer. Both have the ability to enhance an immune response; nevertheless, ECT mainly induces a local immune response but fails to induce an effective systemic immune response, mainly because the stimulation of immune response induced by ETC is due to the release of damage-associated signal which triggers a humoral immune response that lack the strength to affect distant metastasis. Switching this humoral response to a cellular immune response would increase the possibility of an abscopal effect. This possibility could be enhanced by the transfection of canine IL-2 and IL-12 plasmids by GET. The aim of this retrospective study is to evaluate the addition of gene electrotransfer (GET) of canine IL-2 peritumorally and IL-12 intramuscularly for the treatment of inoperable stage III-IV canine oral melanoma.

Materials and methods: Thirty patients were included, of which twenty received only ECT (control group), and ten a combination of ECT+GET (treatment group). For the ECT treatment, intravenous bleomycin was used. Eight minutes after the administration of the drug, the electric pulses were delivered. For the treatment group. after the ECT session, the GET session was performed transfecting canine IL-2 to the periphery of the tumor, and canine IL-12 to the quadriceps muscle. The GET procedure was repeated 28 days later. ECT and GET were performed using an EPV-100 electroporator (BIOTEX, Argentina), delivering 8 100 us long pulses of 1,000 V/cm at a repetition frequency of 5,000 Hz for ECT and 1 Hz for GET.

Results: An objective response (OR) for the local treatment was achieved in 80% of ECT+GET group with a complete response (CR) in 30% of patients and 50% of partial response (PR). In the control group the OR was of 65% (CR of 5% and PR 60%). The results showed a peak of IL-2 and IL-12 in blood levels after 7-14 days of transfection The median survival time (MST) was of 5.5 months (3-32months) for the ECT+GET group. MST for the control group was 6 months (2-17months). The median progression free survival (PFS) was 5.5 moths (3-32months) for the GET+ECT group, while for the ECT group was 4 months (1-16months). A significantly difference was noted for the PFS of the patients in the treatment group (p=0.0284). The treatment was well tolerated with only grade 1-2 side effects presents for all groups.

Conclusion: The addition of GET with peritumoral canine IL-2 and intramuscular canine IL-12 can improve treatment outcomes by increasing the PFS without adding discomfort or side effects.

OR-146

Adjuvant Electrochemotherapy and/or Radiotherapy in Feline Injection Site Sarcoma

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A retrospective analysis of cats with confirmed diagnosis of Feline Injection Site Sarcoma (FISS) was conducted in our clinic between 2020 and 2022. The aim of this work was to evaluate the impact of surgery combined with electrochemotherapy (ECT), or radiotherapy(RTP), or both in the disease free survival (DFS).

The analysis included 85 feline patients with an average age of 10.4 years (3-20). Fourty-one percent (36/85) of the included patients had a recurrence from previous surgery. The analysis included the histological grade (G1, G2, and G3), histological margins (compromised, narrow, and clean), and

anatomical location of the sarcoma (Flank/thoracic and Dorsal/interscapular/spine).

Four treatment groups were defined: 1. Surgery alone (n=21), 2. Surgery+ECT (n=25), 3. Surgery+RTP (n=27), and 4. Surgery+ECT+RTP (n=12).

ECT treatment was performed intraoperatively in a single session using plate or needle electrodes depending on the depth of the margins required. Intravenous blemycin was administered followed by the application of electric pulses using an EPV-300 Electroporator (BIOTEX SRL, Argentina). Pulse trains consisted of 8 square wave pulses of 100us long 1000V/cm at 5KHz. In the case of RTP, it was performed after the surgical wound healed, using an orthovoltage unit in 12 fractions of 3.3-4Gy, with a total accumulated dose of 40-48 Gy.

Results showed that when considering the histological grade, no differences was found between grades G1, G2, and G3, regarding DFI in any of the treatment's groups. When considering patients with clean surgical margins, none of the treatment groups differ significantly in terms of DFI p>0.05. However, patients with compromised and narrow surgical margins that received additional treatment after surfery had a higher DFI when compared with patients that received surgery alone (tp=0.05, p=0.0063, and p=0.05).

Side-effects in group 1 were one case of delayed healing related to the polypropylene mesh used. In group 2, two patients presented partial dehiscence of the surgical wound in the flap that resolved without problems. In group 3, we observed two cases of dry radiodermitis that resolved quickly after 7 days. All the patients in groups 3 and 4 presented alopecia and change in hair color of the irradiated area. All side-effects were categorized as mild and were well tolarated by the patient.

In conclusion, additional treatment with ECT, RTP or both in patients with compromised or narrow surgical margins improved DFI with acceptable side-effects.

OR-147

Retrospective analysis of the outcome and survival time of dogs with mast cell tumors with different degrees of malignancy treated with electrochemotherapy

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Electrochemotherapy (ECT) has shown good response in low-grade mastocitomas (MCT), either as the main or adjuvant therapy after surgery.

A retrospective analysis from data of the medical records of patients with MCT without metastasis and treated with ECT was performed (2018-2023). In total, 29 patients and 39 tumors were analyzed. According to the histopathological analysis (Kiupel grading) 28 tumors had low grade and 11 tumors had high grade. For all patients, ECT with bleomycin was used at a dose of 15IU/m2, using a Veterinary Cellporator VetCP 125 device with pulse needle electrode: 8 monophasic square; pulses duration of 100 μs ; frequency of 5 Hz and pulse amplitude was 800-1200 volts/cm. The evaluation time for response to treatment for each dog was at least 6 months with routine monthly controls. The size of the tumors was recorded prior to ECT. The average size of the tumors was 4.6 cm3. According to the location, 10 were located in the head (25.6%), 12 in the limbs (30.7%), 5 in the thoracic area (12.8%), 4 in the pectoral area (10.2%). To a lesser extent, the tumors were located in zone 3 of mammary glands (7.6%), 1 in the neck (2.5%), 2 periscrotal (5.1%) and 2 perivulvar (5.1%).

The evaluation during the period it was determined that 23 patients (79%) had complete remission. Of them, 18 patients had low-grade, and 5 patients had a high degree of malignancy. It was determined that 6 patients (21%) had disease progression, all of them had high-grade mast cell tumors. Complete remission was achieved in 27 low-grade tumors and 6 high-grade tumors. The remaining 6 tumors had disease progression, having a high degree of malignancy.

According to the survival time analysis of the treated patients and their histopathological grade, it was determined that low-grade MCT patients had a survival of 1101 days on average compared to

high-grade MCT patients where the average survival was 210 days ($p < 0.05$). Considering the average tumors size of 4.6 cm³, the survival curve for those patients with tumors larger than 4.6 cm³ was 124 days with a significant difference with the survival of patients with tumors < 4.6 cm³, which was 900 days ($p<0.05$).

The results concluded that ECT is a method that can achieve complete remission in low-grade MCT. Positive outcome were achieved in neoplasia that had a size less than 4.6 cm³ . On the other hand, with high degree of malignancy of MCT, therapeutic responses will depend on the size of the tumor. This is the first study that evaluates the ECT for the treatment of low and high histopatological malignancy MCT.

OR-148

Evaluation of the safety and feasibility of electrochemotherapy with intravenous bleomycin as local treatment of bladder cancer in dogs

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Local treatment of canine urothelial carcinoma (UC) of the bladder is a challenge. More than 90% of the cases invade the muscular layer, more than 50?velop on bladder sites with a difficult surgical approach and often requiring radical surgical procedures. This study aims to evaluate the safety and feasibility of electrochemotherapy (ECT) with intravenous bleomycin (BLM) as a local therapy for bladder UC. This prospective study included 21 dogs with spontaneous bladder UC. Regional/distant

metastases and neoplastic infiltration of the serosa was considered the main exclusion criteria. We had no deaths during ECT or in the immediate postoperative period, and no suture dehiscence. Most dogs (19/21) developed mild adverse effects, whereas two dogs developed ureteral stenosis. Complete response (CR) was achieved in 62% of the cases (13/21), while partial response (PR) was achieved in 24% (5/21). The median survival and disease-free survival times were 284 and 270 days, respectively. Overall survival was significantly better in the dogs who achieved a CR. In conclusion, ECT was welltolerated in dogs with UC, demonstrating its safety and feasibility. These data pave the way for new

studies aimed at evaluating the effectiveness of ECT in canine bladder UC as a translational model for human disease.

P7 - Electroporation for clinical use

Wednesday morning Track E

Sep 18, 10:40 - 12:10

OR-149

Enhancing sensitivity to radiation therapy using electroporation in a radio-resistant model of oesophageal cancer

Aoibhín Woods[1], Aisling Uí Mhaonaigh[1], Aisling Heeran[1], Lorraine Smith[1], Stephen Maher[1], Niamh Lynam-Lennon[1], Declan Soden[2], Jacintha O'Sullivan[1]

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Oesophageal cancer is a poor prognostic cancer with a five-year survival of less than 25%, and accounts for over 500,000 cancer-related deaths annually. A significant number of patients fail to respond to conventional treatments, chemotherapy and radiation therapy, where only approximately 30% of patients achieve a pathological response. Despite advancements in the multi-armed treatment approach of oesophageal cancer, numerous initial responders to conventional therapies acquire treatment resistance, which poses a particular clinical challenge as their tumours become increasingly refractory to treatment.

As such there is a pressing need for new therapies to improve the survival and quality of life of these patients and technologies to enhance the efficacy of current standard of care treatment regimens.

As an anti-tumour strategy, electroporation has shown promise clinically in the treatment of cutaneous malignancies, but it's therapeutic benefit

could extend to several solid malignancies. Technological advancements in the endoscopic delivery of electroporation will facilitate the treatment of gastrointestinal malignancies (EndoVE, Mirai Medical, Galway).

We hypothesise that electroporation could function as a treatment-sensitizer to enhance the response of resistant oesophageal tumour cells to ionizing radiation.

Using an isogenic model of radio-resistant oesophageal adenocarcinoma developed within our lab, we investigated if electroporation, using the ePORE electroporator (Mirai Medical, Galway), could enhance treatment efficacy in both radiosensitive and radio-resistant cells. We examined the surviving fraction of cells using a clonogenic assay, as a measure of the cell's reproductive capacity.

We identified that electroporation treatment prior to exposure to 2Gy irradiation significantly reduced tumour cell viability, in both the radio-sensitive, and radio-resistant cell lines, $p = 0.0333$ (*) and $p = 0.0033$ (**) respectively, compared to irradiation alone, with the effects more pronounced in the radio-resistant cells.

This work provides novel insight to the potential of reversible electroporation as a radio-sensitizer, using an isogenic model of radio-resistant oesophageal cancer. To interpret this response, we are validating these findings at alternative voltages and testing DNA damage and repair mechanisms to help explain mechanistically this significant radiosensitizing effect by electroporation. Currently we are also examining the potential of electroporation to enhance chemosensitivity using a novel isogenic model of cisplatin-resistant oesophageal cancer treated with an IC50 dose of cisplatin.

OR-150

Electroporation treatment alters the inflammatory tissue microenvironment in the human inflammatory condition, Barrett's Oesophagus

Lorraine Smith[1], Cian Gargan[1], Aisling Uí Mhaonaigh[1], Irene Narinda[1], Aoibhín Woods[1], Aoife Kilgallon[1], Matthew McElheron[1], Meghana Menon[1], Fiona O'Connell[1], James Phelan[1], Declan Soden[2], Jacintha O'Sullivan[1]

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Barrett's Oesophagus (BO) is an inflammatory condition thought to be caused by chronic gastric acid reflux. It is the only known precursor for oesophageal adenocarcinoma (OAC) increasing the risk by 30-125 fold. Approximately 12% of OAC patients when diagnosed had a previous diagnosis of BO but approximately 57% have concurrent BO. Current treatments for BO include Radio Frequency Ablation (RFA) and cryotherapy for patients who have progressed to dysplasia. However, these treatments are not without side effects, RFA is a thermal treatment while accuracy with cryotherapy is difficult. Thus, we hypothesise that electroporation is a feasible new treatment option.

Using a novel human ex vivo Barrett's explant model, we treated 10 matched Barrett's and adjacent normal tissue ex vivo biopsies (five short and five long segment patients) with various electroporation (EP) conditions- EP only, calcium EP (5 mM CaCl2) and Irreversible Electroporation (IRE) using the ePORE electroporator (Mirai Medical). After, untreated and treated biopsies were cultured for 24 hours. The tissue cultured media was then used to analyse inflammatory secretions using the MSD multiplex platform (54-plex). Biopsies were histologically categorised according to the Vienna grading system.

Results confirm at baseline the ex vivo Barrett's tissue explants were indeed more inflammatory compared to their matched normal tissue, with significantly higher levels of IFN-γ, IL-6 and IL-8 ($p < 0.01$). Compared to untreated Barrett's tissue, EP only and CaEP did not significantly alter the levels of any cytokines apart from CaEP significantly reducing levels of VEGF ($p<0.05$). In con-

trast, IRE significantly reduced the levels of different cytokines compared to untreated tissue ((GM-CSF, IL-8, MCP-1, IL-10, IL-4, IL-6, TNF-α, IL-2, VEGF and IFN-γ) ($p<0.01$)). This may be a consequence of IRE directly killing the tissue. Then, clinical variables such as Vienna Grade and length of disease segment were correlated with cytokine profiles. With increasing disease severity, Barrett's tissue treated with CaEP negatively correlated with most cytokines while IL-1B, TNF-a, IL-8, MCP-1 and MIP-1a were statistically significant ($p<0.05$). A similar trend was observed for the same cytokines with increasing segment length. Contrastingly, Barrett's tissue treated with IRE with increasing disease severity positively correlated with most cytokines with IFN-γ statistically significant ($p<0.05$). This trend was also observed with longer Barrett's disease segment length with IL-22, IL-15 and IL-23 statistically significant ($p<0.05$).

Future work will analyse how these treatments affect immune cell activity namely T cells and Dendritic cells. We will also incorporate more patients, especially those with advanced disease and include a HFIRE treatment condition to further test the validity in using electroporation as a treatment for BO.

OR-151

Intraoperative electrochemotherapy of the posterior resection surface after pancreaticoduodenectomy: Preliminary results of a hybrid approach treatment of pancreatic cancer

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Background: Despite extensive research in recent decades, pancreatic cancer remains one of the deadliest forms of cancer, with survival rates showing little improvement. Local recurrences account for approximately 30 per cent of all disease recurrences. With the intent to improve survival, we designed a novel, hybrid treatment strategy consisting of surgical resection and additional intraoperative electrochemotherapy of the posterior resection surface. We outline the study protocols and initial findings from a prospective pilot study investigating this approach.

Methods: Patients meeting the inclusion criteria and consenting to participate, with resectable pancreatic head ductal adenocarcinoma, were enrolled in the study. Following surgical resection, electrochemotherapy with bleomycin was performed using plate electrodes to cover the area between anatomical landmarks.

Results: Electrochemotherapy of the posterior resection surface was feasible in all 7 patients. Pancreatic fistula grade B occurred in only one patient; other observed complications were predominantly Clavien-Dindo grade 2 or lower. Hospital mortality was at 0%.

Conclusions: Our preliminary findings indicate that the hybrid approach combining surgery with intraoperative electrochemotherapy appears to be both safe and feasible.

OR-152

Novel Synergistic Electric Pulses and First Human Cancer Clinical Trials: Towards the Balance between Negligible Muscle Contraction and Enhanced Ablation

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Irreversible electroporation (IRE), a nonthermal therapy for solid tumors, has been implemented in clinical applications and made significant advancements in recent years. However, there is still an extremely urgent for novel pulses that strike a balance between effective tumor ablation and minimizing side effects, especially the occurrence of muscle contractions during this treatment, necessitating the use of deep muscle anesthesia and adequate muscle relaxants. This study focuses on the development and clinical evaluation of a new IRE protocol modality involving synergistic electric pulses for cancer therapy, aiming to achieve both enhanced tumor ablation and negligible muscle contraction.

Here, we introduced a novel IRE strategy combin-

ing optimized high and low-voltage pulsed protocol (S-IRE) to study the ablation effect and muscle contractions with monopolar and bipolar electrodes. Through numerous experiments on cells and animals, we validated the benefits of S-IRE in inhibiting nerve stimulation-induced muscle contractions and killing tumor cells compared to traditional IRE, allowing applied under local anesthesia during ablation. A device Wknife based on the S-IRE was developed and underwent successful safety testing in preclinical trials involving various tissues (liver, thyroid, prostate, and lung tissue), showing promising outcomes and providing a strong rationale for exploring the efficacy of this technique in human subjects. Following successful electrical & EMC testing, the Wknife device received approval from the NMPA (National Medical Products Administration) for clinical trials. The first human clinical trials on liver, thyroid, prostate, and lung cancers, had been conducted in collaboration with renowned oncologists and medical centers respectively. Preliminary clinical data has supported the feasibility of the S-IRE in reducing muscle contractions and local anesthesia perforation, it also primarily demonstrated the safety and efficacy of Wknife in tumor ablation. Now, a multi-center randomized controlled clinical trial is being conducted for comprehensive assessment and monitoring of patient outcomes, along with long-term follow-up, which is expected to provide valuable clinical evidence on the feasibility and potential of this novel cancer treatment approach.

In conclusion, this study offers valuable insights into the novel S-IRE ablation technique, which effectively balances minimal muscle contraction with enhanced tumor ablation. It has the potential to greatly improve the operability and safety of IRE treatment, offering a less invasive/side effect and effective treatment option for cancer patients. This advancement is expected to build an important basis for its integration into cancer treatment protocols in the future.

OR-153

Bleomycin based electrochemotherapy with standard electrodes for advanced stage, recurring vulvar/cervix carcinomas

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Recurrent vulvar cancer occurs in an average of 24% of cases after primary treatment after surgery with, or without radiation. It is often treated with concurrent or sequential multimodal therapies, however, despite the use of multimodal treatments, relapses are recorded in approximately one third of patients with limited salvage options.

In a 2-year period (Dec 2020- Dec 2022), five surgically inoperable V/C cases were treated during six sessions of ECT at the University of Szeged, Department of Surgery, Hungary. After preoperative MRI, each ECT was carried out under general anesthesia, using standard finger electrodes and intravenous bleomycin (15.000 IU/m2) administered 8 min before first pulse generation. Prospective data collection was carried outat 1 week, 1-2-4-6 months. Patient health status and QoL was assessed at each follow-up. Tumor response was evaluated through MRI, and gynaecological examination 2 months after ECT as per RECIST 1.1, adverse events were evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Quality of life was assessed using the EQ-5D (Euro Quality of Life - 5 dimensions) questionnaire.Three squamous cell vulva-, 1 squamous cell cervix- and 1 columnal cell cervix carcinoma were treated, with median age of 70 (64-85) years. Median operative time was 23 min (7-32), median hospital stay 2 days (2–6). Mean BMI (body mass index) was 29.7, mean ASA 2, mean CCI (Charlson Comorbidity Index) 5. Two tumors were located in the cervical stump (after hysterectomy), 3 at the vulva. Previous treatments included surgery (5 cases: 2 hysterectomies+ 3 vulvectomies), preop radiotherapy (RT) in 3, postop RT in 2 cases, postop chemotherapy in 1 case.

Follow-up MRI at two months confirmed 1 CR (complete response), 1 PR (partial response), 2 SD (stable disease) and 1 PD (progressive disease).

No major adverse events were observed, novum atrial fibrillation occurred in 1 case median postop pain level (VAS) was 2. Previous odour.

OR-154

Electrochemotherapy: from palliation to important player in the multidisciplinary management of the cancer patient

Antonio Bonadies[1], Tiziano Pallara[1], Marinella Tedesco[1], Paola Parisi[1], Michela Battista[2], Flavio Andrea Govoni[3], Gennaro Ciliberto[4], Emilia Migliano[1]

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In these past 15 years we have seen the evolution of electrochemotherapy (ECT) from palliation of inoperable tumours to treatment of locally advanced non-melanoma skin cancers, multiple unresectable in transit melanoma metastases and advanced skin recurrences of breast cancer. From the beginning, we performed ECT also as a curative treatment of a skin cancer with a multifocal/multidistrict primary presentation such as cutaneous Kaposi's sarcoma. The effective results achieved in this type of cancer patient led to the inclusion of ECT in the European Guidelines for its treatment. Today, we use ECT as a part of a multidisciplinary skin cancer management providing a spot treatment while continuing ongoing systemic therapies, in case of disease progression or local cutaneous or subcutaneous recurrences. Regarding the management of cutaneous tumours of the head and neck (HN) region, the surgical treatment requires achieving safety margins. This endpoint often demands a large demolition and a complex reconstruction with long operating times that are sometimes not compatible with the frequent comorbidities of elderly and frail patients. Furthermore, some functional and aesthetic disfiguring results are not easily accepted by the patient. In this setting, ECT offers several advantages over surgery including higher preservation of normal surrounding tissues, decreased morbidity, and shorter hospitalization. The purpose of our clinical investigation was to address the ECT antitumor efficacy, organ preservation and aesthetic outcome in the management of skin metastases of HN cancers.
A retrospective outcomes analysis of HN skin cancer patients who underwent ECT at San Gallicano Dermatological Institute IRCCS was performed. Treatment consisted of intravenous bleomycin infusion followed by locally delivered electric pulses. Loco-regional tumour response and side effects were evaluated. Post ECT evaluation of scars' appearance and aesthetics were assessed using the Vancouver Scar Scale (VSS) and the Manchester Scar Scale (MSS) in complete response patients.
Among 33 selected patients, 27 (82%) reached a complete response (CR) and 6 (18%) a partial response (PR). Local control of the disease was maintained for a median time of 9.5 months (range 0.3 - 52.7). All side effects were easily managed and resolved spontaneously. Among 26 CR patients evaluated for cosmetic outcome, at a median time of 7 months (range 4 - 16), the VSS and the MSS total scores were 4 or less and 10 or less, respectively, in 25 patients (96%).
In conclusion, for the treatment of skin cancers of HN, ECT represents a favourable local anticancer procedure with minimal side effects and excellent aesthetic and functional results. Overall, ECT can be regarded as an effective and safe adjunct to the armamentarium of the interdisciplinary oncological team and a feasible curative tool for uresectable primary skin cancer lesions.

S21 - Cardiac ablation by irreversible electroporation - pulsed field ablation (PFA)

Wednesday afternoon Track A

Sep 18, 14:20 - 15:20

OR-155

Protocol-specific modelling of cardiac pulsed field ablation

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Pulsed field ablation (PFA) is a recent procedure for the treatment of cardiac arrhythmias. The procedure selectively targets cardiac tissue, creating nonthermal damage to cardiomyocytes through electroporation, while sparing other types of adjacent tissue structures. Despite the promising clinical and experimental results, no mathematical model is capable of simulating all the different ablation protocols used. This work introduces a protocol-specific framework for the modelling of PFA procedure. The cardiac bidomain equations are coupled with an electroporation term which accounts for the number of membrane pores per unit area. A physiological model tracks pore generation at the cellular membrane and allows for the simulation of pore resealing. After model calibration and comparison against experimental results available in the literature, the impact of two different protocols, the bipolar interlace and the circular bipolar methods, is explored in terms of the acute lesion characteristics.

OR-156

Endocardial or Epicardial Delivery of Pulsed Field Ablation of Ganglionated Plexi? Assessment and Quantification from An In-Silico Modelling Study

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Background: Pulsed Field Ablation (PFA) has recently been proposed as a non-thermal energy to treat atrial fibrillation by selective ablation of ganglionated plexi (GP) embedded in epicardial fat. While most PFA-technologies use an endocardial approach, the use of epicardial access has given promising pre-clinical results. However, as each technology employs a different and sometimes proprietary pulse application protocol, comparing the endocardial vs. epicardial approach is nearly impossible in experimental terms. Therefore, the aim of our study was to compare the electric field distribution and the thermal-side effects of both approaches under equal conditions in terms of electrode design, pulse protocol, and anatomical characteristics of the tissues involved (epicardial fat and myocardium).

Methods: Two-dimensional computational models with axial symmetry were built for endocardial and epicardial approaches using a conventional cardiac ablation catheter (7 Fr – 4 mm). Atrial (1.5−2.5 mm) and fat (1−5 mm) thicknesses were varied to simulate a representative patient cohort treated with PFA using different applied voltage values (1000, 1500 and 2000 V) and number of pulses (30 and 50). The sequence of pulses was monophasic with a width of 100 μs and 1 Hz frequency. The PFA-induced electric effects were assessed by the volume of fat above 1000 V/cm, as well as the collateral volume of electric field damage in the myocardium. Thermal-side effect provoked by PFA was evaluated by the evolution of maximum temperature in the fat and the myocardium and the quantification of thermal damage lesion by using the Arrhenius Equation. The normality of the data was checked using Shapiro-Wilk Test. The comparison of outcomes between both approaches was performed using the paired t-test for normally distributed data. In case of non-normal distribution, the comparison was carried out using the Wilcoxon Signed-Rank-test.

Results: The epicardial approach was superior for capturing greater volumes of fat when the applied voltage was increased: 231 mm3/kV with the epicardial approach vs. 182 mm3/kV with the endocardial approach. In relation to collateral damage to the myocardium, the epicardial approach considerably spared the myocardium, unlike what occurred with the endocardial approach. Although the epicardial approach caused much more thermal damage in the fat, there was not a significant difference between the approaches in terms of the size of thermal damage in the myocardium.

Conclusions: Our results suggest that the epicardial PFA ablation of GPs is more effective than the endocardial approach. The proximity and direc-

tionality of the electric field deposited using an epicardial approach are key to ensuring higher electric field strengths and increased temperatures within the epicardial fat, thus contributing to selective ablation of the GPs with minimal myocardial damage.

OR-157

Modeling the long-term effects of Pulsed-Field Ablation including comparison with Radio-Frequency Ablation

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In healthy hearts, the propagation of electrical waves follows a predictable pattern, whereas in people suffering from a cardiac rhythm disorder, the electrical wave can become chaotic and directly affect the pumping function of the heart. Isolation of the pulmonary veins (PVs) by catheter ablation has become the treatment of choice for atrial fibrillation (AF). The main goal is to isolate the PVs from which the fibrillation is supposed to originate by physical procedures such as the RadioFrequency Ablation (RFA) [1] - a thermal ablation that is currently the most commonly used technique - and the novel Pulsed electric Field Ablation (PFA) [2], which is based on non-thermal irreversible electroporation.

Despite the great interest that RFA and PFA have generated in the treatment of AF, there is still a lack of understanding – and thus modelling – of the underlying biophysical phenomena of these different therapies. On one hand, it is well known that RFA ablation leads to coagulation necrosis with complete loss of cellular and vascular architecture by leaving a scar composed of a fibrotic tissue. On the other hand, PFA is known to destroy mainly the cardiomyocytes, but the tissue scaffold is preserved. Therefore, the physical properties of the cardiac tissue after RFA and PFA are very different, although they have the same goal, which is to isolate the PVs.

This work aims to modify the classical bidomain model – which describes the propagation of intracellular and extracellular potentials in the heart – to introduce a region ablated by RFA or PFA, and study the propagation of the electric signal after the ablation, over the long term. Both types of ablation involve isolation of a pathological area, but we describe them differently by using appropriate transmission conditions at the interface between the ablated area and the healthy heart.

We propose numerical simulations in the context of AF and we consider the isolation of one PV of a synthetic geometry of the left atrium. Both the RFA and PFA models lead to the isolation of the PV. The main difference between the two models results in the isolation of the extracellular potential in the case of RFA being continuous for PFA. Our modeling also enables to propose a mathematical explanation for the lower recurrence of AF after PFA compared with RFA. Partial disconnection of the PV can be seen as the emergence of electrical pathways between the atrium and the PV. It could be caused by the development of necrotic fibrosis after RFA leading to conduction pathways, which is not the case in PFA.

[1] J. Joseph and K. Rajappan. Radiofrequency ablation of cardiac arrhythmias: past, present and future. QJM, 2012.
[2] G. Caluori et al. AC pulsed field ablation is feasible and safe in atrial and ventricular settings: a proof-of-concept

OR-158

Multiscale Simulation of Calcium-Mediated Cardiac Lesion and Stunning in Pulsed Field Ablation

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Electroporation is the basis of cardiac Pulsed Field Ablation (PFA). Although it has been proven as an effective and safe technique clinically, the cell death mechanisms and physiological effects remain not fully understood. Computational modeling has become a powerful tool in the field of electrophysiology and can also be used to elucidate the alterations induced by electroporation pulses.

In this study, the permeabilization of the cell membrane in myocytes has been simulated at different electric fields using Krassowska's model (Biophys J. 2007 Jan 15;92(2):404-17). According

to that, the induced flow of ions (Na+, K+, Ca2+ and Cl-) through the permeabilized membrane have been incorporated into an electrophysiological model (Biophys J. 2000 May;78(5):2392-404.) which considers key ionic channels and pumps (e.g. RyR, SERCA and Na+/K+). Considering the solute changes induced by electroporation, the osmotic pressures and water flow have also been incorporated. The resulting model can estimate the temporal distribution ionic concentrations and predict how the electrophysiologic behavior of the cell is affected. Considering the critical role of calcium in the regulation of cardiac myocytes, its intracellular concentration has been linked to the different effects observed in the cardiac cells after PFA pulses.

The results at cellular level show that for low concentration changes, the cardiomyocytes interrupt their conduction capability showing a transient stunning effect. For larger ionic disturbances, the cell is not able to rapidly restore the physiological levels, and this will result in a long-term cell death. For higher concentrations, the calpain proteases are activated, disrupting large proteins and cardiomyocyte structure which will lead a rapid cell death.

The microscopic simulations of ionic distributions and the triggered pathways have been employed to predict the spatio-temporal effects at the macroscopic tissue level. A 3D numerical model of a clinical PFA procedure with a focal catheter in contact with the myocardium has been employed to deliver the therapeutic pulses and evaluate the extension of the induced effects.

The results of this study predict the appearance of a large area around the treatment location where the cardiomyocytes are stunned and unable to perform their physiologic electric conduction. Within the ablated section, the zone closer to the catheter electrode, with higher homeostatic disturbances, presents a fast structural change induced by calcium-activated calpain. These results are in agreement with preclinical and clinical observations, including the recent observation of fast structural changes after PFA (Circ Arrhythm Electrophysiol, 2024, 17(3):e012255). The presented model with a multiscale approach is able to estimate the spatial and temporal evolution of both electrophysiologic and structural effects induced by electroporation in myocardial tissue.

S01 - Medical applications of nsPEFs

Wednesday afternoon Track B

Sep 18, 14:20 - 15:20

OR-159

Investigating the mechanism and dynamics of Ca2+-mediated pore expansion after nsPEFs in healthy and cancerous urothelial cells

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[3]Vilnius Gediminas Technical University, Lithuania

A major effect on the cell-killing efficiency of pulsed electric fields (PEFs) has been attributed to an excessive influx of extracellular Ca2+, with some studies suggesting a greater effect against cancer cells. Earlier in vitro research has shown that Ca2+ triggers abrupt pore expansion leading to early cell death. Our studies investigate the link between pore expansion dynamics and susceptibility to PEFs, as well as the underlying mechanism, focusing on urothelial cancer cells.

We compared the response to an electric field measured in monolayers and 3D cultures of normal and malignant human urothelial cell lines (SV-HUC-1, T24, UC3, RT4). We used a robotic system to precisely place needle electrodes orthogonal to the monolayer and plate electrodes on both sides of the spheroid. Imaging was performed using an inverted fluorescence microscope configured for high throughput screening with automatic stage shift and autofocus. The area of cell death and permeabilization after application of 300ns PEFs at 10 Hz was measured by staining with propidium iodide (PI) and YO-PRO-1 dye. Dose-response curves were obtained by fitting the stained areas of cell monolayers to the simulated electric field strength. We showed that the presence of Ca2+ in the extra-

cellular medium resulted in a decrease in the immediate membrane permeabilization of various urothelial cancer cell lines measured with YO-PRO-1 Uptake during PEFs delivery. Subsequently starting from 20 min after exposure, Ca2+ caused an abrupt increase in cell permeability to PI and YO-PRO-1 dye suggesting the expansion of cell membrane pores.

Interestingly, healthy SV-HUC cells, which demonstrated weaker YO-PRO-1 uptake at the time of PEFs delivery, exhibited an earlier Ca2+ mediated abrupt permeabilization compared to other cancer cells. The time course of pore expansion does not correlate with the sensitivity of the cells to the nsPEFs. Due to calcium-mediated pore expansion, early cell death can be achieved at lower electric field intensities. The LD50 (electric field dose that kills 50% of the cells) for SV-HUC cell death was 1.2 times lower in 2mM Ca2+ and 1.5 times lower in 5mM Ca2+ compared to the Ca2+-free medium ($p<0.001$). For the UC3 cancer cell line the LD50 was 1.3 and 1.5 times lower and for T24 1.4 and 1.9 times lower in 2 mM and 5 mM Ca2+ respectively ($p<0.001$). The underlying mechanism of pore expansion remain to be explained using Raman microspectroscopy and fluorescence lifetime imaging.

OR-160

Synergistic effects and mechanisms of nanosecond pulsed electric fields and cold atmospheric plasma to treat pancreatic cancer

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A pulsed power technology generating nanosecond pulsed electric fields (nsPEFs) has been demonstrated to effectively treat many types of cancer in animal models. However, there are some limitations of nsPEF cancer treatments. It requires high-electric fields for cancer ablation which limit its application and effectiveness. Our group and others have proposed and investigated several approaches to overcome this limitation. Here, we present data to demonstrate synergistic effects of nsPEFs and cold atmospheric plasma (CAP) for pancreatic cancer and further explore the mechanisms behind this synergy.

Mouse Pan02 pancreatic cancer cells were treated with nsPEFs (60 ns 50 kV/cm, 1 Hz and pulse numbers varied), CAP (9 kV, 200 ns pulses, 2 kHz and treatment times varied), or the combination of these two treatments. Cell viability was examined with WST-1 assays. Cell membrane permeabilization was monitored with Electric Cell-substrate Impedance Sensing (ECIS). Generation of reactive oxygen species (ROS) was quantified with H2DCFDA, MitoSox, and Amplex red for cytosol ROS, mitochondrial superoxide, and H2O2, respectively. Mitochondrial membrane potential was determined with TMRE. Cell death pathways including apoptosis and pyroptosis were examined with caspase activity assays and/or Western Blot. Additionally, the changes of signaling pathways and up-/down-regulation of gene expression were analyzed with proteomic analysis as well.

We have found that nsPEFs at a low dose sensitize cancer cells for CAP treatments in terms of cell membrane permeabilization and cytotoxicity. Both nsPEFs and CAP induce ROS generation including cytosol ROS, mitochondrial superoxide and H2O2. However, it seems that nsPEFs are more effective to elicit the drop of mitochondrial membrane potential than CAP does. CAP leads to apoptotic cell death with the upregulation of Caspase3/7 activity whereas nsPEFs induce non-apoptotic, non-pyroptotic cell death with a negative Caspase3/7 and Caspase1 activity. Notably, a mild dose of nsPEFs can significantly augment apoptotic cell death induced by CAP. Proteomic analysis shows remarkable differences between nsPEFs and CAP treatments in terms of gene expression changes and signaling pathways involved.

Our discoveries suggest distinctive cell death mechanisms involved in nsPEFs and CAP, which contribute to the synergistic effects of the combination treatment.

OR-161

Nanosecond Bursts of Ultra-High Frequency for Electrochemotherapy and Gene Delivery

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Currently, the field of reversible electroporation (associated with molecular delivery) is dominated by the microsecond pulses, while the irreversible electroporation (associated with tissue ablation in the biomedical context) is far more flexible with nanosecond protocols being present on the market for several decades. One of the reasons could be the lack of sufficient electrophoretic component and inferior pore size following nano protocols, which negatively affects molecular transfer. As a result, the gene delivery or drug delivery was not covered at all in the sub-microsecond pulse range with the first works starting to appear only recently. In both application cases, if the bursts are delivered with common 1 Hz – 10 kHz frequencies, the nano-pulses have significant limitations in terms of electrotransfer efficiency. However, with the development of silicon-carbide MOSFET technology, high power and high frequency generation of pulses became possible. It was shown that there is a threshold pulse repetition frequency (500+ kHz), when the depolarization of the cell membrane is longer than the delay between the pulses, which enables sustaining the cells in a polarized state throughout the whole burst without triggering irreversible electroporation. This polarization phenomenon positively affects electrotransfer and efficacy of nano-electroporation, enabling cell plasma permeabilization at significantly lower thresholds. As a result, the nanosecond protocols, but delivered at ultra-high frequency (1+ MHz) are competitive and in many cases superior to conventional microsecond pulses for drug & gene delivery, which was confirmed both in vitro and in vivo.

This presentation is dedicated to provide a quick critical overview of the current status of the technology, benefits and limitations incl. a vision for further development.

Acknowledgement: The research was supported by the Research Council of Lithuania (Grant nr. S-MIP-19-13, S-LL-21-4, S-MIP-23-124).

OR-162

Characterizing the Immune Response Following High Frequency Nanosecond Bipolar and Unipolar Calcium Electrochemotherapy

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Calcium electrochemotherapy (CaECT) is a new and effective cancer treatment approach enabling the replacement of standard chemotherapeutic drugs with non-toxic calcium. Recently, it was shown that the application of sub-microsecond pulses, which are compressed into a high frequency (MHz) burst, significantly boosts the efficacy of the CaECT methodology. However, this is true only for monophasic procedures, while in the case of bipolar nanosecond pulses, a bipolar cancellation (BPC) phenomenon can be triggered. Most of the effects of BPC are reported in the context of tissue ablation, while data on bipolar ECT are hardly available. At the same time, the application of bipolar pulses potentially reduces muscle excitation and associated pain, ensures a more homogeneous spatial electric field distribution within the tumor, and makes the treatment less likely to induce electrolysis and pH changes. Therefore, in this work, we have compared the feasibility of unipolar and bipolar sub-microsecond pulses (7 kV/cm x 300 ns x 250, 1 MHz) in the context of CaECT and compared them to ESOPE: European Standard Operating Procedures for Electrochemotherapy (1.5 kV/cm x 100 μs x 8, 1 Hz). Characterization of the immune response following the treatments was of particular interest.

A luminescent murine breast cancer (4T1-Luc)

cell line was used to induce tumors in Balb/C mice (n=60). When tumors reached the needed volume (˜50 mm3), the CaECT (250 mM) treatment was performed with various electric field protocols. Afterward, volumetric measurements and bioluminescence tumor imaging were used to characterize the tumor response. Also, at the end of the experiment, mice spleens, lymph nodes and blood samples were isolated and used for further analysis with flow cytometry.

Our study demonstrated that both uni- and bipolar nanosecond pulses can be used in CaECT context resulting in partial or complete response of the tumors. The treatments also triggered an increased percentage of CD4□ memory T cell subsets in spleens and tumor-associated lymph nodes. Also, increased helper T cells and decreased CD4□ regulatory T cells were detected in the lymph nodes after CaECT treatment. Moreover, modulation of humoral immune response (levels of antitumor IgG antibodies) post calcium ECT treatment was detected.

Interestingly, the effects of BPC were not profound for the bipolar 7 kV/cm x 300 ns x 250, 1 MHz protocol, which was predicted by the in vitro viability and permeabilization data supporting the research.

Acknowledgment: The research was supported by the Research Council of Lithuania (Grant Nr. S-MIP-23-124).

S13 - High voltage electrical discharges: principles and applications

Wednesday afternoon Track C

Sep 18, 14:20 - 15:20

OR-59

High-Performance Solid-State Generator for nsPEF Applications

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Nowadays, nanosecond pulsed electric fields (nsPEF) have an extensive scope of applications, ranging from many scientific disciplines to industrial fields. Although all these applications are based on the same basics, pulse shape, frequency and patterns, voltage, and current requirements can be very different for each specific scenario. These requirements can range from a few V and mA up to hundreds of kV and A. Furthermore, the effects of shorter pulses are currently being studied due to their potential advantages over longer and lower voltage pulses. In this context, there is a current challenge to develop generators for scenarios where pulses with widths in the range of tens of ns, several kV and hundreds of A are needed. In this context, the impedance of the biological target plays a key role in the design of such pulse generators. There are cases where the impedance of the biological target can be well characterized, but it is also common to cope with unknown impedance targets. Moreover, the impedance of biological targets varies over time during the electroporation process. For those reasons, achieving low output impedance is critical in these devices.

Considering this challenging context, this paper proposes a versatile modular high-performance pulse generator intended for nsPEF based treatments. This paper focuses on detailing the design and its implementation, as well as the main experimental results. The design is based on series-connected half-bridge inverters featuring 650-V Gallium Nitride (GaN) transistors. These are wide bandgap devices with fast switching capabilities, i.e., with rising and falling times even under 1 ns. In the proposed topology, each module conducts all the current delivered to the load but only needs to withstand a fraction of the applied voltage, depending on the number of modules employed. Besides, very low output impedance is achieved, making this pulse generator suitable for working with a broad range of biological targets, independently of their impedance.

By using this approach, a test prototype consisting of 4 modules has been developed, capable of generating pulses down to 5 ns long, and up to 2 kV and 200 A output voltage and current, respectively. Moreover, the output voltage can be regulated adjusting the number of active modules and DC bus voltage. Finally, there is an optically isol-

ated control board to manage the device. The applied pulse pattern can be arbitrarily configurable to match the requirements of the application, and the dv/dt is also controllable.
The final version of this paper will include detailed design, implementation and experimental measurements, including key waveforms.

OR-164

A Synergistic Bipolar Pulse Power Generator for Expanding Ablation Area and Inhibiting Muscle Contraction

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Pulse power technology has a wide range of applications in the field of biomedicine, especially in tumor ablation scenarios based on irreversible electroporation technology. Previous studies have shown that exponential decay pulses result in their energy utilization rate being too low to completely kill tumor cells.

The exponential decay pulse causes its energy utilization to be too low to kill tumor cells completely. The ablation area of the tumor is large when unipolar rectangular pulses, are applied to the target, but the DC component contained in unipolar rectangular pulses will induce strong muscle contractions for the patients. Muscle contraction can cause discomfort to the patient and even displace the electrode needle, which affects the accuracy of the treatment. The bipolar rectangular pulses have no DC component and can suppress the intensity of muscle contraction. However, the ablation area of bipolar rectangular pulses is smaller compared to unipolar rectangular pulses. A two-stage combined pulse, also called synergistic pulse, can improve the ablation efficacy.

It utilizes a dual Marx structure with nested high-voltage short pulse modules and low-voltage long pulse modules. This structure uses two different sets of storage capacitors, charging sources and solid-state switches that can respectively control the parameters of the high-voltage long pulse and the low-voltage short pulse. An experimental prototype is built to verify the scheme which can generate high-voltage short pulses with a maximum output voltage of ±12 kV, adjustable pulsewidth from 100 ns to 1 μs. Additionally, it can generate low-voltage long pulses with a maximum output voltage of ±3 kV, adjustable pulsewidth from 10 to 100 μs. The combination of high-voltage short pulses and low-voltage long pulses is adjustable in various forms. In addition, we conducted further clinical trials.

OR-165

Pulsed Atmospheric Pressure Plasma for the destruction of emerging contaminants and the inactivation of bacteria in water

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Environmental pollution of aquatic systems is a persistent concern for societies, with detrimental effects on human health, biodiversity, and socio-economics. Various chemical compounds, originating from human activities, are extensively used and enter the aquatic environment through wastewater. These compounds ultimately contaminate surface water bodies, groundwater and drinking water reservoirs. Additionally, a significant issue regarding water quality is sanitation, as pathogens such as bacteria, viruses, and protozoa are introduced into water bodies, resulting in the proliferation of numerous waterborne infectious diseases. Water bodies harbor a multitude of pollutants, including pharmaceuticals and, more recently, so-called ”forever chemicals” known as per- and polyfluoroalkyl substances (PFAS). Undigested pharmaceuticals and their metabolites pose a threat to food chains and must be addressed. PFAS, extensively utilized in various products, are of significant concern due to their persistence and propensity for bioaccumulation in the environment, rendering them emerging contaminants of high priority. Numerous conventional methods have been employed for water remediation. However, many of these methods suffer from drawbacks such as limited pollutant/pathogens removal, high energy consumption, prolonged treatment times and the risk of secondary pollution. In recent years,

cold atmospheric plasma has emerged as a highly promising water treatment method due to its cost-effectiveness and environmentally friendly nature. Cold plasma operates by generating highly reactive oxygen and nitrogen species, UV photons, and hydrated electrons, which effectively degrade organic pollutants and inactivate pathogens in water.

In the present study, a comprehensive investigation was conducted to assess the efficacy of pulsed atmospheric plasma in the degradation of various organic pollutants, namely valsartan (a pharmaceutical compound) and perfluorooctanoic acid (PFOA), a member of the 'forever chemicals' family. Additionally, the study examined the inactivation of Escherichia coli, a bacterium commonly found in contaminated water. Two plasma reactors were designed, constructed, and compared: (i) a gas-liquid dielectric barrier discharge and (ii) a plasma bubbles reactor. Both reactors were energized by high-voltage microsecond and/or nanosecond pulses to investigate and compare their effectiveness and energy requirements for the destruction of pollutants and pathogens in water. Various critical parameters such as treatment time, pulse voltage, plasma gas, water matrix, and initial pollutant concentration, were also investigated. Optimal operational conditions with the highest energy efficiency were determined. Additionally, detailed physicochemical characterization and analysis of plasma species formation under different conditions were performed. The results of this study suggest that pulsed plasmas offer a promising, environmentally friendly, and cost-effective solution for the destruction of pollutants and pathogens in water.

OR-30

Degradation of pesticide atrazine in water by high voltage electrical discharges

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Atrazine is an herbicide used primarily for the control of grassy and broadleaf weeds such as maize, sugar cane and sorghum. Atrazine pollutes surface water because it is relatively soluble in water. Atrazine is also found in underground waters, due to its low absorption from the soil. The chronic toxicity of atrazine is of concern. Atrazine interferes with the endocrine system, causing a series of pathological changes and reproductive abnormalities. Atrazine causes breast, ovarian and uterine cancers as well as leukemia and lymphoma. Therefore, it is very important to find clean and economical processes to degrade atrazine.

In this study, high-voltage electrical discharges (HVED) were used for the degradation of atrazine. This process was compared to chemical treatment with oxidation Fenton as well as physical treatment with high-frequency sonication. An analytical monitoring of the degradation process was carried out, by High-performance liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LC-MS). Degradation metabolites were identified and compared for each process.

The work carried out as part of this study has shown that the degradation rate of atrazine by Fenton oxidation is the highest (around 95% of atrazine degradation was observed after 2h of treatment). The use of low-frequency ultrasound at 50 kHz slightly degraded atrazine (around 23% of atrazine degradation was observed after 2h of treatment). The use of high-frequency ultrasound at 525 kHz degraded 87% of atrazine after 2 h. The HVED treatment was much more efficient, as 10-ms electrical shocks degraded atrazine by 83%. During the degradation of atrazine, eleven compounds were identified. The formation kinetics of these compounds were studied for all the treatments applied. The result showed that the degradation products of atrazine are related to the nature of the treatment applied.

The study showed the feasibility of high voltage electrical discharge (HVED) treatment, as an alternative technology, for the degradation of atrazine. Hydroxyatrazine (HA) is the main degradation product in HVED treatment, and it has a lower toxicity than other degradation metabolites of atrazine. This research paves a way for the development of a new clean and economical technology for wastewater treatment.

S11 - In vivo delivery of genetic medicine through gene electrotransfer

Wednesday afternoon Track D

Sep 18, 14:20 - 15:35

OR-166

Exploring gene electrotransfer as a DNA vaccination strategy: insights from a COVID-19 vaccine study

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DNA vaccination is a promising strategy for the treatment and prevention of cancer and infectious diseases. The aim of this study was to evaluate the feasibility of gene electrotransfer (GET)-mediated DNA vaccination on a model of COVID-19 vaccine.

Plasmids encoding the SARS-CoV-2 spike (S) or nucleocapsid (N) protein (pUNO1-SARS2-N, pUNO1-SARS2-S-d19, InvivoGen) were used as antigen source and an interleukin 12 (IL-12)-encoding plasmid (pORFmIL-12(p40:p35), InvivoGen) as an immunological adjuvant. The expression of the S and N antigens was first assessed after in vitro GET (8 × 1300 V/cm, 100 μs, 5 kHz) to murine myoblasts (C2C12) and fibroblasts (L929) using qRT-PCR and ELISA assays (Abcam, Krishgen Biosystems). Vaccination was performed in the skin (right flank) or muscle (right anterior tibialis muscle) of female C57BL/6J mice on days 0 and 14 (booster dose) using previously optimized transfection protocols for skin (MultiElectrode Array, 24×170 V/cm, 150 ms, 2.82 Hz) and muscle (plate electrodes, 1×HV: 600 V/cm, 100 µs, 4×LV: 80 V/cm, 100 ms, 1 Hz). Two weeks after vaccination, blood, spleens, and transfected tissues were collected from vaccinated mice. The expression of S, N, IL-12, and interferon γ (Ifn-γ) in the transfected tissue was determined by qRT-PCR, and serum IFN-γ by ELISA (R&D Systems). The induction of humoral immunity was evaluated with ELISAs for S- and N-specific IgG antibodies (Krishgen Biosystems) in blood serum, and cell-mediated immunity with a tetramer assay (Tetramer Shop) on isolated splenocytes.

The expression of S and N antigens was confirmed both in vitro and in vivo by qRT-PCR. The N antigen was also confirmed at the protein level, while S was undetectable with the available ELISAs, indicating issues with its correct expression. Consequently, vaccination with S did not result in a significant induction of anti-S antibodies. Nevertheless, a significant induction of S-specific cytotoxic T cells was observed. Both skin and muscle vaccination were well tolerated. However, muscle vaccination led to a significantly higher expression of antigens and a stronger induction of antigen-specific humoral and cell-mediated immune responses. Interestingly, the adjuvant and booster did not significantly enhance the immune responses.

Our study reaffirmed the feasibility of GET for DNA vaccination. It showed that muscle delivery outperforms skin delivery in terms of antigen expression and induction of humoral and cell-mediated immunity. The study also provided valuable insights into the role of presentation pathways for inducing cell-mediated immune responses and demonstrated that both immunological adjuvant and booster may be redundant after vaccination with GET. Overall, the results support the use of GET for vaccination against infectious diseases and solidify its role in therapeutic vaccinations for cancer treatment.

OR-167

Enhancing molecular cargo electrotransfer by modulating vesicular transport in cells

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We have demonstrated previously that treatment of cells with non-reducing sugars (NRSs), such as sucrose, trehalose, and raffinose, can significantly enhance the efficiency of electrotransfer (ET) for delivery of various molecular cargo (plasmid DNA, mRNA, and ribonucleoprotein) into cells. The enhancement has been observed in more than 20 cell lines and primary human cells. To achieve

the enhancement, the cells are pretreated with an NRS. After a certain period (6-24 hours), the cargo is electrotransferred into the treated cells. At 24-48 hours post ET, the gene delivery efficiency and cell viability are quantified. However, the same treatment led to minimal improvement in the ET for cargo delivery in mouse muscle. We hypothesized that the lack of improvement was due to short half-lives of NRSs in tissues because they are small, hydrophilic molecules that can be quickly cleared through vascular systems and diffusion into surrounding tissues. As a result, the treatment of cells was reduced. To test the hypothesize, we encapsulated sucrose in biomimetic lipid nanoparticles (sucrose-LNPs) for targeted delivery into muscle cells, and used it to treat the mouse muscle of the hind limb prior to the electrotransfer of plasmid DNA (pDNA) into the same tissue. At 1-7 days post ET, the gene delivery efficiency was quantified. Our data showed that the pretreatment with sucrose substantially increased and prolonged the transgene expression in mice. The amount of increase at 2 days post ET was equivalent to increasing the dose of pDNA by approximately 3000 folds. Mechanisms of the enhancement were associated with the formation of large vesicles elicited by the sucrose treatment. These vesicles included amphisome□like bodies (ALBs) and enlarged lysosomes. The ALB formation hindered vesicular transport of pDNA to lysosomes, while the lysosome enlargement was correlated to the reduction of its acidity, which in turn inactivated nucleases. The observation of large vesicle formation and alterations in vesicle trafficking could be explained by the data from analysis of transcriptomic profiles in sucrose treated cells. The changes in vesicular transport and inhibition of lysosomal function collectively decreased pDNA degradation, thereby increasing the efficiency of ET. The study concluded that the NRS could be used to improve electrotransfer of molecular cargo in vitro and in vivo.

OR-168

LiveGT Enhances Skeletal Muscle Reprogramming and Physiological Levels of Insulin Production

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Introduction: Gene electrotransfer is an established physical method for delivery to various tissue and organs in vivo including melanoma tumors, skin, cardiac muscle, and skeletal muscle. GET advantages include nonimmunogenic and nonintegrative gene delivery to targeted tissues. Disadvantages include low expression levels, short-lived/transient expression, and muscle stimulation. Skeletal muscle, localize in vivo electro gene therapy (liveGT) as a next generation GET, was utilized with a monopolar electrode and optimized pulse sequences to determine longevity of expression and feasibility of reaching and maintain physiological levels of blood circulating protein replacement therapy. Maintain expression at physiological serum levels for extended periods of time is an important step in moving liveGT toward clinical translation as a protein replacement alternative. Gene delivery of plasmid DNA encoding human insulin was used to correlate pulsing parameters to serum levels in the physiological range, as cannot be done with reporters.

Methods: Various LiveGT pulsing parameters were administered directly to the skeletal muscle of Sprague Dawley rats, with plasmid DNA encoding luciferase or human insulin and glucokinase co-delivery. Expression levels were monitored with bioluminescence imaging as well as directly from serum samples via ELISA's over nine months post initial liveGT. Additionally, to blood glucose measurements were also monitored. Two-way ANOVA and Tukey multiple comparison tests were used to determine statistically significant differences, with $p<0.05$ considered significant. Control groups received vehicle, or no delivery at all.

Results: Reporter expression was observed to be maintained for over six months with significantly higher (over 100-fold) than respective controls. Exogenous human insulin expression and

blood glucose modulation were maintained over three months, with significant reduction in mean circulating glucose compared untreated animals.
Conclusions: Our preliminary findings implicate liveGT skeletal muscle reprogramming as a viable target for secretory protein therapies. Physiological levels of insulin as a surrogate for other blood circulating proteins can be achieved and maintained in serum for several months without risks of integration and immunogenicity.

OR-169

Magnetoporation: A novel method of molecular delivery for cell and gene therapies

Zachary Rapp

Sigma Genetics, United States

In the field of gene-modified cell therapies, successful intracellular gene delivery remains a significant challenge. Sigma Genetics offers an innovative solution to this critical issue using proprietary magnetoporation technology. Magnetoporation uses external, pulsed magnetic fields to efficiently introduce foreign molecules and genes into cells. Magnetoporation induces an electric current across each individual cell leading to transient poration of the cell membrane. Cells are placed within a cuvette inside a fixed and cooled coil. Magnetic fields generated by pulsed power transmit through the coil which amplifies the magnetic field and also acts as a reservoir when saturated. The core's passage into and out of saturation causes the magnetic permeability of the sample volume to change rapidly, which in turn causes a higher rate of change in the local magnetic field. The electric current induces transient poration of the cell membranes and entry of extracellular material. The higher the rate of change of the magnetic field, the higher the transfer efficiency. Importantly, the electromagnetic circuitry dynamics of the magnetoporation process are uniquely suited to scalability; as sample volume increases, the uniformity of the magnetic field and the efficiency by which it interacts with the sample both increase. The result is an increase in transfer efficiency with higher processing volumes without additional stress on cells, unlike electroporation and sonoporation, where higher volume processing increase cellular stress. Further, magnetoporation eliminates electrical arcing within the sample and the need for special conductive buffers. The process can be fine-tuned to the magnetic field requirements of a particular cell, minimizing manipulation and physical stressors to the cell, and has shown high efficiency of intracellular transfer into a variety of immortalized and primary cell types including CD4 T cells and red blood cells. It has also proven effective for various sizes and types of payloads, including DNA, RNA, and carbohydrates. ' The hardware is composed of commercial off-the-shelf, custom-order, and internally manufactured components in the categories of hardware, electronic components, and electronic circuitry. The software and user interface are exclusively proprietary. The company is presently working towards a cGMP-compliant flow-through instrument, while also exploring other potential applications for the technology including in the rapidly advancing gene-modified cell therapy space. Our technology is the first to apply pulsed power at the cellular level and improve the manufacturing of clinical cell-based therapeutics; our first commercial application.

OR-237

Development of in vivo-launched synthetic DNA-encoded antibodies employing CELLECTRA® electroporation technology

Trevor Smith[1], Paul Fisher[1], Ami Patel[2], Elizabeth Parzych[2], Kevin Hollevoet[3], David B. Weiner[2], Laurent Humeau[1]

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Monoclonal antibodies (mAb) have proved a simple passive immunization strategy to provide protection against infectious disease. However, access to potentially life-saving mAb biologics faces multiple challenges for broader population coverage including high doses (in milligrams/kilogram), shelf-stability, temperature stability and distribution barriers for low-/middle-income countries and resource-limited settings. Therefore, additional strategies that can further facilitate mAb uptake and global availability would be valuable for

infection control. DNA-encoded monoclonal antibodies (dMAb™) offer a possible alternative technology to traditional recombinant mAb. Synthetic DNA constructs are delivered to muscle tissues with in vivo electroporation to permit local tissue expression of the antibody transgene and transient production and secretion of mAb into circulation. dMAbs have the potential to be a transformative technology, allowing sustained trough levels of bioavailable mAb, flexibility for administration of multiple constructs, rapid production at dramatically lower costs, and long-term drug product stability. Through a combination of in vivo electroporation and drug formulation circulating levels of mAbs providing disease protection can be achieved by this technology platform. Here we discuss the nonclinical path for development of DNA-encoded monoclonal antibodies. Through case studies we investigate the delivery conditions, modeling and understanding the pharmacokinetics of in vivo mAb expression, and identify the requirements for scaling up the technology into clinically relevant models. This process has resulted in the successful translation of multiple dMAb candidates as medical countermeasures into clinical testing.

P11 - Electroporation modeling and mechanisms

Wednesday late afternoon Track A

Sep 18, 16:50 - 17:50

OR-170

AC electrodeformation studies on Compound Giant Unilamellar Vesicle as a model of eukaryotic cell

Rupesh Kumar, Rajarshi Chakrabarti, Rochish Thaokar

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Insights gained from studies on the electrohydrodynamics (EHD) of Single Giant Unilamellar Vesicles (sGUVs) have significantly contributed to our understanding of how biological cells react to electric fields, leading to the advancement in the understanding of cell dielectrophoresis and cell electroporation. The deformation of single Giant Unilamellar Vesicles (sGUV) under alternating current (AC) electric fields has been extensively investigated through a combination of experimental and theoretical approaches in literature. Both analytical and numerical models have been established to understand the membrane's electro-mechanical properties, including parameters such as bending rigidity, tension, and capacitance. These studies have provided an understanding of the electromechanical response of sGUVs, as well as anucleate cells such as RBC , to electric fields.

However, eukaryotic cells have a nucleus whereby Compound Giant Unilamellar Vesicles (cGUVs) emerge as an appropriate biomimetic model of biological cells. cGUVs have a vesicle-in-vesicles in structure, where outer and inner vesicles represent a biomimic, eukaryotic cell and nuclear membrane, respectively. In this work, a method for cGUV synthesis is improved for forming well formed cGUVs, allowing for modification of the electrical conductivities in their inner, annular, and outer regions. A thorough experimental study on the EHD of cGUVs demonstrates promising experimental findings with an agreement with theoretical work. In response to weak externally applied AC fields, the observed deformations of cGUVs—such as spherical, prolate, or oblate spheroidal—depend on the membrane's electromechanical properties and the Maxwell stress, which vary with the relative timescales associated with the frequency of the applied AC field, membrane charging time, and Maxwell-Wagner relaxation time. This work firmly establishes cGUVs as suitable biomimetic models for conducting EHD studies on eukaryotic cells.

OR-171

Suitability (and not) of Giant Unilamellar Vesicles in electroporation studies for biological applications

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Giant Unilamellar vesicles have emerged as very promising systems to investigate biological

processes, including the mechanism of electroporation. The choice of GUVs is attractive because of simplicity and control of the biomembrane composition, absence and choice of internal objects, and ability to manipulate electro mechanical properties of the membrane as well as electrical conductivities of the inner and outer solutions. In this work, I will discuss the reliability and limitations of Giant Unilamellar vesicles as biomemetic electroporating systems, and highlight the similarities and differences between GUV electroporation and cell electroporation.

Unlike biological cells, GUVs show huge electrodeformation over millisecond time scales when subjected to electroporating DC fields, We used a high-speed imaging camera to characterize the electrodeformation of GUVs subjected to electroporating pulsed DC fields, measuring their aspect ratio and shape deformations. The results are presented as a function of amplitude of electric field and conductivity ratio (β) of the enclosed medium to the suspending medium of vesicles.

In our first study, we examined the responses of GUVs synthesized using the gel-assisted method to a dc pulsed electric field. For the low salt case, where the ion concentration in the enclosed medium (Cen) was less than or equal to 0.3 mM, and for the high salt case, where Cen was greater than or equal to 25 mM, we observed that the shape deformation and degree of deformation of the vesicles remained unchanged for both similar electrical conductivities, β=1 and greater inner conductivity β>1, even with an increase in Cen. However, for greater outer conductivity β<1 and Cen=25 mM, we observed a transition from oblate to prolate shape in the GUVs due to a higher differential ionic concentration across the bilayer membrane. Interestingly, the relaxations of the vesicles were not affected by Cen for both β=1 and β<1. Conversely, in the case of greater outer conductivity β>1 and Cen=100 mM, a higher edge tension resulted in inhibited pore growth and faster relaxation of the vesicles.

In our second work, we have studied the effect of waveform on the deformation and relaxations of GUVs where vesicles were subjected to a high-intensity, single cycle of a sinusoidal pulsed electric field (SSPEF) and a square wave pulsed electric field (SWPEF) of same (rms) amplitude electric field. The occurrence of cylindrical deformations in both SSPEF and SWPEF indicates the dependence of deformation on TMP. In both SSPEF and SWPEF, the vesicles deformed into prolate cylinders as a result of Maxwell stress for both similar electrical conductivity β = 1 and greater inner conductivity β > 1, whereas they were compressed into oblate cylinders for greater outer conductivity β < 1. Vesicles subjected to a SSPEF relaxed following the pore closure mechanism for β= 1 and β < 1.

OR-172

Correlation between numerical simulations and clinical outcomes of irreversible electroporation for hepatocellular carcinoma

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Objectives: To assess the clinical relevance of retrospective numerical simulations of irreversible electroporation (IRE) for hepatocellular carcinoma (HCC).

Material and Methods: IRE procedures conducted at our center in 2022 were used for retrospective numerical modeling and treatment evaluation studies. Per-procedural imaging (Cone Beam CT before and after electrodes insertion) was used to create a 3D geometric model of the tumor and its environment using software developed by our team. The spatial coordinates of the electrodes, as well as treatment parameters from the Nanoknife® IRE generator, were used to retrospectively simulate the extent of the electric field (EF). The overall EF at the maximum voltage administered between each pair of electrodes was simulated using a static linear model by the finite element method. The rates of tumor coverage by 3D EF mappings were compared to clinical and imaging follow-up of the patient in terms of primary efficacy (1 month MRI) and

local recurrence (after one month).
Results: Among the 40 IRE procedures, 23 were included as they involved the treatment of one (15 procedures) or two (8 procedures) nodular HCCs <5cm with all parameters available for numerical treatment simulations. The 31 treated HCCs had a mean diameter of 21mm (range: 7mm to 45mm) and 3 to 6 electrodes were employed. The clinical treatment protocol consisted of applying at least 100 pulses of 90µsec between each electrode pair. Numerical simulations revealed a median percentage of tumor segmentation coverage by simulated EF of 100% at 300V/cm, 99,1% at 400V/cm, 94,1% at 500V/cm and 80,9% at 600V/cm.
At 1 month, 29/31 tumors (93,5%) exhibited complete ablation on MRI. Eight out of these 29 initially ablated tumors recurred during follow-up (median delay: 7 months). Overall, 21/31 tumors (68%) remained inactivated after a median follow-up of 17 months. The median percentages of tumor coverage by different EF isolines were significantly lower for the 10 cases of local IRE failure than for the 21 cases of IRE success: 95,1% vs. 100% at 300V/cm ($p<0.001$); 83% vs. 100% at 400V/cm ($p<0.0001$); 72,3% vs. 98,9% at 500V/cm ($p<0.001$) and 61,4% vs. 89,9% at 600V/cm ($p<0.01$). A tumor coverage <95% by the 400V/cm EF isoline predicted a local IRE failure with a positive predictive value >80%.
Conclusion: The results of numerical simulations of IRE conducted on a retrospective cohort of patients are correlated with the local effectiveness of the treatment. This study suggests that, using a static linear model of IRE, the isoline of the calculated EF providing the best prediction of the clinical outcome for the treatment of HCC is around 400V/cm.

OR-173

Simulation study on waveform characteristics of measuring bio-impedance using pulse frequency response method

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Chongqing University, China

Due to the difficulty in assessing the efficacy of pulse electric field tumor ablation technology immediately during the procedure, some scholars have shown that the impedance characteristics of biological tissues can reflect the ablation effect of tumors. Impedance measurement technology of biological tissues is a key technology to assess ablation efficacy. Pulse waveform has rich frequency characteristics, and different pulse waveforms have different amplitude-frequency characteristics. In this paper, pulse frequency response method is used to measure the impedance characteristics of biological tissues. Firstly, the principle of measuring biological impedance from pulse voltage and current waveforms is theoretically analyzed. Secondly, simulation methods are used to set different repetition frequencies, pulse widths, and pulse waveform types to explore the measurement results of biological impedance under different combinations of pulse characteristic parameters. The measurement pulse waveforms in this article include square-wave pulse current, square-wave pulse voltage, bipolar pulse waveform, synergistic pulse voltage waveform, and synergistic pulse voltage and current waveform. By comparing the above results, the pulse current square wave (at burst 10Hz) measurement of biological impedance has the smallest error, with a capacitance measurement error of less than 3%. This simulation lays the foundation for measuring biological impedance using the pulse frequency response method.

S15 - Advanced imaging techniques for visualizing the mechanisms of pulsed electric field interactions

Wednesday late afternoon
Track B
Sep 18, 16:50 - 17:50

OR-174

PEFfect Illumination: Observing Protein Oxidation Effects of Pulsed Electric Field Through Monitoring (Bio)Chemiluminescence

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Pulsed electric field (PEF) technology has become adaptable and useful across a range of research and industrial fields. While its primary effects have traditionally focused on lipid membranes, growing interest surrounds its impact on proteins, essential biomolecules governing cellular processes.

In this study, we utilized a novel and non-destructive endogenous chemiluminescence-based sensing platform to investigate the effects of PEF-generated ROS on a model protein, bovine serum albumin (BSA). Our technique exhibited superior sensitivity compared to traditional assays, enabling the detection of oxidative effects induced by PEF. Additionally, we assessed the influence of prooxidants (such as hydrogen peroxide) and antioxidants (catalase and superoxide dismutase) on PEF-induced protein oxidation, elucidating their roles in modulating oxidative processes.

We proposed a comprehensive reaction scheme describing the pathways through which PEF-generated ROS induce protein oxidation and the modulation by pro- and antioxidants. Our findings provide valuable insights into the mechanisms underlying PEF-induced protein oxidation and highlight the importance of considering pro- and antioxidants in PEF-treated proteins. Understanding these processes holds significant implications for diverse applications, including biomedicine, food processing, and biotechnology.

Acknowledgments: The authors thank the Czech Science Foundation project no. 20-06873X for the support.

OR-175

Electric field effects on human skeletal muscle-derived mesenchymal stem/stromal cells investigated by scanning electrochemical microscopy

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The human skeletal muscle-derived mesenchymal stem/stromal cells (SM-MSCs) possess myogenic differentiation potential and participate in muscle regeneration. However, the question of how to improve human skeletal muscle regeneration remains actual. In this study, the total SM-MSCs population was separated into subpopulations according to the neural cell adhesion molecule (NCAM, CD56), stimulated with an alternating electric field (AC) using scanning electrochemical microscopy (AC-SECM) and the intracellular redox changes, and myogenic differentiation markers were evaluated. The myogenic differentiation of the total SM-MSCs population before and after AC stimulation was evaluated immunohistochemically by the levels of desmin and myogenin. The effect of AC stimulation on the redox capacity of CD56(+) and CD56(-) cell subpopulations, as well as on the level of myogenin on the indium tin oxide (ITO) surface, were also investigated. Results: The total SM-MSCs population grown on a glass coverslip weakly responded to AC stimulus, i.e. the level of desmin was slightly increased by the 3rd day of differentiation, while in the not stimulated cells, it increased only by the 7th day. The level of myogenin did not change after AC stimulation. However, the CD56(+) and CD56(-) subpopulations had different redox activities and myogenic differentiation potential: the CD56(+) cells had stronger natural diffusion and were more redox active compared to the CD56(-) cells; an alternating electric field more actively stimulated the redox activity of CD56(+) cells than in CD56(-) cells; at control level, the CD56(+) cells had more myogenic differentiation-regulating transcription factor myogenin, which AC more intensively stimulated than in CD56(-) cells. Data

show that the total population of human SM-MSCs is heterogeneous, with different regenerating potential cells that do not equally respond to extracellular stimuli. The SECM can be used in both ways: (i) for extracellular stimulation and (ii) for the investigation of intracellular redox changes of the human SM-MSCs or their subpopulations, allowing a deeper understanding of the mechanisms mediating skeletal tissue regeneration both in vitro and in vivo.

OR-176

Effects of Nanosecond Pulsed Electric Field on Cancerous and Normal Cells — Fluorescence Microscopy and Autofluorescence Lifetime Imaging

Nobuhiro Ohta

National Yang Ming Chiao Tung University, Taiwan

Introduction: Electric field effects on dynamics and function of biological systems may depend on the cell membrane capacitance and on the pulse duration of the applied field, which enables selective applications of pulsed electric field in specific organelles and specific living cells. If pulses shorter than the charging time of the outer membrane are applied to living systems, the pulsed electric field may induce changes in subcellular organelles, signal proteins, and biochemical processes, without affecting the outer plasma membrane. Considerable advances have been made regarding the understanding of the pulsed electric field effects on the cellular functions in different cell types. Nevertheless, it is still elusive how the effects of nsPEF on human cancerous cells differ from that on the normal cells of the same origins. This is very important from the clinical point of view because the damage of the healthy cells limits the effectiveness of the cancer treatments. In the present study, effects of nanosecond pulsed electric field (nsPEF) on dynamics and function of live cells have been examined by using fluorescence microscopy including autofluorescence lifetime imaging (AFLIM), with a special attention to the difference of the electric field effects between cancer and normal cells.

Methods: An electrode microchamber has been constructed for applying nsPEF to live cells. Measurements of intensity and lifetime images of fluorescence not only of exogenous fluorophores stained in cells but also endogenous fluorophores such as nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide (FAD) in cultured cells were carried out before, during and after application of nsPEF by using inverted microscope, time-correlated single photon counting system, and steady-state excitation light and femtosecond pulse laser system.

Results and Discussion: The effects of applied electric field having a pulse-width of 50 ns and field strength 20 kV/cm (nsPEF(50)) have been examined for lung cancerous cells H661 and A549 and lung normal cells MCR-5 attached on the glass substrate of the microelectrodes with the following measurements: 1) change in cell morphology; 2) change in cell viability; 3) phosphatidylserine (PS) externalization; 4) AFLIM of NADH and FAD; 5) generation of superoxide anion; 6) generation of singlet oxygen; 7) change in mitochondrial membrane potential; 8) caspase activation: 9) change in calcium ion concentration. The obtained results show that caspase-dependent apoptosis is induced by nsPEF in H661 and A549, whereas such field effect was less or not induced in MCR-5, indicating that cancerous and normal cells show very different electric field effects from each other.

S11 - In vivo delivery of genetic medicine through gene electrotransfer

Wednesday late afternoon
Track C
Sep 18, 16:50 - 18:05

OR-177

Immunomodulatory effects of plasmid DNA following gene electrotransfer in colon cancer utilizing different electric pulse protocols

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Gene electrotransfer (GET) has recently emerged as a promising non-viral approach for delivering plasmid DNA in gene therapy strategies for various pathologies, including cancer, autoimmune, or inflammatory diseases. Exposure of cells to electric pulses in reversible electroporation (RE) approaches induces a transient increase in cell membrane permeability, leading to diverse biological responses and even cell death. Despite multiple mechanisms influencing cell biology impacting the outcome of GET treatments, the molecular effects of in vivo DNA delivery using different electric pulse parameters remain poorly characterized. Therefore, in this study, we characterized, for the first time in vivo, two GET protocols for intratumoral delivery of plasmid DNA, via transcriptomic profiling using RNA sequencing of treated murine colorectal tumors. An electroporation (EP) pulsing protocol based on Short and High Voltage (SH-V) electric pulses and an adapted High Voltage – Low Voltage (HV-LV) pulse protocol were investigated, and the effects compared at three and seven days post-therapy. Although there were no significant effects on mice body weight and tumor growth delay between treatment groups, the SH-V pulse protocol showed better overall survival, with two mice exhibiting a partial response and a steady disease response alongside a prolonged tumor growth delay. Immunofluorescence analysis revealed increased CD4+ T cells and even higher CD8+ T cells recruitment on day seven after both treatments compared to day three, with statistical significance for CD8+ T cells in the SH-V pulse protocol. Notably, the most significant changes were observed in the number of macrophages, which significantly decreased on day three after the SH-V pulse protocol and was restored on day seven, while GET using the HV-LV pulse protocol resulted in the opposite trend. Consistent with these data, RNA sequencing analysis showed that, if apoptosis is the predominant mode of cell death due to the application of SH-V pulses, the altered gene profile following the HV-LV pulse protocol is associated with the activation of cell death signaling represented by immunogenic necrotic pathways, as well as the innate and adaptive immune response. These results suggest that specific pulse parameters can induce distinct immunomodulatory profiles in the tumor microenvironment, highlighting the importance of pulse parameter selection in EP-based treatments. Therefore, this analysis could pave the way to identify information useful for selecting the best EP method or developing new EP-based techniques for an immunization approach.

OR-178

Calcium electroporation and interleukin-12 gene electrotransfer

Barbara Lisec, Boštjan Markelc, Katja Ursic Valentinuzzi, Gregor Serša, *Maja Čemažar*
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Calcium electroporation (CaEP) utilizes electroporation to facilitate cellular uptake of elevated levels of Ca2+, leading to cell death induction. While the efficacy of CaEP has been assessed in clinical trials, additional preclinical investigations are necessary to fully understand its effectiveness and underlying mechanisms. This study assesses the effectiveness of CaEP in two tumor models and compares it with electrochemotherapy (ECT). In addition, the CaEP was combined with gene electrotransfer (GET) of plasmid encoding interleukin-12 (IL-12), aiming to elucidate potential synergistic effects. Our hypothesis was that IL-12 enhances the antitumor efficacy of local ablative therapies such as CaEP and ECT.

The impact of CaEP was evaluated in vitro and in vivo using murine melanoma B16-F10 and murine mammary carcinoma 4T1 models, in comparison to ECT with bleomycin. Various treatment protocols involving CaEP with escalating calcium concentrations alone or combined with IL-12 GET were examined. The tumor microenvironment was extensively analyzed through immunofluorescence staining of immune cells, blood vessels, and proliferating cells.

In vitro, both CaEP and ECT with bleomycin demonstrated dose-dependent decrease in cell viability, with no differences in sensitivity between

the two cell lines. In vivo, a dose-dependent response was also observed, with greater efficacy noted in 4T1 tumors compared to B16-F10 tumors. CaEP with 250 mM Ca resulted in significant growth delay in 4T1 tumors, which was comparable to ECT with bleomycin. Additionally, adjunct peritumoral IL-12 GET following CaEP prolonged survival in B16-F10-bearing mice, but not in 4T1-bearing mice. Furthermore, CaEP with peritumoral IL-12 GET altered tumor immune cell populations, cell proliferation and vasculature.

In conclusion, mice bearing 4T1 tumors exhibited superior response to CaEP compared to those with B16-F10 tumors, suggesting potential immune system involvement, since the difference in sensitivity was not confirmed in vitro. The involvement of immune response in antitumor effectiveness was further supported by enhanced antitumor efficacy observed with CaEP or ECT combined with IL-12 GET. However, the augmentation of CaEP efficacy varied depending on tumor type, with greater enhancement seen in poorly immunogenic B16-F10 tumors compared to moderately immunogenic 4T1 tumors.

OR-179

Unraveling a multifactorial host immune response to intramuscular electrotransfer of dna-encoded antibody therapy

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DNA-based antibody gene electrotransfer aims to administer antibody-encoding nucleotides, rather than the antibody protein, utilizing a non-viral plasmid DNA (pDNA) vector. This strategy aims to achieve sustained in vivo antibody production, addressing challenges inherent to conventional antibody production and delivery. Previously, we demonstrated preclinical proof of concept for diverse DNA-encoded monoclonal antibodies (mAbs) and nanobodies delivered in muscle or tumor using electroporation (EP).

In this study, we aim to delineate the host immune response in mouse muscle following (IM) intramuscular pDNA EP and identify factors directly linked to expressed mAb PK. To achieve this, knockout (KO) models and immune phenotyping are employed, utilizing a validated pDNA encoding a murine 4D5 anti-HER2 mAb. Earlier experiments demonstrated that mAb PK in athymic nude and RAG1 KO mice did not significantly differ from wild-type (WT) mice, suggesting that T and B cells, including antigen-presenting pathways, do not drive the decline in mAb expression. To assess the impact of the innate immune response, we evaluated 4D5 PK in IFNAR1 KO mice, which lack type-I interferon receptor function. After IM p4D5 EP, plasma mAb levels showed no significant difference between IFNAR1 KO and WT mice throughout the 12-week follow-up. This finding diminishes the likelihood that DNA sensing, including cGAS/STING, affects mAb PK.

Concurrently, we ran flow cytometry panels on WT mice muscle, harvested one week post-IM EP. Compared to control muscle (including untreated, vehicle-injected, and EP only), infiltrated T cells showed more of the effector phenotype and dendritic cell (DC) muscle infiltration was increased. NK cell-mediated cytotoxicity was considered, but muscle NK cell infiltration was limited and did not increase after pDNA EP. This suggests that cytotoxic T-cell responses, facilitated by DC-based antigen presentation, may target mAb-producing cells. However, this does not appear to impact mAb kinetics based on earlier observations in RAG1 KO mouse model.

In addition, while assessing the IM macrophage population upon treatment, we found that the percentage of pro-inflammatory macrophages (M1) increased, while the anti-inflammatory macrophages (M2) population remained unchanged. This data, supported by an increased DC infiltration, implies that phagocytic antigen-presenting cells play a role in the decreased mAb production. Potentially, cells transfected with mAb-encoding pDNA increase their “eat-me signals” due to cell stress (EP, protein overproduction, ER stress, etc.), attracting phagocytic cells. Ongoing work investigates whether modulating phagocytosis impacts mAb kinetics.

In conclusion, this work started to unravel a multifactorial host immune response triggered by IM electrotransfer of a DNA-encoded mAb. As we elaborate on these observations, factors demonstrably associated with mAb kinetics will be of particular interest, as modulating these responses could allow for a more robust and prolonged in vivo mAb expression.

OR-180

Enhancing the Therapeutic Benefits of Proteins with Short Half-Lives: Delivery of G-CSF and GLP-1 with DNA-Based MYO Technology

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Numerous recombinant proteins have received clinical approval, drastically transforming the standard of care landscape. However, the therapeutic efficacy of many small biologics is hindered by their relatively short half-life, often due to pharmacokinetic properties, receptor binding kinetics, and disease characteristics that necessitates frequent dosing to achieve and maintain therapeutic effectiveness, thereby causing significant patient discomfort and potentiating nonadherence to the therapy. Additionally, significant challenges like high manufacturing costs and cold chain requirements continue to limit broader access to these life-changing therapeutics, a problem amplified in low- and middle-income countries.

MYO Technology, a platform for the delivery of plasmid DNA (pDNA)-based therapeutics via intramuscular electroporation, offers several advantages over standard delivery of therapeutic proteins. A single administration of pDNA medicine using MYO Technology takes just a few minutes, and the serum level of the expressed therapeutic protein is maintained for many months to years, without the need for redosing, thus alleviating the dependence on patient compliance for durable therapeutic effect. Furthermore, pDNA manufacturing is a simpler and less specialized process as compared to most protein manufacturing processes, and pDNA is very stable and lacks most cold chain requirements.

Here, we report MYO Technology delivery of two molecules from a class of biologics that have a short half-life (about 4 hours): G-CSF (granulocyte-colony stimulating factor) and GLP-1 (glucagon-like peptide-1). We demonstrate the long-term therapeutic effect of G-CSF, used to treat severe chronic neutropenia, when delivered via MYO Technology in both mice and rabbits. Mice were found to maintain durable protein expression and elevated neutrophil counts for over a year. Similarly, proof-of-concept studies in mice demonstrate that expression of MYO Technology-delivered GLP-1/GIP receptor agonists remains stable for over six months and is efficacious in stimulating weight loss in mouse models of diet-induced obesity.

Although initial animal studies have demonstrated the potential of DNA-based platforms for delivering therapeutic proteins, a notable challenge persists in enhancing protein yield to within a therapeutic range in larger animals. The proteins produced and released systemically encounter a dilutional effect influenced by the size and blood volume of each species. To address this, extensive optimizations of the MYO Technology platform have been made that address the disparity in therapeutic protein concentrations achieved between smaller and larger animal models.

Thus, by enabling durable, scalable expression of small therapeutic proteins, MYO Technology has the potential to expand the therapeutic advantages of proteins with short half-lives.

S19 - Pulsed electric fields in meat and fish and their by-products processing

Wednesday late afternoon
Track D
Sep 18, 16:50 - 17:50

OR-182

New advancement on meat processing using Pulsed electric field technology

Indrawati Oey

University of Otago, New Zealand

Pulsed Electric Fields (PEF) is an innovative nonthermal technique where high-voltage electrical energy is briefly applied to food in pulses lasting microseconds to milliseconds. This energy induces various physicochemical changes in meat, particularly enhancing tenderness while preserving attributes such as color, flavor, water retention, volatile profile, and mineral content, and without promoting lipid oxidation. Furthermore, PEF pre-treatment enhances the efficiency, cost-effectiveness, and sustainability of meat processing methods like accelerated ageing, salting, brining, drying, and sous vide (SV) cooking. Particularly in sous vide processing, PEF pre-treatment has been found to reduce electrochemical variability in meat, significantly shorten SV cooking times, and consistently improve tenderness, even in tougher cuts such as brisket and short ribs with bone. Additionally, the subsequent PEF followed by sous vide processed short ribs have shown increased in vitro protein digestibility without compromising the microbial safety of meat during refrigerated storage. In this presentation, a recently built new continuous PEF chamber design will be introduced.

OR-183

Inactivation by Pulsed Electric Fields of Anisakis in naturally infected hake meat.

Vanesa Abad, Javier Raso, Juan Manuel Martínez, Guillermo J. Cebrian, Ignacio Álvarez
Universidad de Zaragoza, Spain

Introduction: Anisakis is a zoonotic parasite found in the stomach of marine mammals. Humans become accidental hosts when they consume raw or undercooked fish, or cephalopods, leading them to suffer from intestinal syndromes and allergic reactions. One of the most parasitized species in Europe is hake. In Europe, the officially prescribed methods for inactivation of Anisakis are heat treatment or freezing, both of which can affect fish quality.

Several studies have demonstrated the effectiveness of Pulsed Electric Fields (PEF) for the inactivation of Anisakis; however, none of them has featured naturally infected samples. Therefore, the objective of this study focuses on Anisakis spp inactivation by PEF in naturally infected hake belly fillets and the evaluation of the quality of fish samples during their shelf life after PEF treatments.

Method: Application of PEF technology (evaluation of field strength – 3 to 5 kV/cm-, pulse width - 10 to 30 µs -, and specific energy - 10 to 30 kJ/kg -) for inactivation of Anisakis spp. from hake (Merluccius merluccius) in naturally parasitized hake belly. The effect of MAP storage on the survivability of Anisakis spp was also evaluated after applying the PEF treatment.

The fish microbiota of control and PEF samples was evaluated during shelf life in MAP (50%CO2-50%N2). Quality tests (drip loss, moisture, water holding capacity, and cooking loss) were also carried out on control, PEF, and freeze/thawed hake pieces to determine the impact of the technology on quality.

Results: Inactivation of Anisakis spp in naturally parasitized hake bellies was highly dependent on PEF parameters, among which the two most important were field strength and specific energy. In addition, Anisakis survivability depended on the parasite's location, as it was more resistant to PEF when located inside the fish meat than when located on the most superficial part of the belly. Almost complete inactivation of the parasite in hake belly was achieved with a treatment of 5 kV/cm and 30 kJ/kg, applying pulses of 30 µs. The degree of inactivation increased over time when the samples were stored after PEF treatments in a modified atmosphere containing 50% CO2.

After PEF treatments, quality analyses during shelf life indicated that fish microbiota evolved similarly to untreated samples; however, the MAP limited the growth of the microbiota. In PEF-treated samples, quality parameters (drip loss, moisture, water holding capacity, and cooking loss) were closer to those of fresh hake and superior, in terms of quality, to the values obtained in frozen/thawed samples during their entire shelf life.

Conclusion: These results suggest that PEF could represent a promising alternative to freezing as a strategy for the elimination of Anisakis spp in fish without affecting fish quality, specifically in hake.

OR-184

Valorization of shrimp by-products: Extraction of high value-added compounds by pulsed electric field (PEF) and accelerated solvent extraction (ASE)

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Shrimp and prawns are one of the most important internationally traded seafood products. Crustacean by-products are important natural sources of high-value compounds. Recently, pulsed electric field (PEF) treatment has emerged as a promising method for the isolation and extraction of various components from seafood by-products such as calcium, chondroitin sulfate, collagen, chitosan and protein. Accelerated solvent extraction (ASE) is considered an environmentally friendly method for the extraction of bioactive and nutrient-rich compounds from plants and food matrices. The main objective of the present study was to apply PEF and ASE to recovery astaxanthin from shrimp by-products and evaluate the effects of these technologies used independently or in combination on the astaxanthin content and antioxidant activities of the extracts. In this study, fresh samples of red shrimp (Aristeus antennatus) and camarote shrimp (Melicertus kerathurus) were obtained from a local market in Valencia, Spain. PEF (3 kV/cm, 100 kJ/kg, 74 pulses) was used as a pretreatment before traditional solvent extraction and before the innovative extraction procedure ASE (50 °C, 15 min, 103.4 bar). The combined and independent effects of the emerging technologies PEF and ASE using different solvents (EtOH and DMSO) on the extraction of astaxanthin were evaluated for each shrimp species (M. keranthurus and A. antennatus). The astaxanthin content in extracts from shrimp by-products was analyzed using spectrophotometric and HPLC methods. The antioxidant capacity of the extracts was evaluated by Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. Solid side streams from astaxanthin extraction were used for the extraction of chitosan biopolymer. The results showed that PEF and ASE increased the astaxanthin content in the extracts for both shrimp species and solvent used, and the higher recovery was obtained using their combination. However, the increase in antioxidant capacity varied depending on the solvent type. Both technologies seem to be an effective tool to recover astaxanthin and antioxidant extracts from shrimp by-products. The techniques for recycling shrimp by-products are not yet well established for industrial use due to a lack of standardization. Further research is needed to confirm these promising results and to explore other valuable compounds in crustacean by-products. This research was funded by the National Recovery and Resilience Plan (NRRP), Mission 4 Com-ponent 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union - NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title ”ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods”.

S10 - Electrochemotherapy of cutaneous tumors

Wednesday late afternoon
Track E
Sep 18, 16:50 - 18:20

OR-185

Electrochemotherapy for Kaposi Sarcoma and Merckel Cell Carcinoma: findings of the InspECT Rare Tumours Working Group

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Introduction: The rare tumors working group of the International Network for sharing practices on electrochemotherapy (InspECT) group has been tasked with reviewing the registry and analysing the information on rare tumors collected by InsPECT centres. Kaposi Sarcoma and Merkel Cell Carcinoma were found in this group of rare tumours. ECT is an alternative treatment option for patients with cutaneous malignancies of skin and non-skin origin, not suitable for conventional treatments. In this study, we aimed to assess outcomes of the use of ECT as a treatment modality for Kaposi Sarcoma and Merckel Cell Carcinoma within the InspECT registry.
Materials and methods: Patients with superficial lesions of Kaposi Sarcoma and cutaneous Merkel cell carcinoma. Data from 15 European centres was included. Patients underwent at least one ECT session with bleomycin, performed following the European Standard Operating Procedures, between March 2011 and October 2021.
Results: The analysis included 61 Kaposi Sarcoma patients (mean age 73 years; median number of lesions per patient 3) and 18 Merkel cell carcinoma patients (mean age 74 years; median number of lesions per patient 1.5). Side effects were reported as mild and easily manageable (hyperpigmentation, 16%; ulceration, 5%; suppuration, 2%). The response to treatment per patient was 85% complete and 11% partial for Kaposi Sarcoma. In patients with Merkel cell carcinoma the response was 45% complete and 45% partial. In the multivariate model, time since diagnosis to ECT and small tumor size showed a significant association with a complete response. One-year local progression-free survival in the whole population was 91%, 2 years LPFS was 87%.
Conclusion: In the present study, ECT showed antitumor activity and a favorable safety profile in patients with cutaneous Kaposi Sarcoma and Meckel Cell Carcinoma, where conventional treatments were unsuitable or refused. Best results were obtained in small tumors (<3 cm) using hexagonal electrodes.

OR-186

Electrochemotherapy in the treatment of cutaneous melanoma metastases – the InspECT experience

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Melanoma is the most dangerous type of skin cancer. The treatment of melanoma skin metastases are challenging despite multimodal available systemic and local therapies. The melanoma working group of the International Network for sharing practices on ECT (InspECT) group investigates treatment outcomes after ECT using a common database with defined parameters.
Objectives: In our four studies the aim was to investigate the effectiveness of ECT in cutaneous metastases of melanoma, the effectiveness of ECT in combination with pembrolizumab and the optimal timing of ECT in combination with immunotherapies

as well as to evaluate health-related quality of life (HRQoL) changes in melanoma patients.

Methods: All analysis included prospective data from the InspECT register. Endpoints included response (RECIST v3.0), local progression-free survival (LPFS), systemic PFS, overall survival (OS) rates, toxicity (CTCAE v5.0), patient-reported HRQoL outcomes ([EQ-5D] and [EQ-VAS]). We also undertook a retrospective matched cohort analysis on the effectiveness of ECT in combination with pembrolizumab in stage IIIC–IV melanoma patients included in the InspECT and the Slovenian Cancer Registry, and compared patient outcomes after the following treatments: (a) pembrolizumab, (b) pembrolizumab and ECT, and (c) ECT alone. Response rates were checked in groups of patients where ECT was performed before, during or after immunotherapy.

Results: The response for MM was with a CR of 64% (600/932) and OR of 82% (768/ 932) and no serious adverse events were observed. The local objective response rate (ORR) was higher in the pembrolizumab-ECT group than in the pembrolizumab group (78% and 39%, $p < 0.001$) amongst stage IIIC–IV melanoma patients.The 1 year LPFS rates were 86% and 51% ($p < 0.001$), and the 1 year systemic PFS rates were 64% and 39%, respectively ($p = 0.034$). The 1 year overall survival (OS) rates were 88% and 64%, respectively ($p = 0.006$). The further investigation of the optimal timing of ECT in combination with immunotherapies showed the best tumor response (CR:80% OR:93%) in small lesions (<3 cm) when ECT was performed during concomitant immunotherapy.Following ECT, both EQ-5D and EQ-VAS scores remained within MID boundaries, particularly among complete responders. Combination with checkpoint inhibitors was associated with better QoL outcomes.

Conclusion: ECT is a highly effective local treatment for melanoma metastases in the skin, with no severe adverse effects and preserves patient QOL. The combined application of pembrolizumab and ECT was safe and more efficacious in preventing further growth of cutaneous metastases than pembrolizumab alone. Interestingly, we observed longer PFS and OS in the pembrolizumab-ECT group than in the pembrolizumab group. Our results suggest that in combined application the best tumor response can be achieved with ECT during concomitant immunotherapy.

OR-187

InspECT database and clinical results of electrochemotherapy

Giulia Bertino[1], Ales Groselj[2], Christian Kunte[3], Hadrian Schepler[4], Julie Gehl[5], Tobian Muir[6], James P. Clover[7], Pietro Quaglino[8], Erika Kis[9], Matteo Mascherini[14], Brian Bisase[10], Giancarlo Pecorari[8], Falk Bechara[12], Paolo Matteucci[20], Joy Odili[13], Francesco Russano[14], Giulia Colavitti[15], Rowan Pritchard-Jones[16], David Mowatt[21], Barbara Silvestri[17], Veronica Seccia[18], Gregor Serša[19]

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The International Network for Sharing Practices on ElectroChemoTherapy (InspECT) is a group of expert clinicians working with ECT formed in 2008 to meet and discuss issues related to the use of ECT. The aim is to bring faster and better treatment to patients by publishing high quality research and by networking for continuous training in the field. The major priority is a web-database, where treatment data from the centres can be collected.

The group is composed by 43 centres and has collected more than 2200 patients over the last 15 years of activity, publishing 20 papers in peer reviewed journals. The most treated hystological types are: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, and breast cancer.

In this lecture we focused on head & neck (HN) cutaneous squamous cell carcinoma (cSCC) and long-term basal cell carcinoma (BCC) data extracted from the database.

A total of 162 patients with HN cSCC and a mean age of 80 years were analyzed: side effects were mainly local and mild (hyperpigmentation, 11%; ulceration, 11%; suppuration, 4%). The response to treatment per patient was 62% complete and 21% partial. In the multivariate model, intravenous drug administration and small tumor size showed a significant association with a positive outcome. One-year local progression-free survival was significantly better in patients with primary tumors (80%, 95% C.I. 70%-90%) than in patients with locally advanced disease (49%, 95% C.I. 30%-68%). Interestingly, ECT showed antitumor activity and a favorable safety profile in patients with complex cSCC for whom there was no widely accepted standard of care.

Among long term follow-up patients in the InspECT registry, a relevant number of patients affected by HN BCC could be retrieved: 129 patients have a follow-up longer than 5 years with a median follow-up time of 7.2 yrs (range 5.0-11.6 yrs). Median age is 74 years (range 41-93 yrs). Most of them were treated for a BCC lesion on the nose (40%), in the auricular region (12%), at the forehead/temple (10%) or in the eye region (10%). Overall disease-free survival at 5 years was 94%, with some slight non-significant differences in primary vs recurrent lesions (95% vs 92%), non-ulcerated vs ulcerated lesions (97% vs 86%) and small (< 3 cm) vs large (> 3 cm) lesions (98% vs 90%).

In conclusion, ECT is a safe and effective treatment for cSCC and BCC with high percentages of complete response lasting over time. It is particularly indicated for lesions located in anatomical sites where surgery could cause aesthetical disfigurement or loss of function and in old people not suitable for standard treatments because of severe general comorbidities.

OR-188

Electrochemotherapy for the treatment of cutaneous metastases from breast cancer

Julie Gehl

Zealand University Hospital, Denmark

This special session presentation aims to discuss clinical indications, results and future directions of the treatment of breast cancer metastases using electrochemotherapy.

Breast Cancer is the most common cancer in women, with around 1 of 8 women receiving a breast cancer diagnosis during their lifetime.

Breast cancer is treated with respectively endocrine treatment with our without cycline kinase inhibitors (for estrogen positive (ER+) cancer only), HER2 inhibitors (for HER2positive disease only), chemotherapy, in some cases immunotherapy and in subset of patients targeted treatments. Surgery remains a very impactful treatment, but is not possible in advanced cancer. Radiotherapy is an important tool but can only be used regionally and with a upper dose limit.

In numbers, cutaneous metastasis in breast cancer patients is one of the most frequent patient groups with cutaneous metastases, due to the frequency of cutaneous metastases multiplied by the population at risk. Tumors may present as smaller lesions, often multiple small lesions, but may also by large confluent areas. Patients are often quite affected by cutaneous metastases, as this may lead to changes in body image, oozing, bleeding and pain.

Electrochemotherapy has a high response rate of 77%, with 62% complete response (Clover et al, EJC, 2020). No difference between breast cancer subtypes (ER+, HER2+, or negative) was observed (Di Prata et al, Cancers 2023). Responses are lower for larger lesions, yet even very large lesions do respond to treatment. Electrochemotherapy may be combined with other treatment modalities, e.g. a patient responding well to endocrine treatment in other disease site, but with progression in skin metastases, may be treated with elec-

trochemotherapy in addition to systemic treatment. Many breast cancer patients have experienced previous surgery and/or radiotherapy to the area affected by breast cancer metastases. A slightly lower response rate is observed after radiotherapy, but still high.

A novel treatment using calcium electroporation is now tested for breast cancer, also showing high response rates. Interestingly, preliminary data indicate that calcium electroporation and electrochemotherapy may be used interchangeably in case of resistance to treatment.

Many patients are referred when tumors are reached a considerable size, so one future goal is to work for earlier referral in order to use electrochemotherapy when the disease burden is more limited.

As electrochemotherapy is increasingly being used across Europe, access to treatment for patients is becoming easier.

OR-189

Differential expression analysis of cutaneous squamous cell carcinoma and basal cell carcinoma proteomic profiles sampled with electroporation-based molecular biopsy

Alexander Golberg[1], Edward Vitkin[1], Ariel Berl[2], Julia Wise[1]

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Clinical misdiagnosis between cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC) affects treatment plans and carries risks of potential for recurrence, metastases, morbidity and mortality. We report the development of a novel tissue sampling approach with molecular biopsy using electroporation. This method, coined e-biopsy, enables non-destructive non-thermal permeabilization of cells in the skin for efficient vacuum-assisted extraction of informative biomolecules for rapid diagnosis. We used e-biopsy for ex vivo proteome extraction from 3 locations per patient in 21 cSCC and 20 BCC pathologically validated human tissue samples. The total 123 extracted proteomes were profiled using LC/MS/MS. In total, we identified 7087 proteins observed with non-zero intensity in at least one dataset samples. The observed intra-patient Pearson correlation of 0.923 ± 0.053 for BCC patients and 0.901 ± 0.060 for SCC patients indicates high consistency of the e-biopsy sampling technique. The obtained mass spectra presented significantly different proteome profiles for cSCC and BCC with several hundreds of proteins significantly differentially expressed in each tumor in comparison to the other. Notably, our study showed that proteomes sampled with e-biopsy from cSCC and BCC lesions are different, and that 7 proteins were significantly overexpressed in BCC in comparison to cSCC even after the Bonferroni correction (FDR=7.1e-03). Our results provide evidence that the e-biopsy approach could potentially be used as a tool to support cutaneous tumors classification with rapid molecular profiling.

OR-190

Electrochemotherapy in the treatment of chronic suppurative benign skin conditions: The St George's Hospital experience

Joy Odili

St. George's University Hospital, United Kingdom

Introduction: Electrochemotherapy (ECT) with Bleomycin is a highly effective local treatment for cancer in the skin, regardless of histology, with no severe adverse effects. It has also been shown to be effective in treating benign skin conditions such as keloid scarring. Bleomycin in isolation is also used to treat benign conditions such as keloid scarring and vascular malformations and is known for its properties as an antibiotic. Acne keloids, folliculitis decalvans, and hidradenitis suppurativa belong to a group of chronic inflammatory skin conditions with limited treatments. Treatment goals include reducing inflammation, improving keloidal lesions, and limiting exacerbating factors. We wondered whether the addition of electroporation would potentiate the action of Bleomycin in chronic suppurative benign skin conditions where all traditional treatments including surgery had failed.

Methods: We identified 9 patients with the following benign conditions were treated: acne keloidalis, folliculitis decalvans, and hidradenitis suppurative

(6 scalp, 2 jaw/ chin, 1 buttock). All were male. All presented with suppuration and pain. ECT was delivered under General Anaesthetic. Bleomycin was administered both Intratumourally and intravenously (combined approach). A linear probe was used in all cases, using the Cliniporator (Igea). Results of the treatments were stored on a shared database (International Network for sharing practices on ECT, InspECT).

Results: all patients reported a significant improvement is suppuration, pain, and itch in the treated areas. Flattening and softening of the scarred areas was a secondary effect which continued to improve several months after the treatment. No serious adverse effects to the treatment were reported. Most of the patients required 2 treatments 6 months apart. None of the treated patients required oral antibiotics or steroid injections after ECT.

Conclusion: Electrochemotherapy is an established treatment for the management of cancers in the skin and deeper tissues. The exact mechanism for the effectiveness of ECT in treating chronic suppurative conditions such as acne keloids, folliculitis decalvans and hidradenitis is unclear. One of the factors may be the role of Bleomycin as a glycoprotein antibiotic. Enhanced extracellular matrix turnover, and the effect on vascularity at the treated areas are also factors which may limit inflammation, making this treatment effective for this group of patients. The preliminary results from St George's Hospital are promising and are presented here.

S24 - Emerging role of Electrochemotherapy in the treatment of GastroIntestinal cancer

Thursday morning Track A
Sep 19, 8:30 - 10:00

OR-191

The Interventional Oncology in the modern interdisciplinary scenario

György Kovács

Università Cattolica Sacro Cuore, Italy

Interventional Oncology is an umbrella term for the practical, minimally invasive treatment of certain types of cancer. The roadmap of Interventional Oncology was first published in 2018. The cooperation of interventional radiotherapy, chemotherapy, endoscopy, and radiology within a dedicated Interventional Oncology Center is the best choice for focal approaches.

By combining these focal expert treatments, we not only enhance local control but also have the potential to significantly reduce toxicity. A dedicated multidisciplinary tumor board further enhances the success of these treatments, offering a truly personalized approach. Moreover, the integration of interventional oncology and immune oncology heralds a new era in minimally invasive personalized treatments.

Recent large cohort clinical trials create the basis for future well-designed and objective multidisciplinary trials in personalized cancer patient care.

OR-192

The role of Interventional Radiology

Laura Crocetti

University of Pisa, Italy

No abstract was provided.

OR-193

The role of Interventional Endoscopy

Fabia Attili

Policlinico Gemelli, Italy

No abstract was provided.

OR-194

The role of Interventional and External Beam Radiotherapy

Bruno Fionda

Policlinico Gemelli, Italy

No abstract was provided.

OR-195

The role of Electrochemotherapy

Martina Ferioli

University of Bologna, Italy

No abstract was provided.

OR-196

The synergistic effect of Electrochemotherapy in the modern Oncology scenario

Attila Kovacs

Klinik für Diagnostische und Interventionelle Radiologie und Neuroradiologie, Germany

No abstract was provided.

P12 - Biomass transformation and biocompounds

Thursday morning Track B
Sep 19, 8:30 - 10:00

OR-197

Non-lethal extraction of phytochemicals and growth promotion of Iris domestica (L.) DC roots enabled by electroporation

Kajetan Grzelka, Joanna Jaśpińska, Adam Matkowski, Sylwester Ślusarczyk

Wroclaw Medical University, Poland

Iris domestica is a perennial herb from Iridaceae family. It has been used for centuries in Traditional Chinese Medicine as an expectorant, antipyretic and anti-inflammatory agent, however active compounds isolated from its rhizome were found to possess antimutagenic, antioxidant and antidiabetic properties. These substances are classified as polyphenols, and among them isoflavones such as tectoridin, tectorigenin, iridin and irigenin are the most abundant and also show promise in developing new phytoestrogenic drugs. Our aim was to extract these compounds without killing the plants, which is an innovative and much more eco-friendly approach when compared to methanolic extraction of dried material. In our previous study we found that applying PEF to plant roots can promote plant growth due to induced abiotic stress and modulate its phytochemical profile. This observation paired with low mortality rate of plants suggested that after providing them with optimal growth conditions it is highly probable to increase the yield of compounds of interest by applying PEF several times throughout the plants' life.

After 2 years of empirically adjusting experimental conditions and refining our methodology, we can conclude that PEF-assisted extraction of phytochemicals from living roots was a success. Plant survival rate was 92,4% and the acquired extracts were largely synonymous with control quality-wise. Six-month-old plants cultivated in aeroponic systems were separated into control (sham treatment) and study groups, paired with a specific Natural Eutectic Solvent (NES): choline chloride : xylose (1:2) + 30% water, choline chloride : glucose (1:2) + 30% water, choline chloride : ethylene glycol (1:4) or tap water. Each specimen was put into a cuvette filled with designated solvent for 5 min and afterwards subjected to PEF of the following parameters: E = 0.3 kV/cm, t = 50 μs, f = 1 Hz, N = 33. Such treatment was then repeated once (Ntotal = 66) or twice (Ntotal = 99) in 5 min intervals. Solvents were then collected, purified and analyzed using HPLC-MS technique. Conductivity, temperature and mass of the media were measured before and after treatment. The current and pulse parameters were recorded on an oscilloscope. Based on these data, specific energy input (Wspec) was calculated and correlated to root growth (measured weekly over 6 weeks) and concentrations of compounds found in the NES extract (calculated based on peak intensity and standard curve). Finally, the plants were harvested and methanolic root extracts were prepared and analyzed in the same way in order to evaluate changes in I. domestica phytochemical profile.

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OR-198

Bioactive Potential of Yeast Proteins Extracted with HPH and PEF

Javier Marín-Sanchez, Alejandro Berzosa, Ignacio Álvarez, Ana Cristina C. Sánchez Gimeno, Javier Raso

Universidad de Zaragoza, Spain

Yeast is a valued source of proteins and peptides with bioactive potential, garnering interest in the food industry and nutraceuticals. Extraction

methods from microorganisms mainly involve cell disruption and cellular permeability modification, yielding products with unique properties.

This study aimed to compare protein and peptide extraction from yeast using High-Pressure Homogenization (HPH) and Pulsed Electric Fields (PEF) and examining their antihypertensive and antioxidant capacities.

S. cerevisiae brewing strain underwent HPH treatment (2 passes at 100 MPa) disrupting over 90% of the population and PEF treatment (15 kV/cm for 100 µs) electroporating over 90%. After treatment, samples were incubated at 37°C and filtered using 3kDa and 100kDa Amicon® Ultra Centrifugal Filters. Amino acids, glutathione, proteins, and antihypertensive and antioxidant capacities were monitored over time.

HPH treatment resulted in the extraction of 610.29 mg/g of proteins after 1 hour, accounting for 85% of those proteins over 100 kDa. After the same incubation time, the extraction of proteins from the electroporated yeast was lower (30.4% of the total amount of proteins). In this case, the extracted proteins corresponded to proteins with a molecular weight lower than 3 kDa. As incubation time increased, proteolytic phenomena led to decreased large protein concentrations in HPH-treated samples, resulting in peptide and amino acid formation. Conversely, proteolysis in electroporated cells facilitated protein extraction over time.

The highest anti-hypertensive capacity in yeast extracts obtained after HPH or PEF was observed in the unfiltered fractions after 1 hour of incubation (IC50 of 0.55 and 0.89 mg/mL for HPH and PEF, respectively). Extending the incubation time led to a decline in this bioactive property, suggesting degradation of bioactive compounds over time. Antioxidant capacity varied significantly depending on the method of analysis. The DPPH method showed also maximum efficacy in both extracts within the first hour, decreasing by approximately half after 48 hours. No differences between fractions of different molecular sizes were detected concerning the antioxidant activity determined by the DPPH method and antioxidant capacity correlated with concentration of glutathione, a widely studied tripeptide known for its antioxidant capacity. Conversely, the ABTS method showed the highest antioxidant capacity in extracts from yeast treated with PEF after 48 hours of incubation. This phenomenon may be attributed to the formation of peptides during incubation that exerting the antioxidant capacity through a different mechanism than those involved in the DPPH method.

The extraction kinetics and properties of yeast-derived proteins and peptides varied depending on the treatment applied to improve the extraction. Proteins and peptides extracted demonstrated significant bioactive potential, making them an intriguing source of bioactive compounds for the industry.

OR-199

Influence of Pulsed Electric Fields in combination with other processes on the extraction of valuable compounds from brewer's spent yeast cells

Sofie Schröder[1], Jan-Michel Schulte[2], Corinna Stühmeier-Niehe[1], Claudia Siemer[1], Stefan Töpfl[1]

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In this study, the influence of pulsed electric fields (PEF) on the extraction of valuable components, such as proteins and minerals from brewer's spent yeast cells was investigated. In addition, the influence of PEF in combination with high pressure homogenization (HPH) and an incubation time after treatment was also studied as a next step. For this purpose, the yeast cells were treated with PEF, HPH, an incubation time or a combination of these methods to increase the permeability of the cell membrane and extract valuable ingredients. Different electric field strengths (5 to 18 kV/cm), specific energies (80 and 90 kJ/kg), pressures (50 to 800 bar) and incubation times (one or two hours, each at 30 °C and 60 °C) were set in order to investigate the effects on serum released, degree of disintegration, soluble extract content and protein content. The comparison of PEF treated and untreated yeast cells showed an increased serum released percentage from approx. 42 % to up to 65 %, higher degree of disintegration from 55 % to up to 100 %, and an increased soluble extract content,

which rose from 17 % to 30 %, after treatment with electric pulses. In addition, analysis of the extract after PEF treatment revealed higher protein content, compared to the untreated one. The study also shows a further increase in the yield of extracted ingredients with a combination of PEF and HPH or an incubation time. HPH and incubation at 30 °C showed a synergistic effect in combination with PEF treatment. While incubation at 60 °C alone also resulted in an increase and thus more of an additive effect was seen in the increase in protein yield. The cell disintegration itself seems to be mainly attributable to PEF, while 60 °C retention time also tends to show an effect on permeabilization. The results show that PEF in combination with other procedures has great potential with regard to the extraction of valuable components from brewer's spent yeast cells.

OR-200

Solvent Lipid Extraction from Oleaginous Yeast assisted by Pulsed Electric Fields (PEF)

Carlota Delso, Nataljia Nazarova, Wolfgang W. Frey
Karlsruhe Institute of Technology, Germany

Oleaginous yeasts are considered a promising renewable source of lipids that can be transformed into various oleochemicals, such as biodiesel or biolubricants. Yeasts' ability to use low-cost carbon sources and produce lipids at high rates, combined with their short cultivation times and independence from arable lands and climate conditions, make them strong competitors to vegetable oils. However, the downstream processing of oleaginous yeast remains expensive and unfeasible. The high energy demand of the pretreatments (for cell disruption) and the recycling costs and sustainability concerns of the organic solvents represent the major drawbacks. The present study aimed to address these challenges by exploring the use of PEF as a low-energy pretreatment and identifying more sustainable solvents that are effective in lipid extraction from yeasts.

Three strains of oleaginous yeasts (Cutaneotrichosporon oleaginosum, Saitozyma podzolica and Apiotrichum porosum) were grown in a bioreactor under nitrogen limitation and aerobic conditions achieving cell dry weight (CDW) concentrations of 20 to 35 g/L and lipid concentrations of 30 to 50 % of their CDW. The biomass harvested was pretreated by PEF (40 kV/cm, 150 kJ/kg) before undergoing solvent extraction (20 h of solvent-mixing). Various solvents or solvent systems including ethanol-hexane, methyl terbutyl ether (MTBE), ethyl-acetate or 2-methyltetrahydrofuran (MeTHF) were tested. Lipid yields were calculated gravimetrically after solvent evaporation. Whether an incubation time after PEF (24 h) and medium osmolarity during this incubation could contribute to increasing lipids yield was also studied. Additionally, the integrity of plasma membrane was evaluated by staining with the fluorescent dye YoPro and flow cytometry techniques.

Results showed that great extraction yields of 80-99 % of the total lipids were reached in all the strains evaluated, although by different strategies. The successful lipid extraction on S. podzolica and A. porosum required PEF plus an incubation period, while no pre-treatment was necessary for C. oleaginosum. This observation pointed out that solvent lipid extraction did not require plasma membrane permeabilization for C. oleaginosum- what was confirmed by neglectable Yopro uptake (< 10 % of permeable cells). Incubation after PEF processing of S. podzolica in the same treatment media increased the percentage of permeable cells (from 10 to 99 %) and the lipid yields (from 23 to 62.8 % of total lipids). However, the lipid yields improved even further (up to 99 %) when the incubation after PEF was performed in a low osmolarity medium. In the case of A. porosum, only PEF plus incubation in a low osmolarity medium was efficient for lipid extraction (83 % of total lipids). The successful of MeTHF, considered a green solvent and easy to recycle, in extracting lipids from C. oleaginosum and S. podzolica opens a promising approach for further optimization and study for industrial application.

OR-201

PEF treatment for the enhancement of microalgae cultivation

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Today's society is urged to find solutions that ensure global food security while maintaining planetary health. Technological advancement, coupled with the exploration of innovative raw materials, holds promise for establishing more resilient food systems. Value chains based on single-cell organisms are experiencing increased traction. However, the economic viability and sustainability of these value chains encounter obstacles owing inefficiencies routed in the up- and downstream processing. This talk highlights the potential of emerging pulsed electric fields (PEF) for establishing more sustainable multi-product biorefineries aiming to foster continuous and circular bioprocessing with microalgae as a case study.

Upstream, nsPEF processing is a technology-driven and resource efficient approach for single-cell bioprocess optimization. Applying treatments during the early exponential growth phase significantly ($p < 0.05$) increased the biomass yields of industrially relevant Chlorella vulgaris (360 ± 114 J kgsus−1). The biomass yield was increased in axenic (100 ns, 5 Hz, 10 kV cm-1) and non-axenic (100 ns, 7 Hz, 10 kV cm-1) cultures by up to 17.5 ± 10.5% and 50.1 ± 12.2%, respectively. In non-axenic cultures, nsPEF-treatments posed a powerful tool for triggering cell-type specific differentiation and selective inactivation. A treatment window of 100 ns, 7 Hz, 10 kV cm-1 decreased microalgae counts (-17.1 ± 13.8%) but had a stronger effect on prokaryotes (-82.7 ± 14.6%). Quantitative assessment of manually obtained data from FCM-based analyses showed that nsPEF treatments enhanced cell proliferation of C. vulgaris. Growth stimulation was also obtained across other organism domains repeatedly treated with 100 ns pulses at 10 kV cm−1: Cyanobacteria (Arthrospira platensis SAG 21.99, 256 ± 67 J kgsus−1), heterotrophic C. vulgaris CCALA 256 (227 ± 60 J kgsus−1), and yeast (Saccharomyces cerevisiae DSM 70449, 173 ± 55 J kgsus−1). Evidence suggests that this enhancement is based on the induction of intracellular abiotic, sub-lethal stress related to transient, cytosolic surges in Ca2+, and effects on plasma membrane level. Downstream, microsecond PEF (80.72±1.90 kJ kgsus-1) enhanced mild biocompound extraction from microalgae while preserving cell morphology. Under the condition applied, PEF was 1.67x more energy-effective than traditional unit operations such as high pressure homogenization for the same extracted biocompound.

PEF has the potential to emerge as a high-impact technology that addresses challenges impairing the economic viability of single-cell-based value chains by tackling primary bottlenecks related to enhancing upstream efficiency and recovering downstream biocompounds. It underpins the development of future food systems rooted in more sustainable paradigms capable of supporting a growing population.

S07 - Potential applications of PEFs technology in vegetable and fruit processing

Thursday morning Track C
Sep 19, 8:30 - 10:00

OR-202

Understanding of the applicability and the mechanism behind pulsed electric fields (PEF) as an alternative peeling method

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Over the last decades, numerous strategies have been implemented in the food industry to prevent soil and water contamination and limit the exploitation of water and energy resources. One approach was using electrotechnologies to improve or replace existing industrial processes. However, several applications rely on empirical process conditions, and systematic approaches are still restricted due to the unclear mechanisms generated by PEF at the intracellular level. Although PEF-assisted peeling is of great interest for industrial applications, the mechanism that drives the peeling process is still unclear. A study on the impact of PEF treatment on the peeling ability of tomatoes and kiwi fruits has been performed to investigate the underlying mechanisms. This study used

monopolar exponential decay pulses with an electric field intensity of 1.0 kV/cm, resulting in total energy inputs ranging from 0.6 to 5.0 kJ/kg for tomatoes and 1.2 to 12.6 kJ/kg for kiwi fruits. Two procedures were utilized for comparing the efficacy of PEF treatments to typical peeling methods: hot-water blanching (98 °C for 60 s) and lye peeling (98 °C for 45 s in 2% NaOH solution). The peeling efficiency was assessed using manual and mechanical methods. Texture, colour (L*, a*, b* scale), ascorbic acid concentration, chlorophyll a and b, carotenoids, total polyphenol content, and antioxidant activity were used to evaluate the quality of the final peeled product. Compared to traditional techniques, the PEF treatment revealed similar or superior peeling capabilities while dramatically reducing product loss. Additionally, to investigate the mechanisms of PEF-induced peeling ability, the microscopic structure of tomato pectin has been further studied. Atomic force microscopy (AFM) and high-performance liquid chromatography (HPLC) analysis demonstrated that the ripening stage and the intensity of PEF treatments affect the physical properties (fiber length and aggregate area) of water-soluble pectin, chelator-soluble pectin, and alkali-soluble pectin. The study has demonstrated that electroporation-induced changes in plant tissue may be used to improve or replace traditional food processes. Despite the promising findings, this study provides awareness of how the advantages can only be converted to effective industrial strategy when the specifics of raw material structure are considered.

OR-203

Effects of pulsed electric field pre-treatment on the heating uniformity and final product quality of ohmic cooked vegetables

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Healthy and sustainable food is an important consumer trend these days. In order to provide nutrient-rich and safe food, innovative techniques for gentle processing are required. In the case of vegetables in particular, thermal processing can lead to loss of nutrients and product quality. Ohmic heating (OH) provides the potential to optimize conventional thermal processes in terms of resource efficiency, process efficacy and product quality retention. However, inhomogeneities in the heating behavior of vegetables, caused by different tissue structures, can lead to overprocessing of product parts. Therefore, this study investigated the use of pulsed electric fields (PEF) as pre-treatment to disintegrate the plant cells and to minimize non-uniformities in the electrical conductivity of the tissue that affect the heating homogeneity. PEF treatment at a pulse repetition frequency of 2 Hz was performed on peeled and unpeeled whole beet root and turnip cabbage with an electric field strength of 0.5 – 4.0 kV/cm and a number of 25 – 500 pulses, resulting in a specific energy input of 0.5 – 150 kJ/kg. The OH was then carried out with 3 kW/kg at two different pulse repetition frequencies (12 kHz and 300 kHz) until a temperature of 90 °C was reached with a subsequent holding time of 5 min. Temperature kinetics and thermal imaging were used to characterize the heating rate and uniformity. The product quality and nutritional value were evaluated by analyzing weight loss, texture, color and vitamin C content. The results of the thermal analyses showed a significant reduction (up to 46 %) of the required heating time to reach 90 °C for both unpeeled and peeled samples pre-treated by PEF. In addition, thermal imaging revealed an improved heating homogeneity within the different tissue regions due to prior cell disintegration of up to 85 % of the vegetable tissue. The faster and more uniform cooking performance resulted in enhancement of the final product quality in terms of improved texture uniformity and better retention of vitamin C. This study demonstrated the potential of using PEF as a pre-treatment to improve thermal processing of vegetables by OH. The synergistic effects of combining both electrotechnologies lead to a rapid, homogeneous and energy-efficient cooking performance with improved quality retention of the final product.

OR-204

Germination and stress tolerance of oats treated with pulsed electric field at different phases of seedling growth

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This study explores the impact of pulsed electric field (PEF) application on oat seedling growth and stress tolerance. PEF treatment (99 monopolar, rectangular pulses lasting 10 µs each, with a frequency of 13 Hz and a nominal electric field strength of 2250 V/cm) was applied at two growth stages: (i) when the seedlings had 0.2 cm roots emerging from the kernel, and (ii) when they had a 0.4 cm shoot emerging from the kernel. Post-treatment, the seedlings were hydroponically grown for 8 days. To induce stress, the hydroponic medium was augmented with PEG (15 %) to induce drought stress and NaCl (150 mM) to induce salinity stress. Results demonstrate that applying PEF improved the growth of the root and shoot of oat seedlings. This effect was more pronounced when applied to more developed seedlings. When PEF was applied during the later stage of germination, seedlings exposed to salinity stress showed enhanced shoot growth compared to the control. Under the studied conditions, the application of PEF had no impact on the growth of seedlings under drought stress.

OR-205

Pulsed electric field, a possible strategy for mitigation of process contaminants in vegetable snacks.

Stefan Toepfl

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Pulsed Electric Field induced electroporation of vegetable tissue results in release of intracellular liquid and a reduced turgor pressure. This affects subsequent processing steps such as cutting, washing, cooking or frying and allows mitigation of process contaminants. The effect of PEF on final product quality has been evaluated for sweet potato, carrot, red beetroot and cassava. After a PEF treatment with a field strength of 0.5 to 1.5 kV/cm and a specific energy input of 1 to 3 kJ/kg the possibility to extract (reducing) sugars as well as anti-nutritive substances has been observed. Subsequent washing has shown to reduce levels of precursors for process contaminants such as acrylamide, the cell opening induced allows lower frying time and temperature and hence reduced formation of thermal reaction products. Dependent on product type and raw material quality a reduction potential of up to 50 % has been observed. The presentation will discuss mitigation strategies considering raw material composition and PEF impact on product quality during various processing steps.

OR-206

Sustainable extraction of plant-based food colorants with Pulsed Electric Fields

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Food colorants are widely used for all types of products in the food industry. Due to an increased consumer demand for natural ingredients, artificial colorants are being replaced more and more with extracts from plants like carrots, red beet, grapes, or spinach. For the production of natural colorants, rough methods, such as enzyme addition or massive disruption of the plant material, are used to obtain high yields. The enzymes break down the cell walls und facilitate the extraction of intracellular components, including pigments, but prolonged exposure times at enzyme specific temperatures are needed to reach the desired effect. The physical process of electroporation by Pulsed Electric Field (PEF) is an alternative to replace elaborate methods currently used in the industry. The increased color extraction after the PEF treatment is caused by pore formation in the cell membrane. Optimal PEF parameters, such as electric field strength and energy input, are crucial for maximizing dye yield while minimizing resource consumption. As a non-thermal alternative, the extraction with PEF is particularly advantageous for heat-sensitive compounds. In this work, colors from the four main groups (anthocyanins, betalains, carotenoids, chlorophylls) were extracted with PEF

parameters relevant for industrial applications. The color yields, quality and stability are compared to those of control samples and of enzymatic treated samples, allowing to focus on the cost and resource efficiency of different extraction processes. Comparative analyses highlight the advantages of PEF over enzymatic methods in terms of higher extraction efficiency, reduced processing time, and lower resource utilization. Further research is warranted to optimize PEF conditions for each color compound and further types of raw materials. Harnessing the potential of PEF for extracting dyes from food sources could offer sustainable and cost-effective solutions for the food industry's colorant needs.

OR-207

Biospeckle activity: New electroporation assessment method for treated fruits and vegetables

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Each applied process causes stress to the plants, which as a consequence induces physiological and metabolic changes. A full understanding of the mechanism of stress-induced changes would enable developing a method that allows to reduce its effects. The pulsed electric field (PEF) may act as an abiotic stressor, influencing the metabolism of the treated tissue. Considering that the treatment with PEF, as well as all the methods of assessing the effects of electroporation, are based on the necessity to interfere with the treated tissue, a method of measuring the obtained effects in a non-invasive manner is essential, so that the obtained result actually relates to only one, specific process. Measurement of biospeckle activity is a technique used to analyze the life processes taking place in the cell. Its non-invasiveness results from illuminating the tested sample with coherent light. An interference pattern is created on the detector, the dynamics of which depend on the rate of metabolic processes. Apparent activity of biospeckle is the result of the physical movement of particles within cells, e.g., the movement of organelles, cytoplasmic flow, and biochemical reactions. Therefore, this method makes it possible to obtain information about the life processes occurring inside a given cell. This research aimed to evaluate the impact of PEF treatment on the biospeckle activity of apple and carrot tissues.

Both studied materials were treated with PEF (electric field strength 1 kV/cm, electrode voltage 24 kV, pulse duration 7 µs, pulse frequency 20 Hz). For each material three different specific energy inputs were analyzed. In addition, untreated apples and carrots were also evaluated. Right after PEF treatment, both plant materials were subjected to biospeckle activity analysis. Measurements were performed with 10-minutes intervals throughout 1 hour.

It was observed that treatment of the tested materials (i.e., apples and carrots) with a PEF caused changes in the metabolism of these tissues. These changes were evaluated on the basis of Tau coefficient - parameter determined from the autocorrelation graph of frames from a video sequence. The slower the observed process, the higher value of this parameter. For 30 min after PEF treatment, all samples exhibited similar values of the Tau coefficient, which constitutes a similar rate of processes occurring in the treated tissues. After 40 and 50 minutes, there was a clear decrease in the Tau coefficient value for the most processed samples (the highest specific energy input), which indicates a significant slowdown in their metabolism.

Measurement of biospeckle activity can be used as a new non-invasive method for assessing the level of electroporation of treated fruits and vegetables.

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S12 - Numerical modelling as an essential tool in electroporation research

Thursday morning Track D
Sep 19, 8:30 - 10:00

OR-208

Electrodissociation of cytoskeleton proteins by intense electric field: in silico

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Although the effects of pulsed electric field (PEF) on biological membranes are being widely explored, the effects of PEF on proteins are still understudied. Existing works on PEF effects on proteins often focus on the effects on protein secondary or tertiary structures. Here we present our molecular dynamics simulation-based perspective on the PEF effects on protein interactions, focusing on selected cytoskeletal proteins such as microtubule-forming tubulin and kinesin. We demonstrate in these examples that intense PEF can lead to protein electrodissociation, i.e., electric field-catalyzed disconnection of interacting proteins.

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OR-209

A coarse-grained lattice model of PEF Inactivation kinetics from percolation theory

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Inactivation kinetics not only reflects the biological lethality of PEF but guides dosage settings in clinic. Despite our insight in single cell death, unfortunately, inactivation kinetics of a multicellular system depends on a myriad of factors which has so far lacked a theoretical framework. Inspired by percolation theory from statistical physics, we propose a lattice model to reconstruct the inactivation kinetics of cell suspension. Coarse-grained parameters can simultaneously describe the disorder distribution of cell collectives, the randomness of discharge in liquid, and membrane rupture of a single cell. We uncover the typical features of three-staged inactivation kinetics and study the universal scaling law of PEF settings on the survival rate based on extensive simulations. This model helps us to quantitatively predict inactivation kinetics in complex settings without resorting to empirical data or FEM analysis with huge computational burdens.

OR-210

Quantum chemical simulations of the interaction of Fe2+ with glycerophospholipids

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Glycerophospholipids are the main component of any cell plasma membrane. Under normal physiological conditions, most molecules cannot directly permeate through the membrane without physical or chemical perturbation of the bilayer. Pulses of electric field, acidic and/or alkaline media, and localization of aggressive metal ions cause cell membrane damage through lipid conformational and destructive processes. Since the membrane structure is a layered lipid, it is necessary to evaluate not only the physical but also the chemical aspects: the redox reactions taking place at the membrane create many ionized components that essentially catalyze the above-mentioned factors. Metal ions belong to these factors.
To understand the dynamics of the formation of the pore in the lipid bilaer and its subsequent closure at the molecular level, modeling of the structure of phospholipids and iron ion complexes by quantum molecular theory methods was performed. Quantum chemical simulations were run using Gaussian16 package.
Optimization of associate geometry was performed by Gaussian16 program, using the semi-empirical density function method B3LYP by means of Gaussian basis set 6-31G. Influence of the solvent media (water) was evaluated by Polarized Continuum

Model (PCM).
It has been found that metal ion fixation to the lipid chain is insignificant to cause lipid conformational movement. Similarly, metal ion fixation in the case of the –N–3(CH3)3 head in the lipid head group was not observed. The iron ion binds two lipid molecules in the orthophosphoric region, forming an energetically stable bridge between orthophosphoric fragments. Both saturated and unsaturated phosphatidylserine (PS) have been reported to be similarly resistant to iron-dependent lipid peroxidation. It is believed that the PS head group is responsible for this effect, as it binds the iron ion, reducing the concentration of free iron ions. As a result of this process, the lipid aliphatic chains change their conformation - a curved chain around the metal ion centre is formed from a straight structure.
A typical molecular charge redistribution during excitation was determined and described. It is stated that due to the energetically favorable Fe2+ ion position, one lipid becomes a charge donor and the other – a charge acceptor. A typical associate contains two phospholipids bridged by Fe2+ ion in the orthophosphoric region. Molecular orbitals correspond to a forbidden electronic transition of 0.50 eV from the ground state (MO 210,211) to the first excited state (MO 226). The iron ion binds two lipid molecules in the orthophosphoric region, forming an energetically stable bridge between the orthophosphoric fragments. As a result, the lipid aliphatic chains change the conformation - a curved chain about the centre of the metal is formed from the straight structure.

OR-211

Modelling the impact of electroporation on spheroid growth and the release of damage-associated molecular pattern molecules

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Electroporation is a technique in which electric pulses are applied to cells in order to increase the permeability of cell membrane. In reversible electroporation (RE), the pulse duration is sufficiently short to ensure that the cell membrane reseals within several minutes. In irreversible electroporation (IRE), however, the pulses are more numerous or their amplitude is higher so that the cell membrane is irreversibly destroyed, and the cells are killed. While the cells are destroyed, the integrity of adjacent tissue remains preserved, making IRE very appealing for ablation of tumours. Recent studies have shown that IRE used for cancer treatment also induces immunogenic cell death (ICD), a form of cell death resulting in a regulated activation of the immune response. In particular, damaged or dying tumour cells release damage-associated molecular pattern molecules (DAMPs) which may ultimately trigger an immunological response.
In this talk, we present a hybrid model to investigate the ICD and regrowth of tumour spheroids exposed to IRE. In this model, a stochastic individual-based model tracking the dynamics of single tumour cells is coupled with a partial differential equation describing IRE dynamics. Here, the death of tumour cells and the release of DAMPs correlates with the intensity of the IRE electric pulses. The model is confronted to biological measures of DAMPs release and volume evolution of tumour spheroids submitted to electric pulses with different intensities. The results of computational simulations obtained from the proposed model shed light on the way in which the intensity of the IRE electric pulses may affect the regrowth of tumour spheroids, as well as their release of DAMPs.

OR-212

Skeletal muscle anisotropy from the perspective of experimental and model-based electrical impedance spectroscopy

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Muscles are frequently the target tissue in electroporation-based therapies. In particular, skeletal muscles are often chosen as the target tissue for gene electrotransfer therapies, owing to

their capability to both express transgenes and release proteins into the bloodstream. In addition, the cardiac muscle is a tissue of interest in a novel therapeutic approach for the treatment of atrial fibrillation that utilises the phenomenon of irreversible electroporation, known as pulse field ablation. The anatomy of the skeletal muscle, which comprises elongated fibres, results in anisotropic electrical properties of muscle tissue. This means that the tissue properties (e.g. electrical, mass transfer, etc.) parallel to the fibres differ from those perpendicular to them. The aim of our study was to investigate the frequency-dependent properties of skeletal muscle tissue using electrical impedance spectroscopy, complemented by numerical modelling to substantiate our findings.

Four electrode electrical impedance spectroscopy experiments were conducted ex vivo in porcine biceps femoris skeletal muscle tissue. These experiments were performed between an hour and several hours post-mortem, ensuring that tissue properties were assessed before as well as after significant changes due to cell death occurred. To investigate the effects of electroporation pulse delivery on tissue properties, we measured the impedance both before and after pulse delivery. Our experimental setup involved the delivery of 8 pulses with a duration of 100 µs and a pulse repetition rate of 1/s. We cut and divided the tissue samples in two orientations, firstly such that the fibres were oriented in the same direction as the applied electric field, and secondly such that the fibres were perpendicular to the direction of the applied electric field. Voltage and current measurements were also carried out and the pulse shape was analysed based on the orientation of the fibres.

The numerical model was built using the Electric Currents interface and a Frequency Domain study within the COMSOL Multiphysics software. We developed a detailed numerical representation to simulate the muscle tissue, incorporating individual fibres in a three-dimensional geometry to accurately capture the anatomical complexity of the tissue. The model was used to determine the electrical impedance values both parallel and perpendicular to the fibres, at the same frequencies used in our experimental setup. The calculated results of the model support and confirm the experimental findings and demonstrate the importance of considering the anisotropic electrical properties of muscle tissue in electroporation-based therapies.

P6 - Calcium electroporation

Thursday afternoon Track A
Sep 19, 14:10 - 15:40

OR-213

Calcium Assisted Irreversible Electroporation Treats Early-Stage Bladder Cancer by Uniformly Ablating the Urothelial Layer

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Non-muscle invasive bladder cancer (NMIBC) has a high rate of local recurrence despite treatment with standard of care tumor resection and adjuvant intravesical chemo or immunotherapy. The objective of our work was to develop technology for whole bladder urothelial cell ablation using calcium assisted irreversible electroporation (CAIR) as a novel form of adjuvant therapy for NMIBC.

Contrast enhanced computed tomography images of a rat bladder were used to construct finite element models in Comsol Multiphysics that guided the design of a novel catheter electrode (configuration and sizing) and electric pulse parameters (intravesical conductivity and voltage) required for whole-bladder CAIR of the urothelial layer. Simulation results were validated in-vitro by performing IRE with prototype catheters in a 3D rat bladder gel phantom embedded with murine bladder cancer (MB49) cells, using PI staining and microscopy to map the penetration and distribution of ablation. Efficacy and safety of CAIR was tested with healthy Sprague Dawley (n = 20) and cancer (n=22) bearing Wistar rats (by exposure to N-butyl-N-(2-hydroxylbutyl) nitrosamine in water).

The bladder was surgically accessed for placement of the electrode catheter and was filled with 5mmol CaCl2 + 0.9% saline solution prior to treatment (1000 V/cm, 100 μs pulse length, 100 pulses, 1 Hz, 2x pulsing with repositioning of the electrodes). Rats were clinically monitored prior to sacrifice at 1, 7, and 10 days post-treatment. Bladders were processed for histology with Hematoxylin and Eosin, Masson's Trichrome, and TUNEL staining to assess morphology, extracellular matrix status, and ablation status respectively.

Simulations showed that intravesical conductivity (.1 – 1.75 S/m) was inversely proportional to penetration and intensity of electric field strength (EFS) in the urothelial layer and the EFS gradient between electrodes (17% and 100% reduction, respectively), and directly proportional to current drawn (35%). In-vitro results demonstrated that depth and penetration of ablation increased with PEF dose and intravesical conductivity (2.4:1) was linked to heterogeneity in the depth of penetration. Ultrasound measurements demonstrated CAIR treatment did not impact bladder volume or voiding in healthy or cancer bearing rats. In healthy rats, acute samples showed complete ablation of the urothelial layer on Day 1 followed by recovery to baseline status by Day 10. In rats with bladder cancer, the use of CAIR resulted in rapid, predictable debulking of the urothelial layer within the entire bladder without injury to the underlying muscularis and submucosa. CAIR treatment of rats with bladder cancer elicited regeneration of a healthy urothelial layer by Day 10 post-treatment.

Rapid, whole-bladder focal debulking of urothelial cancer with CAIR is feasible and safe. This may create a new adjuvant therapy for the treatment of NMIBC in patients.

OR-214

Characterization of two distinct immortalized endothelial cell lines, EA.hy926 and HMEC-1: Exploring the impact of calcium electroporation, Ca2+ signaling and transcriptomic profiles

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Disruption of Ca2+ homeostasis after calcium electroporation (CaEP) in tumors has been shown to elicit an enhanced antitumor effect with varying impacts on healthy tissue, such as endothelium. Therefore, our study aimed to determine differences in Ca2+ kinetics and gene expression involved in the regulation of Ca2+ signaling and homeostasis, as well as effects of CaEP on cytoskeleton and adherens junctions of the established endothelial cell lines EA.hy926 and HMEC-1.

CaEP was performed on EA.hy926 and HMEC-1 cells with increasing Ca2+ concentrations. Viability after CaEP was assessed using Presto Blue, while the effect on cytoskeleton and adherens junctions was evaluated via immunofluorescence staining (F-actin, α-tubulin, VE-cadherin). Differences in intracellular Ca2+ regulation ([Ca2+]i) were determined with spectrofluorometric measurements using Fura-2-AM, exposing cells to DPBS, ionomycin, thapsigargin, ATP, bradykinin, angiotensin II, acetylcholine, LaCl3, and GdCl3. Molecular distinctions were identified by analyzing differentially expressed genes and pathways related to the cytoskeleton and Ca2+ signaling through RNA sequencing.

EA.hy926 cells, at increasing Ca2+ concentrations, displayed higher CaEP susceptibility and lower survival than HMEC-1. Immunofluorescence confirmed CaEP-induced, time- and Ca2+-dependent morphological changes in EA.hy926's actin filaments, microtubules, and cell–cell junctions. Spectrofluorometric Ca2+ kinetics showed higher amplitudes in Ca2+ responses in EA.hy926 exposed to buffer, G protein coupled receptor agonists, bradykinin, and angiotensin II compared to HMEC-1. HMEC-1 exhibited significantly higher [Ca2+]i changes after ionomycin exposure, while responses to thapsigargin, ATP, and acetylcholine were similar in both cell lines. In a Ca2+-free medium, ATP induced a significantly higher [Ca2+]i rise in EA.hy926, suggesting metabotropic P2Y receptor activation and Ca2+release from intracellular stores. RNA-sequencing analysis showed significant differences in cytoskeleton- and Ca2+-related gene expression, highlighting upregulation

of ORAI2, TRPC1, TRPM2, CNGA3, TRPM6, responsible for Ca2+ import in EA.hy926 compared to HMEC-1. Moreover, KEGG analysis showed upregulated Ca2+ import and downregulated export pathways in EA.hy926.

Our finding show that significant differences in CaEP response and [Ca2+]i regulation exist between EA.hy926 and HMEC-1, which may be attributed to distinct transcriptomic profiles. EA.hy926, compared to HMEC-1, displayed higher susceptibility and sensitivity to [Ca2+]i changes, which may be linked to overexpression of Ca2+-related genes and an inability to mitigate changes in [Ca2+]i. The study offers a bioinformatic basis for selecting EC models based on research objectives.

OR-215

Calcium Ascorbate delivered by Electroporation as a novel effective strategy for colorectal cancer treatment

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Introduction. Over the last two decades, electroporation of the tumor has emerged as a valid approach to deliver chemotherapy or other therapeutic agents in areas where surgical resection is not possible or chemotherapy proves ineffective. Calcium chloride (CaCl2) is a very promising treatment because, unlike other chemotherapy agents such as bleomycin, is not genotoxic and is associated with less severe side effects. The main mechanisms of calcium toxicity include ATP depletion, production of ROS, and activation of hydrolytic enzymes. Many in vitro studies indicate the potential antitumor properties of ascorbic acid, or vitamin C, including among others ATP depletion and ROS production. Furthermore, preliminary clinical studies have demonstrated the efficacy of ascorbic acid, when combined with first-line therapies. Here we describe the use of a novel calcium formulation, calcium ascorbate (CaAsc2) and the synergistic effects of ascorbic acid combined with calcium, both in vitro and in vivo. Methods. MC38 or CT26 colon cancer cell lines were in vitro electroporated with a solution of either CaCl2 or CaAsc2 5 mM in a 0.4 cm cuvette with a BTX square wave technology. Post-treatment cell viability was assessed using the MTT assay. Apoptosis and cell cycle assays were also performed, and results were analyzed by flow cytometry. For in vivo studies, C57Bl/6 and Balb/c mice were challenged with MC38 or CT26 colon cancer cell lines, respectively. A solution of either CaCl2 or CaAsc2 168 mM was administered in the tumor via electroporation, as tumors reached 4-8mm diameter, by means of Cliniporator technology with the following electric conditions: 8 pulses with a duration of 100 µs – 1000 V/cm.

Results. Our study showed that both CaCl2 and CaAsc2 had comparable effects on cell viability, inducing early and late apoptosis as well as necrosis in MC38 cell line. Interestingly, treatment with CaAsc2, with or without electroporation (EP), resulted in a slight increase in the number of cells arrested in the G2 phase compared to CaCl2-treated cells, thus preventing cells from replicating. Additionally, both treatments led to a slight increase in HMGB1 levels, a marker of immunogenic apoptosis. Treatment with either CaCl2 or CaAsc2 significantly delayed tumor growth in MC38 mouse model and induced partial tumor regression in CT26 mouse model. In MC38 model, treatment with CaAsc2 led to increased survival rates compared to CaCl2 by the end of the study. Finally, the analysis of cytokines in sera of CT26 mouse model revealed a slight increase in the levels of MIG and IL17A, responsible for T cell activation, after treatment with CaAsc2. Conclusions. In summary, our findings demonstrate that both calcium formulations exhibit similar effects on tumor growth both in vitro and in vivo and indicate calcium ascorbate as a promising alternative to calcium chloride for cancer electrochemotherapy.

OR-216

Modeling the Calcium Oscillations Response to Pulsed Electric Fields for Spinal Cord Regeneration

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Recently, a growing number of biomedical applications focuses the attention on the possibility of using pulsed electric (E) fields to obtain cell regeneration and differentiation [1], [2], on the bases of the well-established effect of cell membrane electropermeabilization. In particular, the European RISEUP project [3] explores the possibility of achieving neuronal regeneration after Spinal Cord Injury by delivering microsecond pulsed E fields (μsPEF) to induce and control stem cells differentiation. Indeed, the change in ionic fluxes across the electroporated cell membranes may alter the intracellular calcium concentration which plays an important role in proliferation and differentiation of mesenchymal stem cells (MSCs) [2].

In this study, a multiphysic and multiscale computational model was developed to understand and predict changes in calcium oscillations observed in in vitro experiments on MSCs exposed to μsPEF.

The computational model was developed in Comsol Multiphysics v. 6.1 by coupling four different physics: (i) the quasi-static EM problem to calculate the induced transmembrane potential (TMP); (ii) the pore density equation, implemented as a PDE applied to the cell membrane; (iii) the transport of diluted species module to calculate the calcium leakage flux through the cell membrane as a function of the pore density; (iv) the dynamics of the cytoplasmic calcium concentration described as PDEs [4] applied to the cell domain. This latter dynamics depends on the equilibrium of the incoming calcium flux through the inositol triphosphate (IP3) receptors on the endoplasmic reticulum, the outgoing fluxes through the pumps on the reticulum and on the plasma membrane and the leakage flux from the extracellular medium [4]. Depending on the concentration of IP3, the calcium dynamics exhibits a Hopf bifurcation, showing periodic oscillations [4]. Calcium flux due to the membrane permeabilization induced by the applied μsPEF represents an additive term acting on the calcium equilibrium.

Preliminary results on a simplified elliptical MSC exposed to a classical biphasic pulse of 100+100 μs duration and 30 kV/m intensity showed that, despite the short duration of the μsPEF with respect to the typical calcium dynamics (order of a few minutes), its application can modulate the calcium oscillation period, accordingly with experimental results [2].

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P8 - Electroporation in veterinary oncology

Thursday afternoon Track B

Sep 19, 14:10 - 15:40

OR-217

Electrochemotherapy (ECT) with intratumoral and intravenous chemotherapy for the treatment of equine skin neoplasias

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Skin neoplasias are the most common neoplasias in horses. Surgical excision is generally successful in treating small and less invasive neoplasias (e.g. sarcoids). Nevertheless, in advanced stages or with aggressive tumors (e.g. squam-

ous cell carcinoma, malignant sarcoids, advanced melanomas) ECT has been lately used for intralesional or margin treatments. Typically, 1-3 treatments, 2-4 weeks apart are required. More treatments (6-12) are needed with chronic/severe presentations [personal-experience]. Thus, the use of ECT with intratumoral and total intravenous chemotherapy was used to increase treatment success.

Medical records from horses treated in 2023 with ECT using intratumoral and intravenous chemotherapy were collected and analyzed. Cases were admitted and treated at the Veterinary Teaching Hospital of Universidad Austral de Chile. Data included were: age, sex, weight, breed, tumor location, tumor size, diagnosis, prior and concurrent therapies, number of ECT sessions, clinical signs, physical exam findings, follow-up, and disease progression.

Four horses and 10 neoplasias were included. Three horses were diagnosed with malignant sarcoids and 1 with equine melanoma. Age ranged from 5 to 15 years, 54% were males and 46?males, all mix breeds.

In each patient chemotherapy with 90UI of bleomycin was administered IV, 8-10min later, intratumorally cisplatin (0.5mg/cm2) and ECT with biphasic-electrical-pulses was applied (700volts, 200ms, 5KHz; VETCP 125-electroporator).

Surgical resection of ulcerated-bulging-masses was performed before ECT.

All cases remained clinically healthy, after each ECT session treated areas showed local inflammation which resolved within 10-14d.

CBCs performed for 7d after treatment showed mild leucopenia and neutropenia 3-4d after treatment.

Number of treatments ranged among 2 to 10 sessions, depending on case severity. All horses were discharged, 100% of the sarcoids and 70% of melanomas showed complete remission.

Intravenous-chemotherapy in conjunction with intratumoral ECT was well tolerated and successful in treating aggressive-chronic-sarcoids and melanoma in horses. This is the first report of total intravenous chemotherapy with ECT in horses, improving treatment-efficacy when compared to the use intratumoral ECT alone.

OR-218

Safety of concurrent administration of electrochemotherapy with intravenous bleomycin and intravenous carboplatin or vinblastine in tumour-bearing dogs and cats: a case series

Chiara Penzo, Jinjing He, Sarah Jayne Baker, Daisy Trewin, Stephen John Baines

Willows Referral Service, United Kingdom

Introduction: Electrochemotherapy (ECT) with intravenous (IV) bleomycin (BLEO) is an effective treatment (TX) for local control of neoplasia in dogs and cats with some patients requiring additional systemic chemotherapy. We wanted to establish the safety of such a combination.

Methods: Client-owned dogs and cats with malignant tumours that underwent ECT with concurrent IV BLEO and IV carboplatin (Group CARBO) or vinblastine (Group VINBL) at our institution from March 2021 to March 2024 were included. Signalment, tumour type, location, histological grade and margins, clinical stage, presence of microscopic or gross disease, ECT modality and parameters, adjunctive medications, clinical history and results of haematology, biochemistry and urinalysis were retrospectively reviewed. Reversible electroporation (EP) was performed with one train of 8 biphasic electric pulses per site at 800-1150 V/cm lasting 50+50 µs each (interpulse 1ms) delivered with a veterinary electroporator (Onkodisruptor EXP-Vet; Biopulse) 5 minutes after IV BLEO using double-plate (60mm length) and double-needle (45mm length) stainless-steel electrodes. Irreversible EP was performed with 5 trains of pulses per site at 1000 V/cm. Pets were treated under general anaesthesia or deep sedation. Blood/bone marrow, constitutional and gastrointestinal Adverse Events (AEs) were graded according to the Veterinary Cooperative Oncology Group's common terminology criteria for AEs (VCOG-CTCAE). Local toxicity was assessed by a published 5-point scale grading score for tissue necrosis.

Results: Five dogs and 6 cats met the inclusion criteria (total 21 TXs). Group CARBO (1 dog, 6 cats, all carcinomas) received IV carboplatin at 200 mg/m2 (total 15 TXs). Group VINBL (5 dogs, all mast cell tumours) received IV vinblastine at 1.8-

2 mg/m2 (total 6 TXs). Intralesional (IT) medications included cisplatin (1 cat, 1 TX), BLEO (1 cat, 1 dog, 3 TXs) and calcium chloride (7 pets, 12 TXs) in the Group CARBO and IT calcium chloride (2 dogs, 3 TXs) in the Group VINBL. Adjunctive medications for all pets included meloxicam (Group CARBO), prednisolone and paracetamol (Group VINBL) and maropitant and metoclopramide (both Groups). Some pets received additional analgesia or antibiotic prophylaxis (both Groups). Irreversible EP was performed in 5 patients in the Group CARBO (10 TXs). AEs included Grade 1 neutropenia (2 cats, 2 TXs, Group CARBO), Grade 1-2 gastrointestinal signs (9 pets, 17 TX, both Groups) and Grade 2 skin toxicity (pyoderma)(1 dog, Group VINBL). Local toxicity score was 1-2 (5 pets, 10 TXs), 3 (2 pets, 2 TXs) and 4 (1 TX) for the Group CARBO and 0 in Group VINBL.
Conclusions: Combining ECT with IV BLEO with IV carboplatin or IV vinblastine was well tolerated. Larger prospective studies are needed to confirm the safety and efficacy of these combinations.

OR-219

Electrochemotherapy of Cutaneous Tumors in Exotic Pets

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Electrochemotherapy (ECT), a new antineoplastic treatment for locally accessible tumors, has been introduced with promising results in veterinary medicine for exotic pets. The aim of this study was firstly to clinically observe the safety aspect of using ECT in exotic animals and secondly to evaluate the efficacy of ECT treatment based on histological tumor type. In our study, ECT with bleomycin was used in eight patients: seven ferrets and one rabbit. Four cutaneous mast cell tumors, one squamous papilloma, one sebaceous gland adenoma, two sebaceus epitheliomas and one basal cell tumor were treated in ferrets and papilloma in one rabbit. On the other hand, ECT with cisplatin was used in four patients: a cutaneous fibroma in a cockatiel, a squamous cell carcinoma and a hemangioma in a leopard gecko and a chomatophoroma in a bearded dragon. A complete response of the tumors was achieved in almost all patients, with the exception of a partial response of the chromatophoroma in a bearded dragon. No clinically visible side effects were observed in any of the treated patients. In conclusion, ECT with cisplatin or bleomycin has been shown in our cases to be a safe, effective and appropriate treatment option for the treatment of the most common skin tumors in selected species of exotic pets. However, our study was conducted on only a few cases. Therefore, further studies with more patients and a larger number of different tumors are needed for a wider acceptance of ECT in the treatment of skin tumors in this animal species.

S15 - Advanced imaging techniques for visualizing the mechanisms of pulsed electric field interactions

Thursday afternoon Track C

Sep 19, 14:10 - 15:40

OR-220

Optical streaking microscopy enables visualization of ultra-fast response to charge accumulation from MHz bursts of nanosecond pulsed electric fields

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Nanosecond electric pulses (nsEP) have been recognized as a versatile tool due to their ability to elicit subtle changes in cellular permeabilization1. It has been suggested that MHz nsEP trains could offer an efficient way to regulate the delivery of energy such that control can be established between

excitation and electroporation2. Testing pulse protocols at the MHz burst frequencies requires measurement equipment that can validate membrane responses at time scales ranging from microseconds to milliseconds depending on the length of the burst. Previously, our team used streak camera microscopy to capture sub-microsecond changes in the membrane potential. While streak cameras can capture sub-microsecond biophysics3, they are exceedingly expensive and cannot capture slow biophysical responses beyond a few milliseconds. We developed an optical streaking microscope4 that can acquire single-shot membrane voltage response down to microsecond temporal resolution, with an unrestricted sequence depth. We used this system to capture the membrane potential from CHO-K1 cells labeled with FluoVolt voltage sensitive dye exposed to MHz nsEP trains using bipolar electrodes. Optical streaking microscopy was validated by visualizing the membrane charge accumulation as demonstrated in Figure 1, which shows that the membrane response can be affected by the repetition rate of the 100-pulse train (500 ns in duration and 1.2 kV/cm in electric field amplitude). Optical streaking microscopy's unrestricted sequence depth enables analysis with microsecond to millisecond temporal resolution, thereby offering a method for studying both microsecond biophysical and millisecond physiological effects.

OR-221

Visualization of Sub-microsecond Changes in Plasma Membrane Potential After Exposure to a Single Microsecond Electric Pulse, or 5 MHz Burst of Low Energy Nanosecond Electric Pulses

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Low energy micro- and millisecond electric pulses (EPs) charge and depolarize the cellular plasma membrane (PM) below the electroporation threshold. On the contrary, nanosecond electric pulses (NSEPs) are too brief to charge the PM of cells to initiate depolarization. It is believed that a single NSEP depolarizes the cell via PM electroporation after the application of high-power EPs. Pakhomov et al. proposed using low-energy (0.01–0.2 kV/cm) NSEPs combined with a very high pulse repetition rate (PRR) to prevent electroporation. This approach allows temporal summation of NSEPs and consequent PM charging without damage. However, the exact mechanisms of this stimulation remain to be elucidated. It is challenging to assess PM charging kinetics directly due to the rapid time scale of microsecond and nanosecond EPs. Recently, we conducted a series of electrophysiological patch-clamp experiments to measure the degree of cellular PM depolarization directly after exposure to 5 MHz trains of NSEPs of 100 ns duration compared to single microsecond and ms pulses. However, the electrical stimulation artifact prevented direct measurements of PM depolarization during exposures. To overcome these limitations, we employed optical measurements of PM depolarization using an organic fluorescent reporter of membrane potential (MP) (Fluovolt) and an in-house custom-made streak imaging system. Ultra-fast (2 ms long) streak kymographs of the MP changes were obtained after exposure to a single 200 microsecond EP and 5 MHz trains of 1000 and 2000 NSEPs of 100 ns duration. The electric field was constant between all exposures (˜0.01 kV/cm). Immediately after exposures, a Fluovolt response (% fluorescence change) was observed in the PM areas facing electrodes. The duration of the response is directly correlated with the pulse width (PW) or duration of the NSEPs burst. During exposure, the PM near the anode electrode experienced hyperpolarization, while the PM near the cathode electrode became depolarized. The MP changes were as follows: 6.7±0.04% and -3.4±0.03% (n=8) after 200 microsecond EP; 3.7±0.04% and -1.4±0.04% (n=9) after 5 MHz train of 1000 NSEPs of 100 ns duration; 4.1±0.05% and -1.6±0.05% (n=9) after 5 MHz train of 2000 NSEPs of 100 ns duration. These results demonstrate that a single 200 microsecond EP at ˜0.01 kV/cm is more effective in charging PM than an equivalent

energy 5 MHz burst of 2000 NSEPs of 100 ns duration. Additionally, close amplitudes of PM fluorescence changes between ~0.01 kV/cm bursts of 1000 and 2000 NSEPs suggest that an increase in either PW or applied voltage is required to increase the extent of PM depolarization.

OR-222

Changes in hydration of cell membranes exposed to pulsed electric fields detected by wide-field Coherent anti-Stokes Raman microspectroscopy

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The application of short (from hundreds of milliseconds down to few nanoseconds) and intense (from few kV/m up to tens of MV/m) electric pulses (EP), known also as electropulsation [1], facilitates biomolecules, peptides, proteins and/or nucleic acids to cross the cell membrane. So far electropulsation initiation mechanisms have been studied only with molecular dynamics simulations or with indirect experimental approaches in living cells or artificial models. In a recent paper [1], we demonstrated the interest of wide-field Coherent anti-Stokes Raman Scattering micro-spectroscopy (CARS) to study directly phospholipid and water molecular responses after and during electropulsation. This work [1] was carried on artificial lipid vesicles, in the absence of fluorescent or molecular markers. Indeed, CARS is a non-linear optical technique enabling the label-free and fast time monitoring of the vibration modes belonging to specific chemical bonds in bio-samples.

In the present communication, we will report the Electro-CARS platform used in [1]. It integrates a CARS setup with microwave-based engineering techniques to deliver microsecond (µs) and nanosecond (ns) duration EP. We exposed living cells: human mesenchymal stem cells HuMSC, murine lung fibroblasts DC-3F, and murine erythrocytes. These cells present different levels of biological complexity and very different diameters, from tens of µm for HuMSC to a few µm for the erythrocytes. Electro-CARS micro-spectroscopy enables the recording of precise spatial and temporal signals originating from chemical vibrational modes of the molecules constituting real cell membranes which we expected they would change in response to electropulsation. We paid attention to the phospholipids C-H and water O-H vibrational modes located in the 2900-3480 cm-1 range. Thanks to the special geometrical arrangement of the illuminating laser beams in our set-up, the probed water vibrations corresponded to the molecules of water located at the cell membrane surface (identified as interfacial water molecules) or confined in proximity of the phospholipid heads (identified as interstitial water molecules). We thus report novel observations of spectral features originating from cell phospholipids, interfacial and interstitial water in the CARS spectra of cells after their exposure to µs and ns EP of high amplitude (few MV/m). To link these changes to the membrane permeabilization levels, we also measured them using pulses of different durations and fluorescent markers such as YOPRO-1 and Fluo-4. Thanks to the capabilities of our ElectroCARS platform, CARS signatures of single relevant Raman shifts associated to water molecules and lipids were also followed in real-time during the exposure to the high amplitude EP lasting µs and ns.

This work is thus a valuable contribution to decipher the mechanisms of the membranes electroporation/electropermeabilization.

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OR-235

Identifying the Differences of Nanosecond Pulsed Electric Field Effects on Intracellular Functions among Breast Cancerous and Normal Cells through Real-time Monitoring

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Nanosecond pulsed electric fields (nsPEFs) have garnered significant interest in biomedical re-

search due to their ability to modulate cellular function by electrostimulating intracellular organelles, functions, and signaling processes without interacting with the plasma membrane. However, nsPEF faces significant challenges in clinical application because the effects of nsPEFs on living cells depend on experimental conditions, such as field strength and pulse duration, pulse rise and decay time, and cell type. Here, another challenge is to identify the nsPEF-induced changes in intracellular space and interpret their relation to cell survivability and death. Therefore, in-depth studies are required for the future development of nsPEF-based modalities for biomedical applications. From this point of view, we have developed a real-time monitoring system by fabricating a 3D microelectrode chip with a 200 µm electrode distance on the microscopic cover glass and incorporated it with a fluorescence microscope.

The differences in nsPEF effects, with the same pulse duration, field strength, and frequency, on intracellular functions including cell death, among breast cancerous (MCF7 and MDA-MB-231) and normal (MCF10A) cells have been identified through real-time microscopic monitoring of nsPEF-induced changes in mitochondrial membrane potential, superoxide anion concentration, intracellular calcium ion concentration and mobility, autofluorescence lifetime of NADH, caspase-3/7 activity, phosphatidylserine externalization and the cell viability, without changing the cell culture condition. The results show that the nsPEF-induced changes in intracellular function and dynamics depend on the nature of the cells. The nsPEF application to subtypes of breast cancerous and normal cells shows different natures of cell death. The survivability of MCF10A cells was much higher than that of MCF7 and MDA-MB-231 cells. We have also identified that the mode of cell death was different among MCF7, MDA-MB-231, and MCF10A cells. The MCF7 cells died in a caspase-dependent manner, whereas MDA-MB-231 and MCF10A cells died in a caspase-independent manner under the application of nsPEF.

S14 - Electromagnetic modelling for pulsed electric fields

Thursday afternoon Track D

Sep 19, 14:10 - 15:40

OR-224

A coplanar waveguide picosecond pulsed electric fields (psPEF) delivery system for the electro-permeabilization of biological cells

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Over the past few decades, the bio-electrics field has been deeply engrossed in studying the effects of high-voltage, ultra-short pulsed electric fields (PEF) on biological cells and their potential applications. When cells are exposed to high-voltage PEF of short duration (ranging from microseconds to milliseconds) within an intensity range of kV/cm to MV/m, one main outcome emerges, termed ”electroporation” or “electropermeabilization”. This phenomenon is defined by the formation of nano-pores and the permeability increase in the cell’s membrane. This leads to an exchange of molecules between the cytoplasm and the extra-cellular environment, either temporarily (reversible electroporation) or permanently (irreversible electroporation) if higher intensity PEF are applied, which may cause cell death or cell apoptosis. Both reversible and irreversible electroporation have demonstrated utility across various therapeutic and biological applications, including electrochemotherapy and electrogene therapy. As for shorter duration of pulsed electric fields, e.g., nanosecond (nsPEF) and picosecond (psPEF), they were proven to electroporate or permeabilize intra-cellular organelles membranes. The efficient delivery of short, picosecond-duration, intense PEF requires generators designed to meet specific pulse characteristics like shape, duration, and amplitude. Additionally, there is ongoing investigation into delivery devices with reduced electrode gap distances, directly in contact with biological samples, to enhance the generation of high electric field intensity. In this paper, we propose

a miniaturized coplanar waveguide (CPW) transmission line designed as a delivery system for high intensity picosecond-PEF. The delivery system main characteristics are: i) a wide frequency band performance allowing the delivery of picosecond pulses, ii) a miniaturized structure, especially the gap distance between electrodes which is equal to 130 µm insuring high intensity electric fields (˜MV/m), and iii) a transparent substrate to enable real-time visualization of bio-effects on the samples. The numerical assessment of the psPEF delivery system is ongoing, with plans for experimental validation aimed at future utilization in in vitro experiments to explore the impacts of high-intensity psPEF on the intra-cellular organelles.

OR-225

A few hundred optoelectronic pulsed electric field generator with fully configurable pulse shape, duration and amplitude

Lionel Michard, Hafsa Tjiou, Vincent Couderc, Philippe Leveque, Delia Arnaud-Cormos
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Recent research has shown growing interest in high voltage, subnanosecond (sub-nsPEF) and picosecond (psPEF) pulsed electric fields. For the instance, picosecond pulses might be used to induce cell electroporation without excitation [1], electroporate cell organelles [2] or in neurodegeneration research [3]. Nevertheless, the effects of subnanosecond and picosecond pulses on biological cells, and the underlying interaction mechanisms are yet to be explored to their full extent [4]. Generating subnanosecond pulses is technologically challenging as at such high voltages, the switching element must be able to sustain high voltages while allowing ultrafast switching. The generator we propose is based on photoconductive semiconductor switches (PCSS) in conjunction with a pulse-forming network (PFN) constituted by coaxial transmission lines and optical delay lines (ODL). The PCSS are triggered using an optical pulse delivered by a 35 ps 1064 nm Nd:YAG laser. The proposed architecture can generate single, paired and bipolar pulses with durations of a few hundred picoseconds and amplitudes up to 15 kV. The generator allows precise shaping of the pulses by using optical delay lines allowing to delay PCSSs triggering. The duration of single pulses can be arbitrary varied as well as the temporal asymmetry of bipolar pulses. The inter-pulse delay of paired pulses can also be adjusted by varying the lengths of transmission lines. Furthermore, the generator can absorb reflected pulses due to nonmatched load impedances and facilitates the use of any type of constant-impedance applicator. The entire system has been numerically studied by means of software simulations with the aim to fit the experimental measurements. The presented state-of-the-art performances associated with the high configurability of the pulse shapes enable further exploration of the effects of picosecond pulses on biological cells and could lead to the discovery of new bioelectrical phenomena.

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OR-226

Complex Electrical Impedance and β-dispersion for Electroporation Sensing

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Electroporation holds applications in various fields such as gene transfer, cancer treatment, and industrial pasteurization. However, understanding the phenomenon is challenging due to the nanoscale dimensions and dynamics of 'electro'-pores. Impedance spectroscopy, typically between 1 kHz and 100 kHz, has been used to detect cell membrane alterations. Yet, traditional impedance spectroscopy is not fast enough to analyze electroporation dynamics and is prone to errors due to electrode polarization (up to 10 kHz). Currently, PEF equipment lacks feedback in their control systems. This work investigates the alteration of complex electrical current during the application of pulsed electric fields (PEF). The tested hypothesis proposes utilizing information from β-dispersion changes (intrinsic to biological tissue) to study electroporation during PEF, considering that the sample itself collaborates with the transduction of the effect, i.e., bioelectronic sensing. The method uses the same electrodes as the PEF application for sensing. Two signals are mixed: main PEF protocol and a small signal centered at β-dispersion. By treating electrical current as a complex number, the analysis introduces an additional dimension for studying electroporation and voltage-dependent phenomena. Experimental results using vegetal tissue reveal significant changes in impedance and phase during PEF exposure. The phase is reduced to 0º at the direct current PEF. The project contributes to the development of fast sensors for feedback and control of PEF.

OR-227

Interpulse bioimpedance reading during electroporation as a tool for monitoring ablation completeness

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Introduction: The application of short, high-voltage electrical pulses induce pore formation in the cell membrane, ultimately leading to cell death; this procedure is called Irreversible Electroporation (IRE) [1]. IRE is typically used in the clinic for the treatment of unresectable tumors. Most IRE protocols involve the application of a fixed number of pulses, disregarding the ablation completeness. This could lead to overtreatment, resulting in a higher temperature increase, or to undertreatment, resulting in a higher tumor recurrence [2]. While the impact of electroporation on tissue bioimpedance is well known [3], a fixed way to monitor ablation completeness by means of bioimpedance monitoring has yet to be implemented clinically. Methods: To investigate the relationship between increasing pulse number and interpulse bioimpedance measurements, 500 V/cm IRE pulses (100 μs) were delivered to vegetable tissue samples and stained with a metabolic dye to reveal ablation areas. A 2 ms low voltage pulse (LVP) was used for the bioimpedance reading and was delivered 499 ms before each IRE pulse at 1 Hz. The Fast Fourier Transform was used to analyze the voltage and current data obtained from the LVP. The resulting interpulse bioimpedance was used to calculate metrics for shape and mean value, to reflect the ablation and the temperature changes caused by electroporation. Discussion: Our results show that the LVP doesn't induce electroporation effects and that the LVP method for reading bioimpedance shows comparable accuracy to a commercial impedance analyzer. Our results also suggest that the ablation area saturates concurrently with the attenuation of the beta dispersion, and this attenuation can be measured by means of bioimpedance. This finding can be integrated into a real-time monitoring system utilizing bioimpedance to assess the completeness of ablation.

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OR-228

Optimization of Electrode Arrangement in 96-Well Plates for In Vitro Electroporation Experiments

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Background: Pulsed-field ablation (PFA), a technique based on the application of electric pulses to cells, has emerged as a powerful tool in molecular biology, biotechnology, and medicine. In vitro electroporation experiments, particularly those conducted in multi-well plates are becoming essential that lead to a better understanding of electroporation effects on cell and tissues. The electrodes are crucial part of the electroporation system and are submerged directly into the solution within the well. The shape and placement of these electrodes significantly impact the distribution of the electric field, posing a persistent challenge.

Purpose: This study aims on the task of optimizing the arrangement of the electrodes within the well. Our primary objectives are twofold: firstly, to enhance the uniformity of the electric field throughout the solution, and secondly, to maximize the volume exposed to electroporation. By refining the electrode layout, we aim to achieve a more consistent and efficient electroporation process.

Methods: The impossibility of measuring the electric field distribution of short pulses within the well using conventional methods, make it only feasible through analytical solutions or numerical simulations. In our study we used numerical simulator COMSOL Multiphysics. We evaluate the field homogeneity, employing methods such as spatial averaging and statistical analysis of 2D images to assess uniformity, while also quantifying the electroporated volume by analysing the isocontour of the electric field. Our investigation involved testing over 50 different arrangements of electrodes to comprehensively explore the optimization possibilities.

Results: From our set of models, we discovered that the configuration employing six needle electrodes, each with a radius of 0.2 mm and positioned at a distance of 1.25 mm from each other, achieved the highest levels of homogeneity in the electric field distribution within the well. This arrangement exhibited superior performance compared to other configurations tested, suggesting its potential for optimizing the electroporation process.

Conclusion: In summary, our study demonstrates the significance of electrode arrangement in enhancing the efficiency of in vitro electroporation experiments. Through numerical simulations and rigorous analysis, we identified a configuration with six needle electrodes that achieved superior homogeneity in electric field distribution within the well. This finding highlights the potential for optimizing electroporation processes and improving experimental consistency. Moving forward, these insights may lead to advancements in molecular biology, biotechnology, and medical research. Ultimately, our work contributes to the development of more effective electroporation techniques with broad applications.

S06 - Electrochemotherapy in treatment of vascular malformations

Thursday afternoon Track E

Sep 19, 14:10 - 15:40

OR-229

Principles and mechanisms of bleomycin electrosclerotherapy in treatment of vascular malformations

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Vascular malformations are specific vascular anomalies, either of the venous or arterial vascular system. They often occur in children and young adults. Treatment consists of either surgical intervention or sclerotization of the vessels with various agents, including bleomycin injections. Treatment with bleomycin sclerotherapy is currently the most commonly used treatment approach. The

efficacy is usually good, but recurrence of the malformations is common. The first report on the use of electrochemotherapy in the treatment of capillary malformations dates back to 2019 by Sophie E.R. Horbach, who provided the proof of concept for electrosclerotherapy. Later, this treatment approach was used by several clinical groups and was named Bleomycin ElectroScleroTherapy – BEST. The review of clinical results has shown very good efficacy and no side effects. Several clinical trials are also currently underway. However, standardization of the treatment is required in order to standardize drug dosage and administration as well as the application of electric pulses. Electrochemotherapy has been used in the treatment of tumors for three decades. Its effectiveness depends on the administration of drug to the tumor cells, the induction of an immune response and also on the vascular disrupting effect of this approach. It has been shown that the vascular disrupting effect is an important component of the antitumor effect of electrochemotherapy. Therefore, this mechanism must be involved in the efficacy of BEST in the treatment of venous or arterial vascular malformations. The effect is limited to the tumor vessels in the tumors, indicating selectivity of treatment to physiologically different tumor vessels and possibly to altered vessels in the malformations. Some molecular features of the vessels in malformations have already been identified. However, further studies are needed on the characteristics of malformations and the potential of BEST for their treatment. To this end, we need to develop models and perform preclinical tests with BEST to optimize this treatment in humans.

OR-230

Electrode for the Treatment of Atheromatous Plaques by means of a combination of reversible and irreversible electroporation

Ximena Manglano[1], Jesica Rodríguez Miranda[1], Aldo Perusso[1], Sebastian D. Michinski[2], Antonella María Cilio[1], Ana Laura Campastri[1], Felipe Maglietti[1]

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Introduction: The peripheral vascular disease is a common problem among high risk cardiovascular patients. It can be successfully treated by femoral angioplasty. However, restenosis is a problem that has still to be solved. The use of electroporation for cardiac ablation has been increasing its applications worldwide. We present a novel electrode for treating an atheromatous plaque in the femoral arthery with a combination of reversible and irreversible electroporation.

Objective: Develop and validate a new electrode to treat atheromatous plaques in patients with peripheral vascular disease by means of reversible and irreversible electroporation.

Materials and Methods: The electrode was designed and modeled in COMSOL Multyphisics software (Comsol, INC). It consists of a probe with 4 rings of conductive material separated 4 mm from each other on the tip. Several pulse parameters were tested to achieve an effective electric field that could reach the vessel wall. The prototype electrode was built in plastic and stainless steel. Different pulse parameters were tested in a vegetable model using an EPV-LAB electroporator (BIOTEX SRL, Buenos Aires, Argentina).

Results: The in-silico model showed that when 600V were applied an adequate circumferential electric field was generated. This field theoretically can reach the vessel wall and treat it completely. The prototype developed showed promising results in the in-vitro models when 32 pulses of 600 V were delivered. The effective electric field reached a distance of 2 cm away from each side of the electrode. This spatial distribution is enough for ensuring sufficient coverage of the affected arterial segment.

Conclusions: These results highlight the promise of the designed electrode for interventions targeting peripheral artery disease associated with atheromatous plaque. The ability to deliver targeted electrical pulses with sufficient spatial coverage holds significant implications for enhancing blood flow, reducing stenosis, and promoting vascular remodeling in affected arteries. The developed electrode could be an effective tool for the treatment of peripheral atherosclerotic disease, with translational potential to human medicine. Further experimental validation, including in vivo studies in a suitable translational model is warranted to corroborate

these in silico and in vitro findings and assess the safety and efficacy of the electrode.

OR-231

Development of in vitro and in vivo models of vascular malformations for determining bleomycin electrosclerotherapy (BEST) efficacy

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Institute of Oncology LJubljana, Slovenia

Vascular malformations (VM), a rare condition characterized by abnormally developed blood vessels, can occur anywhere in the body, presenting a spectrum from simple and benign to complex conditions. VM develop due to inherited or somatic mutations that disrupt the major intracellular endothelial receptor signaling pathways, i.e. the PIK3CA-AKT-mTOR, RAS - MAPK - ERK and SMAD signaling pathways. The resulting vasculature in the VM is abnormal, often leaky and highly proliferative, i.e. very similar to tumor vasculature. Vascular abnormalities are classified into two main groups: tumors (true proliferative neoplasms) and malformations (morphological defects). Tumors are further subdivided into benign, locally aggressive/marginal or malignant, while malformations are subdivided into simple, combined or associated with other abnormalities. The selection of optimal treatment strategies for vascular malformations depends on the specific type and anatomical location of the anomaly. Several different therapeutic options are available, including observation, sclerotherapy, laser treatment, embolization and surgical interventions. Although current therapies for vascular malformations have shown efficacy in many cases, challenges and limitations still exist. In the case of ethanol, one of the most potent and effective sclerosing agents, a high complication rate occurs, with skin complications being the most common side effect, occurring in 8% of patients.

Electrochemotherapy is a local treatment that enhances the cytotoxicity of a drug exclusively at the site of application of the electrical pulses. In addition to its direct cytotoxic effect on tumor cells, bleomycin electrochemotherapy also has a specific vasodilatory effect on the tumor's blood vessels, without damaging the normal vasculature around the tumor. Therefore, combining bleomycin sclerotherapy and electroporation, i.e. bleomycin electrosclerotherapy (BEST) for the treatment of VMs, could potentially improve the efficacy of bleomycin sclerotherapy. Bleomycin is already one of the most commonly used sclerosing agents in the treatment of VMs. Therefore, the combination with electrical pulses could only further enhance the efficacy of bleomycin, as it would increase the uptake of the drug into the endothelial cells of the affected blood vessels. In early clinical studies, BEST has already proven to be an effective and safe method for the treatment of VM, but its mechanisms of action are still not yet understood. Therefore, we have developed in vitro murine models of VMs based on the most common somatic mutations found in VMs and are now using them to determine the optimal treatment parameters for BEST in vitro and to evaluate its therapeutic potential in treatment of VMs in vivo. Our research will fill the gap in knowledge of the mechanisms governing the response of VMs to BEST treatment and provide standardized treatment parameters that could help guide the use of BEST in clinics.

OR-232

Use of bleomycin electroscleotherapy (best) in hereditary hemorrhagic telangiectasia patient (HHT): a case report

Marta Minuti, Giulia Bertino, Fabio Pagella, Rebecca Gelli, Marco Benazzo

University of Pavia, Italy

Introduction: Hereditary Hemorrhagic Telangiectasia (HHT) is a bleeding disorder occurring in 1/5000 person. It is an invalidant disease because of frequent and recurrent bleeding episodes of the arterio-venous malformations. Standard treatments (sclerosant agents, Agon-laser, etc.) are burdened by high recurrence rates.

Recently, Bleomycin Electrosclerotherapy (BEST) has shown promising results in the treatment of vascular malformations. We present a case of oral telangiectasia successfully treated with BEST.

Materials & Methods: A 70 year-old man affected

by HHT with multiple telangiectasias of the tongue was treated with BEST in August 2023.
The procedure was performed under general anaesthesia; reversible electroporation was applied with Cliniporator™ immediately after intralesional administration of 1,5 ml of Bleomycin (at concentration of 0,25 mg/ml) according to the ESOPE protocol.
Treatment response was assessed clinically and with Patient and Observer Scar Assessment Score (POSAS) and Global Assessment of Change (GAC) questionnaires.
Results: At the end of BEST, the "vascular lock" of electroporation effect was immediately visible. In the following days, a mild oedema of the tongue was observed which resolved spontaneously. Moderate pain was controlled with paracetamol. At the two-months follow-up, complete remission of the lesion was observed. The scores on the evaluation questionnaires improved significantly with a relative increase in the patient's quality of life. At 7 months follow-up patient is still NED.
Conclusions: BEST is a procedure that significantly increases sclerosis of the vascular malformations. This is the first case of application of BEST in a HHT patient. The safety and efficacy of the procedure could lead to consider BEST a valid alternative in the treatment options of oral vascular lesions in HHT patients.

OR-233

Clinical applications of BEST in treatment of vascular malformations

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Nonsurgical Bleomycin injection (sclerotherapy) of vascular anomalies is an effective and widely used treatment modality. Although this treatment is safe and efficacious, a response is usually only achieved after a series of repeat treatments. (An average of 4 treatment sessions were needed in our series of 890 patients). Bleomycin is a large, charged molecule that does not easily cross into the target cell. The utilisation of the technique of electroporation can however address this issue. In a number of interesting observations, case reports and case series, the benefit of adding electroporation to bleomycin sclerotherapy, coined as BEST (Bleomycin electrosclerotherapy) has induced a significant improvement in responses achieved and a reduction in the number of repeat treatment sessions needed. Achieving a quicker treatment response with less repeat sessions transfers significant benefits to patients, clinicians and institutions. Further exploration has led to the establishment of the BEST multi centre study to assess treatment response and quality of life in a patient and clinician reported outcome study. We will present our current understanding, and up-to-date experience of this exciting novel treatment option.

OR-234

Bleomycin Electrosclerotherapy (BEST): experience with lymphatic malformations

Giulia Bertino, Marta Minuti, Rebecca Gelli, Valentina Ravetta, Marco Benazzo

IRCCS Policlinico San Matteo Foundation, Italy

Introduction: Congenital lymphatic malformations represent 12% of all the vascular anomalies and two thirds of all the reported cases are found in the head and neck, especially in the oral mucosa or the neck. They are usually categorized as macrocystic or microcystic, but both can be found in the same lesion. Intralesional injection of various sclerosing agents, particularly bleomycin and picibanil, is the main treatment option, but multiple sessions can be necessary to resolve or remarkably reduce the lesions. Recently, the association of reversible electroporation to bleomycin sclerotherapy (BEST) has been shown to increase the sclerosing properties of the drug with very high percentages of complete resolution of the lesions and without severe side effects; but up to now no dedicated standard operating procedures (SOP) exist. The International Network for Sharing Practices on Electrochemotherapy (InspECT) is the largest group of experts (42 all over Europe) on electroporation-based treatment and it is working on the definition of these

SOP.
Here we report the experience with BEST of one of these Centers.
Materials and Methods: We tested the efficacy of BEST in three patients with recurrent or non-responsive lymphangiomas, 1 child with intraoral lesions, and 2 adults with neck lesions.
Results: No major or minor side effects were observed. All the patients underwent significant reduction or resolution of the cysts with only one session, and the results are stable after more than 1 year of follow-up.
Conclusions: The association of reversible electroporation to the standard application of intralesional bleomycin enhances the sclerosing effects of the drug because it increases the permeability of the cell membranes to the drug, and the result is acquired most of the time in one single session. Moreover, the treatment is safe, with organ and function sparing. It can be applied both in adults and in children and it can be considered a valid alternative in patients resistant to surgical or other sclerosing treatments.

POSTER PRESENTATIONS' ABSTRACTS

Poster session

Coffee Break and Poster Session

Sep 16, 14:40 - 16:10

PO-01

Surgery and electrochemotherapy: an option for amputation of the posterior limb of a canine with an infiltrating sarcoma

Oscar Pagoto

Sociedad Argentina de Oncología Veterinaria, Argentina

Skin and subcutaneous tissue tumors are the most common type of tumor in dogs (approximately 30%). Soft tissue sarcomas are 15%. All Sarcomas have a common mesenchymal origin and similar behavior, but their differentiation is difficult diagnostic. They can present a pseudo capsule or be very infiltrating and manifest a very aggressive local growth, although those of high grade can also present metastasis rates of between 8-20% in canines. Surgery is the main therapeutic tool. But if the surgery is performed with a narrow margin, the chances of recurrence increase significantly, so other alternatives must be implemented: new surgery, chemotherapy, radiotherapy, cryosurgery and electrochemotherapy.

In the Electrochemotherapy an electric field is generated with predetermined frequency and time, a chemotherapeutic (Bleomycin, Cisplatin) is applied locally and / or intravenously before. The chemotherapeutic enters the cell in greater percentage and induces the apoptosis.

It is possible to apply it on the tumor directly or as an adjuvant after surgery, inside the surgical bed and treat possible cancer cells that could not be treated. It is very useful in those tumors that are very difficult to resects or resolved with little margin or in those cases in the surgical intervention it is very aggressive and may not be accepted by the owner (Mandibulectomies, amputations, rhinotomies).

The case of a 15-year-old female canine with a soft-tissue sarcoma infiltrating the left limb knee of rapid evolution, of very difficult surgical resolution with good margin, is presented. Other veterinarians suggested amputation. But given the amputation alternative, tumor resection, electrochemotherapy and subsequent reconstruction with axial caudal superficial epigastric flap technique are proposed. The procedure was accepted by the owner and due to the high recurrence rate of these types of tumors, the pet was monitored every 15 days for 4 months and then every 2 months to evaluate the evolution of the surgical suture. So far 24 months have passed since the procedure was performed and there is no evidence of tumor recurrence and no evidence of metastasis.

In conclusion, the procedure performed avoiding amputation was satisfactory, without recurrences or metastasis.

PO-02

Outcome of one session of electrochemotherapy with bleomycin as single or adjuvant treatment in equine cutaneous sarcoids and melanoma

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Evidensia Specialist Animal Hospital in Helsingborg, Sweden

The aim of this retrospective study was to evaluate the safety and efficacy of electrochemotherapy (ECT) with bleomycin in cutaneous tumours of the horse. All horses underwent one session of treatment only. Fifteen horses with 49 sarcoids were treated with ECT and bleomycin as single treatment, and two horses with 5 sarcoids were treated with ECT and bleomycin as adjuvant treatment with marginal excision. One horse with 4 melanomas was treated with ECT and bleomycin as single treatment.

For four horses with 18 sarcoids, information for follow up was not available. Of the remaining sarcoids, complete remission was observed in 21/31 (67,7%) tumours, partial remission in 5/31 (16,1%) tumours, and stable or progressive disease in 5/31 (16,1%) tumours. In one horse with 4 melanomas, complete remission was seen in 3/4 (75%) tumours, and stable disease in 1 tumour.

For two horses with 5 sarcoids, after 1 session of adjuvant treatment with ECT and bleomycin intraoperatively in combination with surgical/laser ex-

cision, complete remission was observed in 4/5 tumours and recurrence in 1/5 tumours.
Four horses with 5 sarcoids were treated with sedated, of which one was periocular.
The results of the study show that ECT with bleomycin was safe, and effective in the large majority of tumours, with only one session. Location rather than size was correlated to outcome, with periocular location having a worse outcome.

PO-03

Squamous cell carcinoma treated with electrochemotherapy

Jesica Rodríguez Miranda[1], Antonella María Cilio[1], Ana Laura Campastri[1], Raquel Lertora[2], Sebastian D. Michinski[3], Ana Clara Acosta[4], Felipe Maglietti[1]

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Introduction: Squamous cell carcinoma is a malignant proliferation of keratinocytes of the epidermis, is the second tumor in frequency after the Basal cell carcinoma and represents 20% of the cutaneous tumors.
Risk factors that increase the probability of developing Squamous cell carcinoma include unprotected UV exposure, as well as specific patient characteristics such as age, skin phototype, immunosuppression, chronic inflammatory processes, among others.
In early stages, surgery is the treatment of choice.
Most cases of squamous cell cancer are limited to the skin and are successfully treated with surgical excision, local ablation, or radiotherapy. When these treatments fail or are rejected by the patients, new therapeutics options are needed. Electrochemotherapy has been used in Europe since 2006 and in Argentina since 2020 as a standard of care, being an effective treatment with few adverse effects in patients where other therapeutic options have not been successful.
Patients and Methods: These are the first seven patients recruited from this prospective study with squamous cell carcinoma in the head that were treated with electrochemotherapy. They were not candidates for first line therapies or they rejected them. Intravenous bleomycin was administered at a dose of 15,000 IU/m2 and 8 minutes later the electric pulses were delivered.
They consisted of 8 100 us long 1,000 V/cm pulses at a repetition frequency of 5 kHz by means of the OncoPore (BIOTEX SRL, Buenos Aires, Argentina) using a six-needle electrode.
Prophylactic antibiotics were administered after the treatment.
The patients were followed-up weekly until the final response was obtained.
Results: All the patients presented edema which resolved after the first week. Patients with pain prior to the treatment improved considerably during the first days of follow-up.
According to RECIST criteria 86 % OR (67 % CR + 29% PR) and 14% PD were obtained.
All but one patient required one treatment session. The patient with an ulcerated lesion in the scalp (initially without bone involvement determined by ct scan), required two sessions. Despite having an initial good response, this patient progressed and developed bone involvement requiring additional treatment. The treatment was very well tolerated in all cases, and the patients were willing to repeat it if needed.
Keywords: Electroporation, Cancer, Head and neck, epidermoid carcinoma
Conclusion: In advanced stages of squamous cell carcinoma where first line therapies provide poor response, electrochemotherapy currently represents an effective and safe treatment for patients, being a valuable new tool for the oncologist.

PO-04

Electrochemotherapy as salvage treatment of adrenal metastasis

Barbara Perić, Nina Boc, Maja Čemažar

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Introduction: Electrochemotherapy (ECT) is effective and safe method of treatment for cutaneous melanoma (CM) skin metastases. ECT induces immunogenic cell death and release of tumour antigens. In the era of novel systemic CM treatment,

role of ECT in distant metastases treatment is less clear. Approximately half of the patients don't respond to the systemic treatment or relapse after initial success. Studies have shown, that ECT can act in synergy with systemic immunotherapy. We are presenting immunotherapy and ECT treatment of resistant CM adrenal gland metastases.

Case report: 59-years old patient was diagnosed with systemic, BRAF negative CM metastases in January 2020. Pembrolizumab was introduced combined with RT of the spleen 2021. Upon progression, 4 cycles of ipilimumab + nivolumab were given. In 2022 nivolumab monotherapy was introduced, it is still ongoing. In December 2022 inoperable 4.5 cm tumour in left adrenal gland (LAG) was described among the others. RT (TD 20 Gy) of LAG was initiated.

In September 2023, CT showed 7.8 x 9.2 cm big LAG metastases, new metastatic lymph node in left axilla (3.1 cm) and regression of other lesions. In November 2023 unsuccessful attempt of surgical removal of LAG was performed, followed by open LAG ECT.

Eight minutes after i.v. injection of 34 mg of bleomycin (BMI 30.8, 2 minutes bolus), hexagonal needle electrodes (IGEA, H-30-ST) were inserted in LAG. All together 22 applications of electric pulses were delivered to the LAG, covering 2/3 of the tumour. Upon wound closure patient was moved to surgical department. Patient was released on the second postoperative day with no pain or side effects.

Computed tomography (CT) 2 months after ECT showed partial response with extensive necrosis in the LAG and regression of ECT-untreated metastatic lymph node, S100 and LDH were decreased. 5 months after ECT necrotic post-therapeutic area was smaller on CT with slight increase of S100 and LDH. Patient remains asymptomatic.

Conclusion: ECT can be effectively and safely used as salvage therapy in case of inoperable, immunotherapy resistant CM LAG metastases.

PO-05

The Pancreatic Cancer Cellular Response to Calcium Irreversible Electroporation

Agnieszka Gajewska- Naryniecka[1], Nina Rembiałkowska[1], Dagmara Baczyńska[1], Katarzyna Bieżuńska-Kusiak[1], Anna Szewczyk[1], Vitalij Novickij[2], Julia Rudno-Rudzińska[1], Wojciech Kielan[1], Julita Kulbacka[1]

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Pancreatic cancer is an aggressive disease that spreads early and has a 5-year survival rate of only 10%. It often shows no symptoms until it has progressed significantly. Studies have shown that cancer progression from stage I to IV takes an average of one year. The only curative treatment is surgical resection, which is possible in the early stages of the disease. Irreversible Electroporation (IRE) is an innovative localized treatment that offers new hope for those with unresectable tumors. IRE is a non-thermal ablative therapy that causes cell death by increasing cell membrane permeability and activating apoptosis. It is well-established that alterations in the mitochondria are the primary drivers of apoptosis activation, and calcium plays a crucial role in this mechanism. In our approach, the IRE technique involves the administration of calcium ions during an IRE procedure. CaEP is intended for patients who do not respond to standard treatment methods and holds promise for enhancing treatment outcomes. Our research investigated the efficacy of CaEP combined with IRE and cellular responses of pancreatic cancer cells.

We have studied three immortalized cell lines, PL45, HPAFII, and BxPC3, as models for pancreatic cancer. The cells were trypsinized and exposed to calcium (2,5 mM CaCl2) and IRE monotherapy in combination with a HEPES-based buffer (SHM). All protocols were compared to clinical standard ESOPE (European Standard Operating Procedures of Electrochemotherapy) 1.3kV/cm×100µs×8. The viability of the cells was assessed at 24, 48, and 72 hours after exposure to the electric field. The cell membrane permeabilization rate was measured by flow cytometry using a

cell-impermeant dye Yo-Pro-1. Cellular responses to EP protocols were assessed on gene and protein levels at different time points depending on assay type (RT-qPCR, Western Blot, Miliplex® assay).

Our study on electroporation revealed that the addition of calcium ions to electroporated cells led to a significant decrease in cell viability in all three cell lines compared to IRE alone. As the electric field intensity increased, we observed a corresponding increase in cell permeabilization. There were noticed alternations in the RNA and protein expression levels of inflammatory and migratory markers (IL1a, IL2, IL4, IL6, IL8, IL10, IL17A, TNFa MMP9, Cadherins, F-actin, Integrins, and TGFß. Calcium ions potentiated cell death combined with irreversible electroporation. According to our study, we state that CaEP may potentially alter cellular behavior and immune responses. Our study is particularly innovative since few reports exist on applying calcium ion electroporation (CaEP) in treating pancreatic cancer.

Funding: This study was supported by the Medical Research Agency, Poland, IREC project No. 2020/ABM/01/00098/P/02 (PI: Prof. Wojciech Kielan), and SUBK.D260.24.024 .

PO-06

Tissue-ablation App for Electroporation-based Therapy

Sudip Kumar Das

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Pulse field irreversible electroporation (IRE)-based minimally invasive tissue ablation technique uses high-intensity electric pulses of short duration to cause permanent damage to the cells in the tissue. However, achieving safe and effective tissue ablation depends upon multiple factors, including the tissue properties, applied electric pulse potential, and the electrode configuration. Thus, it demands understanding the insights and optimizing the PF ablation parameters for the target region of interest. In this line of contribution, this work presents the relationship between electrode configurations, electrode geometry and the achievable ablation volume using the developed PF-IRE. Additionally, monitor the tissue temperature, which aids in selecting right IRE parameters in clinical treatment planning, avoiding thermal damages. The computational model of classical electrodynamics for the solution of the electric field, coupled with the statistical model for cell viability, can be very effective in planning electropermeabilization-based tissue ablation. For the numerical implementation of the mathematical model for the IRE, we followed the Finite Difference (FD) formulation in two dimensions, 2D, and three dimensions, 3D, and the model is implemented in an app. The developed mathematical model in our work aims to provide the tissue-specific effective operation parameters with the visual outputs through the app-based user interface of the solver for a better understanding of the clinicians. The tissue-ablation app includes the following concepts/features.

> A mathematical model capable of predicting the tissue ablation volume in IRE by solving the electric field and using the Peleg-Fermi model.

> Estimating the temperature is a necessary part of the treatment procedure; therefore, a module solving Pennes bioheat equation determines the temperature.

> The model can consider electric field- or temperature-dependent electrical conductivity Depending on the treatment parameter set.

> Several treatment options include pulse type, pulse shape, pulse length, and the number of electrodes.

> The GUI of the developed app provides easy access to the selection of treatment parameters, the provision of computation, and the visualization of results.

PO-07

The Effects of High Frequency Nanosecond Pulsed Electric Fields with Calcium on 3D Spheroidal Model of Lung Cancer

Wojciech Szlasa[1], Julia Kucharczyk[1], Eivina Radzevičiūtė-Valčiuke[2], Vitalij Novickij[2], Julita Kulbacka[1], *Nina Rembiałkowska*[1]

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Pulsed electric fields (PEFs) serve as a prevalent method for enhancing the intracellular delivery of diverse molecules, including pharmaceutical agents. Despite widespread use, the precise parameters governing the permeabilization process necessitate further optimization.

Our study aimed to investigate how nanosecond pulses burst of varying frequency and field intensity affect CaECT and cellular response (100 pulses of 8, 10, 12 kV/cm field intensity, 200 ns duration time, and high frequency pulses 10 kHz, 100 kHz and 1 MHz). Also, the study seeks to identify the effects of nsPEF on the 3D spheroidal model of lung cancer (A549 cell line). ESOPE (European Standard Operating Procedures for Electrochemotherapy) protocols were used as a reference.

We have shown that the increase of pulse repetition frequency nsPEFs significantly reduces cell viability and promotes membrane permeabilization. As expected, the addition of calcium ions (5 mM CaCl2) to the nsPEF protocol further amplifies these cytotoxic effects. Morphological analysis of spheroids revealed inhibited tumor growth from 8 kV/cm and 100 kHz pulses and higher parameters with CaCl2. The control group's tumor growth served as a reference. The nanosecond pulses were as effective as the ESOPE sequence. Control cells exhibited normal cadherin distribution on cell membranes, while exposure to different PEF frequencies and calcium altered cadherin localization, impacting cell adhesion dynamics and responses to electrical stimuli. Exposure to PEF at different frequencies shows an increasing p53 fluorescent, indicating a frequency-dependent cellular stress response or activation of the tumor suppressor pathway, modulated by calcium supplementation, as evidenced by increased nuclear fluorescence of p53.

Financing: The research was supported by the Statutory Subsidy Funds of the Department of Molecular and Cellular Biology (UMW) no. SUBZ.D260.24.076.

PO-84

Irreversible Electroporation: Impact of novel multiple train pulse field on non-thermal tumor ablation

Ciara Nulty

Trinity College Dublin, Ireland

Electroporation (EP) therapy, achieved by application of electric pulses, can either be categorised as reversible or irreversible dependent on electric field strength and duration of treatment. IRE is defined by at least 80-100pulses and a high electric field amplitude of up to 3000V. This high magnitude and pulse duration overwhelm the cell capacity to regain homeostasis leading to cell death.

There is an existing clinical need for IRE protocol which is predictable with a larger ablation zone, while constraining the muscular contractions and reducing temperature fluctuations near the electrode site. Clinically, IRE requires an anesthetic regime with paralytic agents to prevent severe muscle contractions associated with strong electrical pulses, and analgesia management during recovery. An electrocardiogram synchronizer should be used with IRE therapy to minimise risk of dysrhythmia. Additionally, although the mechanism of cell death by IRE is non-thermal, it can still create ohmics heating with structures in direct contact with the electrodes where the electrical field line density is highest, resulting in thermal ablation. Here we investigate different IRE parameters to spare surrounding healthy tissue, nerves and blood vessels, leading to tissue regeneration in the treatment area from thermal ablation, while preventing severe muscular contractions.

In this study, development of Mirai Medical's electroporation technology, including electrode design and IRE protocol, aimed to increase the efficacy of IRE treatment, while overcoming the necessity of anesthesia and muscle relaxant. Mirai Medical designed a novel IRE protocol with varied voltage trains, duration and number of electrical pulses to overcome current limitations including severe muscle contractions and thermal ablation. Our analysis showed that high frequency, high and low voltage trains can reduced skeletal muscle con-

tractions. Temperature monitoring during pulsing showed less heating around the electrode sites and we also found alterations in probe design from the traditional needles was more user friendly and reduced superficial damage to tissue of pentretrating needle electrodes.

Therefore, pain reduction in IRE can be achieved by appropriately defining the protocol parameters and electrode design. However, the further development of these alternative protocols remains a crucial point to be assessed for translation into clinically setting as a standardised treatment. While reducing associated IRE-morbidities, patients can undergo IRE therapy without anesthesia, while retaining non-thermal cell death of pulse treated tissue. We suggest these new IRE operating procedures can be used in pre-malignant and malignant patients during a day procedure, reducing hospital wait times and reducing patients' morbidities post treatment and improving ability to withstand treatment.

PO-08

Effects of calcium electroporation (CaEP), electrochemotherapy (ECT) and irreversible electroporation (IRE) in patients with locally advanced pancreatic adenocarcinoma. On-going clinical trial. (IREC)

Julia Rudno-Rudziska, Ewelina Frejlich, Karolina Rodak-Mirocka, Wojciech Kielan, Julita Kulbacka
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Pancreatic cancer is 3% of cancers in Poland. It is located on the 10- th place in man and 12- th place in woman mortality. With it's poor prognosis it is on the 6- th and 7—th place in mortality. The morbidity increases in Poland. Because lack of symptoms pancreatic cancer is diagnosed late and in 80% of the patients is unresectable. For those patient only palliative chemotherapy and radiotherapy is available and the overall survival with palliative systemic treatment is 8-10 months.

IREC study aimed to present and compare the findings and outcomes of patients with locally advanced, unresectable, histopathologically confirmed pancreatic adenocarcinoma (stage III). Another inclusion criteria are age above 18 years old, WHO scale below 2> .Pancreatic adenocarcinoma may be diagnosed as primary tumor or recurrence. Patients excluded from IREC study are patients with severe cardiac arrythmia, WHO above 2 and metastatic disease.

Until the date 10.04.2024 47 patients with stage III of pancreatic adenocarcinoma (unresectable) were included to IREC study and underwent IRE or CaEP or ECT therapy with bleomycin. Open or percutaneous procedure was performed. During one-week stay in hospital the safety of the procedure was examined. Overal survival (OS), progression free survival (PFS) were analysed as well as quality of life (WHOQOL-BREF, EORTC QLQ- PAN 26) Patients were examined one, three, six and twelve months after procedure.

IREC is on-going study and the questions we need to answer is weather this methods are effective, if any of these three methods is more suitable or safe, is there the best moment for the patient to underwent this procedure or is there any specific group of patients who will benefit most from the treatment.

PO-09

2D, 3D and in vivo osteosarcoma models for electrochemotherapy studies

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Osteosarcoma (OS) is one of the most common primary bone cancers in humans and dogs, which are a good model for the human disease. In both humans and dogs, amputation with standard adjuvant chemotherapy has been the established primary treatment for decades. Electrochemotherapy has already proven to be a good treatment option for various tumor types and could also be a good option for OS patients. To evaluate electrochemotherapy as a potential therapy for OS patients, human and canine, we first had to create a predictive model of the disease.

Therefore, we established 2D and 3D cell models from the murine OS cell line K7M2 and an orthotopic in vivo mouse model using the luciferase-expressing cell line K7M2-luc. First, the 2D cell model was used for initial cytotoxicity and prolifer-

ation assays. However, 2D models cannot reproduce important properties of solid tumors in vivo. Therefore, 3D multicellular tumor spheroids were used as their architecture promotes cellular organization, differentiation and communication. These processes lead to the development of a spheroidal microenvironment that recapitulates the in vivo properties of solid, non-vascularised tumors; growth kinetics, metabolic processes and resistance to cytostatic agents. We cultured spheroids with different numbers of seeded cells, incubated them in a Clinostar® rotary incubator and harvested them at selected time points. The percentage of apoptotic/necrotic cells was then determined. Spheroids formed from 5000 cells and cultured for 1 week were selected as a suitable model for further studies.

Since OS tumors usually develop at sites of bone growth and involve specific components of the bone microenvironment, the use of an orthotopic mouse model is required. The implantation of cells at the same anatomical site as the corresponding cancer in humans and dogs (in this case the proximal end of the tibia) better mimics the microenvironment and cellular interactions found in human and canine tumors than the commonly used ectopic (subcutaneous) models. Therefore, for the in vivo studies, we first transduced the K7M2 cell line with the gene for luciferase. Next, the cells were inoculated into the proximal part of the tibia using a 29G needle. With a drilling action the needle passed through the periosteum and once it reached the medullar area, the cell suspension was injected. The growth of the tumors was monitored once weakly with IVIS. The mice were euthanized as soon as bone lysis was pronounced and clinical signs of distress were detected, approximately 6 weeks after inoculation. In addition to the primary tumors, we also detected lung metastases, which are an indicator of disease progression. In conclusion, we have developed a suitable 2D, 3D and orthotopic in vivo model for further studies on electrochemotherapy of OS tumors.

PO-10

Cell death mechanisms detected in cardiomyoblasts after conventional IRE and after H FIRE

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Cell death mechanisms underlying the basis of IRE protocols are still poorly understood. Signs of accidental death (necrosis), regulated cell death (e.g., necroptosis and pyroptosis), and programmed cell death (apoptosis) have been observed. For protocols consisting of high□frequency biphasic pulses (i.e., H□FIRE), our lab reported in vitro data indicating that the portion of the total lesion due to delayed (i.e., non□necrotic) cell death mechanisms increased with frequency and that, among that portion, apoptosis was less prevalent as frequency increased (Ann Biomed Eng. 2020 May;48(5):1451-1462). That situation suggests that other delayed cell death mechanisms must be involved in H□FIRE. The main aim of the present study was to understand which delayed cell death mechanisms are involved, apart from apoptosis, when H□FIRE pulses are applied. H9C2 cells were 2D cultured and treated with a non□uniform field similarly as in (IEEE Transactions on Biomedical Eng. 2022 ;69(4):1318-1327). A thin layer of low gelling agarose with 1 % v/v in DMEM was carefully placed over cells to immobilize the cells after treatment. The treatment consisted of either 60 bursts of biphasic pulses (internal frequencies of 90, 150, 260 and 450 kHz) or 60 conventional IRE pulses, all with a duration of 100 µs repeated at 1 Hz. They were applied using a custom□made electrode in a monopolar configuration. Voltage amplitudes were adjusted to obtain similar areas free of surviving cells after 24 h. Pyroptosis was assessed at 4 h and necroptosis at 18 h using indirect immunofluorescence detection methods. These time points were established in a preliminary study in which it was determined when these two cell death mechanisms were predominantly activated. Membrane permeabilization by PI uptake was assessed at 4, 18 and 24 h. Electric field magnitude across the setup was computed by simulation with COMSOL Multiphysics. An additional simulation study

was computed to neglect possible temperature effects. The areas where pyroptosis and necroptosis were manifested were smaller than the total area of the lesions as assessed by PI uptake at 24 h. We reached a few conclusions. First, by using larger voltage amplitudes, H□FIRE produces similar lesion sizes to those achieved by conventional IRE. Second, H□FIRE lesion size cannot be accurately estimated by PI uptake at 4 h because the final lesion, at 24 h, is substantially larger; as anticipated, there are retarded mechanisms of cell death when conventional IRE, and in particular H-FIRE, is applied. Third, signs of pyroptosis are maximal after 4 h of treatment and of necroptosis after 18 h. Fourth, both pyroptosis and necroptosis are highly frequency□dependent, increasing their importance with frequency. Fifth, in addition to necrosis, necroptosis, pyroptosis, and apoptosis, other undetermined cell death mechanisms must be involved in ablation by conventional IRE and H□FIRE.

PO-11

Lesion Depth Analysis for Pulsed Field Ablation

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Pulsed field ablation (PFA) is a promising alternative therapy to current thermally induced cardiac ablation techniques such as radiofrequency ablation and cryoablation for the treatment of atrial fibrillation (AF). PFA employs irreversible electroporation to induce cell death in specified regions of tissue with short duration (us), high amplitude (kV) electric pulses. Lesion depth, a critical factor influencing arrhythmia recurrence, presents an issue as the lack of deep, transmural lesions allows for irregular cell regrowth. A more efficient set of protocols must be established to create robust lesions, ensuring long-term freedom from fibrillatory symptoms. This problem can be addressed through the use of 3D hydrogels and ex vivo tissue characterization to gain insights into the efficacy of various IRE treatment protocols. Using a high-throughput hydrogel model will efficiently narrow down optimal pulse parameters for ex vivo cardiac tissue experiments. Two-needle electrode configurations will be used for both in vitro and ex vivo experiments for the generation of comparable data between the two treatment platforms. A COMSOL finite element model will incorporate hydrogel experimental data to calculate electric field thresholds to determine the necessary applied pulse parameters for a desired lesion area. Ex vivo tissue characterization will provide us with realistic conductivity and temperature data to incorporate into our finite element model, further improving the accuracy of the modeled lesion dimensions. By establishing mathematical relations between our in vitro and ex vivo data, treatment plans can be optimized, allowing for the rapid refinement of pulsing protocols to minimize in vivo animal experimentation while focusing on investigating lesion depth in porcine cardiac tissue. This comprehensive approach presents a promising strategy for optimizing cardiac ablation protocols to maximize lesion depth, ultimately providing prolonged relief from atrial fibrillation symptoms.

PO-12

Evaluation of collagen role in electroporation: two cell lines compared

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Electrochemotherapy (ECT) uses electroporation to improve drug uptake into cells. This is an established and effective treatment in the care of patients with several types of superficially metastatic tumors like breast cancer recurrences or melanoma. Nevertheless, is applied to other types of tumors.

In general, ECT efficacy is evaluated in vitro on cell suspensions with liquid of low conductivity or using cells in adhesion. In recent years the use of 3D culture models diffused. Cell suspension and adhesion cultures are very easy methods, nevertheless, they lack extracellular matrix components, that are found in 3D cultures or in vivo. Previously, the authors studied the effect of inhomogeneity of the scaffold materials and their electrical properties in the distribution of the electric field when voltage

pulses are applied. When seeded on 3D scaffolds, the breast cancer cells or melanoma cells produced their own extracellular matrix (ECM) that induced an improvement in electroporation. In these cultures, new collagen deposition was found, and authors hypothesized that it played a role in the electroporation process.

To investigate the role of collagen in the electroporation process, HELA and SCOV-3 cells were cultured in two different scaffolds. The first scaffold consisted of hyaluronic acid (HA) and 5% w/w self-assembling peptides (EAbuK) condensed with an adhesive motif mapped on Laminin (IKVAV), referred as HA-EAbuK-IKVAV. The second scaffold was HA enriched with 5% w/w calf Type I collagen (referred as HA-Collagen).

The cell cultures were seeded on the scaffolds and the cultures were characterized at 3 days by cell viability assessment, hematoxylin-eosin staining, and Masson's trichrome staining. Moreover, the gene expression for collagen was investigated.

Electroporation of the cells seeded on the two types of scaffolds was performed using EPS02 EP equipment (Igea SpA, Carpi, Modena, Italy). Different voltage amplitudes, generating different strengths of the applied electric field (0, 400, 600, 800, and 1000 V/cm), were tested. The EP efficiency was evaluated using propidium iodide. Both the 3D cultures, HA-EAbuK IKVAV and HA-Collagen, were electroporated in the culture medium. The cells cultured in the two scaffolds showed a different grade of electroporation improvement with respect to adherent cells.

PO-13

Electro-gene-transfer of a synthetic gene: a possible approach for treatment of Glycogen Storage Disease type III

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Glycogen Storage Disease Type III (GSDIII) is a rare hereditary disease (with an approximate incidence of 1 in 100,000 births) caused by the deficiency of the "Glycogen Debranching Enzyme" (GDE). Mutations within the Agl gene, encoding human GDE, result in the loss of its enzymatic activity. Consequently, abnormal glycogen accumulates in skeletal and cardiac muscles, as well as the liver, causing a spectrum of organ dysfunctions with considerable heterogeneity. Currently, no definitive cure exists, with therapeutic treatments relying solely on stringent dietary interventions.

Our investigation focused on the development of a novel approach utilizing electroporation for efficiently delivering genetic material into cells and restoring missing enzymatic function.

Initially, we employed electro-gene-transfer to introduce of the human Agl gene into GSDIII fibroblasts using the syntethic plasmid pVAX-sGDE, which encodes the human GDE, and optimized electric parameters to enhance transfection efficiency and ensure targeted gene delivery.

Subsequently, we advanced our study obtaining GDE overexpression in vivo in the tibialis cranialis muscle of C57Bl/6 mice using the plasmid pVAX-sGDE and two different electric pulse protocols.

The long-term goal of our work is to contribute to an alternative non-viral gene therapy approach for GSDIII.

PO-14

Comparative Evaluation of Lipofectamine and Electroporation Side Effects on Cellular Functions: Emphasizing IMPDH Regulation

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Lipofectamine and electroporation are widely used for nucleic acid transfection, but their potential side effects on cellular regulatory mechanisms are not fully understood. Considering the crucial roles of inosine 5′-monophosphate dehydrogenase (IMPDH) and its main regulatory form, cytoophidia, in cell proliferation and fate, we examined the impact of these methods on IMPDH cytoophidia in K562 cells. Utilizing a home-made electroporation instrument, our results showed that Lipo-

fectamine treatment significantly decreased K562 cell viability compared to electroporation. Despite both methods demonstrating similar siRNA transfection efficiency with optimized parameters, Lipofectamine induced more pronounced changes in cell cycle distribution and erythroid differentiation in K562 cells than electroporation. Additionally, Lipofectamine affected both the formation and size of cytoophidia, whereas electroporation only influenced cytoophidia size. The localization pattern of cytoophidia remained consistent under both treatments. These findings support the lack of adverse effects of electroporation technique, relative to the Lipofectamine approach, concerning the regulatory function of IMPDH-based cytoophidia and thus, it is a suitable replacement technique for Lipofectamine-based investigations.

PO-15

New microsystem for gradual electroporation of a regular spheroid population and application for protocol comparison

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A microfluidic platform specifically designed by our team for culture, electroporation, and observation of spheroids with regular size and shape has been recently described (WCE22).

We present in this study a microfluidic device derived from this work designed to apply a gradient of electric field to a spheroid population, in order to investigate effect of electroporation (EPN) parameters on a spheroid model. Fluorescence microscopy is used to estimate the efficiency of EPN, it allows for the direct plot of permeabilization-intensity relationship in a single experiment.

The system is centred around a microfluidic chamber, composed of a 1 mm silicone seal between two electrodes, the chamber contains a hydrogel scaffold hosting each spheroid at a specific location, arranged into 16 columns. The bottom electrode is fully coated with ITO (Indium Tin Oxide) while the upper is gold-coated and wet-etched to set up 8 independent linear electrodes above the spheroids, following the alignment of the spheroid columns. 8 variable resistors (0-5kΩ) are added to control the electrical potential on each electrode. A PMMA structure is used to host the PCB and ensure watertightness of the chamber and electrical contacts. The spheroids can thus be exposed to an electric field by applying voltage between the bottom electrode and the resistors shared node. The actual electric field in the chamber depends on the resistor values. The latter was adjusted using COMSOL software (Livelink with Matlab).

EPN was performed on a population of HT-29 spheroids after 3 days of culture, in presence of Propidium Iodide (PI), using eight 100 μs large monopolar pulses, with a 1 Hz repetition frequency. The spheroids were exposed to a solution containing Fluorescein Diacetate (FDA) after the procedure as a measure of cell viability.

As IP dyes enter cells that have lost, temporary or not, their membrane integrity, IP stains cells that have been reversibly or irreversibly electroporated, as well as dead cells. The IP intake thus correlates to the permeabilization of the spheroids.

On the other hand, FDA is converted into green fluorescent fluorescein by living cells, thus assessing the reversibility of EPN at a given intensity.

The plot of IP and FDA intensity along the chamber thus shows the permeabilization as a function of the electric field, as well as the statistical distribution per column (15 to 20 spheroids). A sigmoid function was fitted to those data.

As the protocol used in this experiment is considered a standard for in vivo ECT procedures, we can consider that this experimentation is a solid way to evaluate the potential of other protocols or waveforms in comparison to this standard.

We intend to use the device to compare the permeabilization-intensity relationship in different EPN protocols, in particular to investigate other waveforms.

PO-16

Z-Can modality for remote selective stimulation incorporating bipolar nanosecond cancellation

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The feasibility of using nsEPs alone as a targeted, receptor-independent, non-chemical stimulus has been demonstrated previously. nsEPs have been shown to cause diverse effects, including electroporation, calcium influx, voltage-gated channel activation, and action potential activation. Extending the capability of nsEPs to deep-lying tissues will enable many more therapeutic applications. We propose a modality that can potentially target deep responses while suppressing superficial cell responses (Z-CAN). This concept is derived from CANCAN stimulation [1, 2], exploiting the fact that tissue sensitivity to unipolar pulses (UP) is greater than to bipolar pulses (BP) [3]. The difference could be as high as 10-fold in the nanosecond range [4].

In Z-CAN, nsEPs of opposite polarity are delivered to the tissues by two pairs of electrodes: P1 (with a longer gap distance for deeper penetration) and P2 (with a shorter gap distance for shallower penetration). P1 electrodes are fired ahead of P2 electrodes with a controllable delay to maximize the electrode isolation and cancellation effect. At the tissue surface, the effect of P1 electrodes is canceled by P2 electrodes with opposite polarity. However, this cancellation diminishes as depth increases, allowing the cell response to be retained. Specifically, the pulse waveform in the superficial region is controlled to be bipolar, while in the deep layer, it is unipolar. Only the tissue in the deep region above the threshold will be activated. Other volumes will either be exposed to subthreshold BP or subthreshold UP and will be spared from stimulation.

The Z-CAN concept will be simulated with a 3D electromagnetic simulation (Computer Simulation Technology, CST) and tested on 3D cell tissue. Challenges regarding the pulse generator design and pulsing sequence will be discussed.

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PO-17

Scanning electrochemical microscopy as a tool for microscale imaging after electroporation

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A scanning electrochemical microscope was employed to investigate S. cerevisiae yeast-based biofuel cells. One- and two-electron transfer mediators and Nystatin were chosen to be tested to create a convenient system and an efficient biofuel element. The potential application in small concentrations of Nystatin to improve electron transport across yeast cell membranes by pore-forming, thereby increasing the efficiency of the biofuel cell. Scanning electrochemical microscopy (SECM) has been chosen to capture minimal electrochemical activity in the yeast. Studies on the efficiency of the developed biofuel cell by changing the external loads were also performed.

We compare results with varying Nystatin and non-modified yeast modifications using the same hydrophilic and lipophilic mediators concentrations with different Nystatin concentrations from 0 to 6 μg/ml. The research discovered that the power generated by the microbial biofuel cell, based on Nystatin-modified yeast, was higher than that generated by a non-modified yeast-based biofuel cell. The topographical redox activity maps produced

by the scanning electrochemical microscope have shown that larger electric currents are generated from Nystatin-modified yeast during their cultivation.

PO-18

Development of a high-voltage pulse generator with an integrated function of measuring tissue bio-impedance spectrum

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Irreversible electroporation (IRE) as a novel tissue ablation technique has found widespread applications in areas such as tumor therapy and cardiac ablation. IRE employs high-voltage pulses to create nanoscale defects inducing cell death due to the loss of cell homeostasis. The cell death caused by IRE has a certain time lag in evaluating therapeutic outcomes when using the current medical imaging techniques, presenting challenges for surgeons. To address these issues, in the present study, an optimized topology of a high-voltage pulse generator with impedance measurement was proposed based on the previous studies assessing the outcome of irreversible electroporation using bioimpedance spectroscopy. The impedance measurement was realized by relatively lower voltage pulses extracted from a single-stage charging circuit of the Marx circuit. By doing this, the generator could simultaneously generate high-voltage pulses for irreversible electroporation therapy and produce low-voltage pulse signals for measuring the impedance spectra of biological tissues during high-voltage pulse intervals. By theoretical analysis, simulation, and experimental verification, the prototype of the pulse generator could output pulses with a voltage of ±5kV, a maximum repetition frequency within a burst up to 1MHz, and a pulse duration of 500 ns to 100 μs. For the low-voltage pulses, the pulse width and amplitude could be adjusted from 500 ns to 100 μs, and 10V to 200V, respectively, and the maximum frequency for burst mode is 1MHz, the pulse number could be adjusted as required. In conclusion, by optimizing the circuit of the high-voltage pulse generator, the irreversible electroporation and impedance measurement could be achieved simultaneously, which provides the hardware support for real-time assessment of irreversible electroporation outcomes and has significant implications for advancing the clinical application of IRE.

PO-19

A numerical model of irreversible electroporation at tissue scale specific to cardiac pulsed field ablation

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Cardiac ablation is a technique used to address heart arrhythmias, a leading cause of mortality worldwide. It involves the destruction of dysfunctional cardiac tissue in targeted areas using a catheter inserted into the heart. Irreversible electroporation ablation is increasingly used in the cardiac atrium, under the name of Pulsed Field Ablation (PFA) [1]. However, existing electroporation models, used in oncology for instance, are inadequate to describe PFA as they do not account for the complex geometry of cardiac cells and their excitability. It is therefore a challenge to describe both the pulses at tissue scale and the microscopic electrical phenomena. Some first simulations were presented in this direction, combining the electroporation models and the specific cardiac cells geometry [2]. To go further in terms of modeling, we propose in this work a new model of electroporation at tissue scale that is adapted to cardiac ablation, and the resulting numerical simulations.

We obtain a model, using mathematical homogenization, by considering the cardiac electrophysiology cell-scale equations at the time and length scales of PFA, together with the non-linear resistive term modeling membrane electroporation. Interestingly, a non-linear transport term depending on the electric field appears, which is in accordance with the experimental observations and with the phenomenological models used in oncology [3], and links the electric field with the

transmembrane potential at tissue scale. As a specificity of the homogenization technique, some terms carry microscopic information, namely the geometry of the cells.
We give some numerical illustrations in a realistic context, including the geometry of catheters used in clinical practice. Both bipolar (two electrodes on the catheter) and unipolar (one electrode on the catheter and one on a distant patch) configurations are studied. In the latter, an electrical current is set instead of a voltage, in accordance with the clinical procedure. We investigate the influence of the contact force between the catheter and the tissue and of the cells geometry on the computed electric field and thus on the estimated ablated zone. As our model involves time and has a cardiac-specific structure, the simulations are different from the quasi-static models.

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PO-20

Electrical Equivalent Thermal Modelling of Tissues During High-Frequency Irreversible Electroporation

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High-frequency irreversible electroporation (H-FIRE) is a focal ablation therapy that uses high voltage, short electrical pulses that has been proposed as an ablation technique for colorectal cancer. H-FIRE disrupts cell membranes, leading to cancer cell death, with outcomes influenced by factors like pulsed electric field parameters, cell membrane properties, and electrical conductivity. Although application of H-FIRE pulses produces non-thermal cell death application of any pulse will induce some tissue heating and in evaluating proposed H-FIRE pulse candidates it would be useful to be able to quantify the tissue heating expected from a given pulse candidate before carrying out experimental validation, for this reason a new approach for thermal effect modelling has been developed.
In this work, we propose a novel method to model the thermal response of tissues treated with H-FIRE: Electrical Equivalent Thermal Models (EETM). EETM represents the tissue geometry and its surroundings as an equivalent electrical circuit, enabling simplification of the thermal analysis compared to Finite Element Methods for different combinations of electroporation pulse parameters typically applied during treatment. The circuit elements signify the thermal resistance and specific heat capacity properties of targeted tissues. EETM is helpful for understanding how cells respond to pulsed electric fields, providing a simple way to analyse both electrical and thermal changes during electroporation.
The proposed model includes the EndoVE device (Mirai Medical, Ireland) and utilizing tissue electrical properties from the IT'IS Foundation database. It is implemented in LTspice—a free software for electrical circuit modelling. The equivalent circuit assesses the thermal effect induced by H-FIRE treatment with various amplitudes and frequencies. Temperature analysis of Mirai Medicals proprietary parameters reveals a rise from 37°C to 40.6°C, with a 3.6°C increase, validated through experiments using the EndoVE device on perfused ex-vivo ovine liver.
The alignment of predicted EETM outcomes with experimental results highlights the value of our work, demonstrating the EETM's potential as a reliable predictor for thermal assessment in H-FIRE treatments with variable pulse parameters. This study contributes to the understanding and optimization of electroporation techniques, specifically H-FIRE, with implications for enhanced cancer treatment strategies.

PO-21

Rate of pore formation by electroporation in black lipid membranes

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In a recent study on black lipid membranes (BLM) by C.M. Marques and colleagues (Proc. Natl. Acad. Sci. USA, 2023 Mar 14, 120(11):e2213112120) it has been observed that the frequency of pores appearance by electroporation has a milder dependence on the transmembrane voltage than previously anticipated by standard model of electroporation. We have carried out an experimental study to confirm such observation and, in particular, to explore pore formation when relatively low amplitude voltages are applied. For that, we have acquired a commercial BLM kit (ELEMENTS srl) consisting of a stimulator/current amplifier and a recording chamber where two electrolyte-filled cuvettes are separated by either polytetrafluoroethylene (i.e., Teflon) or polyimide septa with a micrometer hole on which the lipid membrane is created. Voltage waveforms are applied across chlorinated silver electrodes (i.e., Ag-AgCl electrodes) immersed in the cuvettes so that transmembrane voltages are induced, and the resulting currents can be measured. We have tried 80, 100, 110, 150 μm sized micro-holes. For creating the membranes, we have either applied the painting method (Muller - Rudin) or the folding method (Montal – Mueller). The assayed membranes were composed of DPhPC or POPC phospholipids. Two electrolyte solutions were tried: 100 mM KCl, 10 mM HEPES in double distilled water, and 20 μL 1 M NaOH solution to adjust pH 7.4; and a 0.1 M HCl solution. Most of the assays have consisted of delivering a series of rectangular pulses with varying amplitudes, alternating between positive and negative polarities. The applied voltages ranged from 0 mV to 500 mV. The time between consecutive pulses and pulse durations were in the order of seconds. As expected, the implemented membranes acted as a capacitance in the order of 0.1 μF/cm^2. For voltage amplitudes of a few hundreds of millivolts, we have observed sudden peaks in current that randomly occur in between the peaks of current due to the charging and discharging of the membrane at the beginning and end of the voltage pulses. These peaks can be attributed to the formation of short-lifetime pores by electroporation. Under this assumption, our preliminary quantitative analyses of the results seem to confirm that the rate of pore creation seems be proportional to |V|exp(α|V|) rather than to □exp(α|V^2|), where V is the transmembrane potential as α is a constant. Currently, we are conducting further assays and analyses to validate this conclusion and to determine whether it is possible to observe electroporation for very low transmembrane voltages (<200 mV) when the membrane is partially hydroperoxidized.

PO-22

Advancing Electroporation Studies with Automated SECM and Machine Learning

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Scanning electrochemical microscopy (SECM) employs an ultramicroelectrode (UME) as its probe in this scanning probe microscopy technique. It is particularly effective in characterizing the electrochemical properties of surfaces and applying localized electric fields to induce electroporation in cells. However, limitations such as slow imaging speeds and dependence on user input restrict its full potential. To enhance SECM's capabilities, we incorporated visual recognition and machine learning to detect micro-objects within images and determine their electrochemical activity. By reconstructing images from multiple approach curves, we achieved faster scanning and accurate detection of active areas, including sites of electroporation. This approach reduces both scanning time and the need for constant user presence. We developed an automated SECM system with visual recognition, utilizing commercially available modules, low-cost components, proven design and software solutions from other fields, and a custom control and data fusion algorithm to study electroporation and other electrochemical phenomena efficiently.

PO-23

Characterization of Transferred Electrodes obtained with Laser Induced Carbonization Process for Electroporation of Adhered Cells

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Lab-on-Chips revolutionized biology research by consolidating various functionalities into a compact platform. In particular, a lab-on-a-chip device can be a powerful tool for electroporation since with a small distance between electrodes, (hundreds of micrometers), it can generate a high electric field providing a relatively low voltage (hundreds of volts).

In this work, we reported a systematic characterization of a lab-on-chip platform based on electrodes obtained with laser-induced carbonization process, which were transferred on a biocompatible transparent polymer substrate (namely PMMA) for the electroporation of adherent cells. The device comprises multiple cells featuring interdigitated electrodes positioned at varying distances ranging from 200 µm to 2 mm. These electrodes were utilized to generate an electric field up to 6 kV/cm.

The device has been shown to be effective in electroporating U87 glioblastoma cell line looking at calcium intake. So, we thoroughly characterized the electrode from multiple perspectives both pre- and post-pulse delivery. This comprehensive analysis involved scanning electron microscopy (SEM) to examine surface morphology, Energy Dispersive X-ray Spectroscopy (EDS) for elemental analysis at SEM, cyclic voltammetry to assess electrochemical characterization, Raman spectroscopy to investigate carbon bond alterations and Fourier-Transform Infrared Spectroscopy (FTIR) for additional molecular characterization.

Preliminary results indicate that the morphology undergoes slight modifications between pre- and post-pulse applications, even though the electrodes do not undergo oxidation processes or substantial differences at the molecular or bond level. These analyses serve a crucial role in discerning whether the same chip can be reused with consistent performance or to what extent its functionality persists over subsequent applications. Such insights are paramount for assessing the chip's reliability and longevity in practical usage scenarios.

PO-24

Power Supply Chain with Series Insulation Structure for High Voltage Marx Pulse Generator

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Nanosecond pulse shows a wealth of physical effects in cell electroporation and tumor ablation. For solid-state pulse generators (SSPG), insulation power supply (IPS) for multiple switches is a key issue for solid-state pulse generators (SSPG). The existing parallel structure for IPS is short of insulation similarity, which results in low insulation utilization ratio (IUR) and limited adjustability. Here we propose a power supply chain (PSC) with series insulation structure. The insulation circumstance of PSC maintains the same for each stage, which increases the minimum IUR to almost 90%, compared to the existing parallel structures. The proposed PSC requires purely diodes, capacitors and inductors to achieve IPS for switches, thus avoiding the customized cost and enhancing insulation adjustability. After analyzing the working principle of PSC, we evaluate the performance of PSC from the perspective of power supply and insulation. Finally, an 8-staged Marx SSPG prototype is developed to examine the compatibility of PSC and the protection circuit for PSC is implemented. The withstand voltage of PSC can be easily adjusted within 14kV according to the number of diodes. The PSC does not require external control signals and does not interfere with gate driving synchronization or load waveforms, which offers a new solution of IPS for compact Marx generators designed at nanosecond scale.

PO-116

Hybrid Digital-Analogue Square Wave Generator for Bioelectronics Applications

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Short-pulse high-voltage waves are used for a wide range of treatments and research, such as dielectrophoretic (DEP) control and Electroporation. Wave generators that create such waves tend to be expensive, bulky and highly application specialised in terms of delivered wave parameters. By contrast, a low-cost and flexible solution, allowing its output to be adapted for a range of different applications, could be of great value for bioelectronics research teams while democratizing treatment protocols and options.

This report demonstrates a hybrid concept relying on digital synthesis to create square high-low portions of the wave along with analogue summing and inversion to combine them together. This approach allows for a great variety of waveforms to be produced while ensuring fast/clean pulse transitions owing to the inherently high slew rate in digital signals. The design core concept and a packaged prototype which was 3D printed and include an integrated heat sink, appear in Fig. 1 (a) and (b), respectively. The present footprint, height and weight are 140mm x 130mm, 70mm and 1070g (with batteries) respectively.

The design concept consists of synthesizing two digital signals through a microcontroller or equivalent digital circuitry that implement the two phases of a bipolar pulse, while also affording phase delay control. The digital signals can be programmed allowing the user to synthesize a unique waveform according to the application. The achievable peak to peak amplitude is 100 V for a 50 ohm load which is dictated by the analogue inverter and amplifier. The pulse width and delay can be controlled down to 2μs and up to over a second when a basic Arduino Uno is used for digital signal synthesis. The prototype here presented demonstrated easy reconfigurability through the Arduino IDE, including for bursts with an arbitrary number of pulses, a sample of which is reported in Fig. 1(c).

By utilising lower-level programming techniques like port manipulation, faster processing times were possible [1], [2] allowing for pulses to be as short as 2μs. Realistic developments could additionally target Field Programmable Gate Array (FPGA)-based digital synthesis to achieve much shorter pulses. Overall, the hybrid concept herein demonstrates a viable and cost-effective method for flexible waveform generation in bioelectronics settings. The output waveform parameter range achievable through the present approach comfortably lay in the realm of on-chip DEP trapping, sorting and High-Frequency Irreversible Electroporation (H-FIRE).

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PO-26

Irreversible electroporation of tethered bilayer membranes by scanning electrochemical microscopy

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This study investigates the electroporation process of tethered bilayer lipid membranes (tBLMs) using ultramicroelectrodes and scanning electrochemical microscopy (SECM). The effects of varying the potential applied to the ultramicroelectrodes, relative to a silver/silver chloride (Ag/AgCl(sat)) reference electrode, on the formation of pores within the tBLMs are elucidated through the experimental findings. In particular, it has been observed that applying relatively low potentials, ranging from 1 to 2 V, results in the formation of pores of varying sizes within the tethered bilayer lipid membrane. For ultramicroelectrodes with a diameter of 10 µm, the resulting electric fields are estimated to be extremely high, approximately 100 kV/m at 1 V and 200 kV/m at 2 V. These high field strengths are responsible for inducing significant variations in pore diameters within the tBLMs,

ranging from 5 µm to 140 µm.
The study examined how pulse duration and the number of impulses affect pore formation. Pulse duration's impact on pore formation was studied using durations of 120, 60, 30, and 5 seconds (one impulse). Furthermore, the impact of varying the number of impulses from one to eleven, with durations ranging from 0.1 seconds to milliseconds, was investigated. It was observed that reducing the impulse duration resulted in a decrease in pore size. Other factors affecting the size of the pores in tBLMs after electroporation include the distance between the ultramicroelectrodes and the surface of the tBLM (from 1 to 30 µm), which are critical determinants in modulating the pore size within the lipid membrane.
In conclusion, the use of tBLMs in experimental techniques holds great promise for advancing our understanding of the mechanisms of electroporation and the factors that influence the properties of pores and their ensembles under electric field conditions. The research demonstrates that the combination of tBLM with scanning electrochemical microscopy has the potential to greatly enhance the applicability of electroporation processes in biotechnology and biosensing.

PO-25

Scaling of continuous PEF processes by means of dimensionless numbers and computational fluid dynamics (CFD)

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The colinear arrangement of electrodes and insulators for continuous PEF treatment chambers is currently state of the art. This configuration enables higher field strengths to be generated at relatively low power and current. This design has already been implemented in the industry for continuous treatment of liquid foods, e.g. to inactivate microorganisms or enhance mass transfer of valuable metabolites.
However, scaling up poses challenges due to the dependency of electric field distribution on insulator geometry. Existing models cannot predict energy input and field distribution for different chamber geometries, impeding process optimization. This knowledge gap hinders the full potential of PEF in the food industry, as safe processes can only be designed with extensive experiments. Computational fluid dynamics (CFD) simulation has been proven to be a suitable tool for visualizing local physical phenomena and therefore, to investigate the influence of the geometry of the treatment chamber. This helps to reduce the number of experiments, necessary to implement a new process. However, a mechanistic approach to transfer the PEF process to other scales by means of CFD simulation is still missing.
The presented work aims to fill this gap by deriving dimensionless numbers based on the relevant balance equations. These numbers include the Reynolds and the Prandtl numbers as well as a newly developed dimensionless factor which scales the energy source term of the heat transport equation. CFD-Simulations on five different scales with five different insulator geometries were performed using Ansys CFD. A relevant parameter space with Reynolds numbers from 200 to 3000, average field strengths from 5 to 26 kV/cm and specific energy inputs from 0,7 to 200 kJ/kg was investigated. The obtained data were used to investigate the dependency of relevant process parameters like the specific energy input and the average electric field strength on the derived dimensionless numbers.
The results reveal that the specific energy input can be adequately described by the newly defined factor (R2=0.995). However, to determine the average electric field strength, more factors like the Reynolds and Prandtl numbers as well as dimensionless geometry factors for the insulators need to be considered. To ensure the correctness of the simulations, validation experiments were performed on two of the five investigated scales with insulators of at least two different length to diameter ratios.
In conclusion, the study shows for the first time how the specific energy input and the field strength can be described independently of the treatment chamber geometry. This is an important contribution to

simplifying process design and enabling safe processes.

PO-27

Potential use of pulsed electric field (PEF) treatment to increase the concentration of bioactive compounds during fermentation of Clementina peel pomace: conversion of waste into food additives

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The effects of pretreatment of Clementina peel, inoculated with a yeast strain as starter for its fermentation, with pulsed electric field (PEF) at 0.75 to 1 kV/cm on sucrose content, antioxidant activity and yeast growth during fermentation were studied. The results showed that permeabilization of Clementina peel by PEF treatment at 22°C caused an increase in sucrose content and antioxidant activity compared with the control during the incubation period. Obtained results showed that the previous trituration of Clementina peel promoted the highest conductivity when the electric field strength increased from 0.75 to 1.25 kV/cm. Treatments at 1 kV/cm resulted in an immediate reduction of yeast viability which however recovered over 3 days fermentation attaining final cell loads similar (around 6 Log CFU/g) to the control and PEF-treated ones at 0.25-0.75 kV/cm. During fermentation total polyphenol, soluble solids (°Brix) and β-carotene content significantly ($p \leq 0.05$) increased when PEF treatment was applied at 1 kV/cm after 1 hour of treatment. However, the DPPH index significantly ($p \leq 0.05$) increased after 1 hour of PEF treatment at 0.75 kV/cm. Further increase in electric field strength did not significantly ($p \geq 0.05$) modify this value. Thermograms obtained by using isothermal calorimeter (TAM-Air) were analyzed according to the modified Gompertz equation to obtain the growth parameters (lag phase (λ); maximum growth rate (μ max) and maximum density (Amax) of the cells). The predicted curves well fitted experimental points (R2=0.996). Increasing PEF intensity, the lag phase decreased by about 6.3 hours without significantly ($p \geq 0.05$) affecting the maximum growth rate. These results suggest that PEF is a promising mild pretreatment technique which can increase the efficiency of the industrial process by promoting the reduction of fermentation time and increasing the amount of recovered bioactive compounds. Clementine peels, a waste product of the citrus processing industry, thus offer potential economic benefits by returning to the food processing chain as an additive and providing sustainable and creative approaches to food waste reduction.

PO-28

Study on the functional properties of starch regulated by pulsed electric field assisted esterification

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Starch plays an important role in human life, it is the main source of human energy and one of the important industrial raw materials. However, due to some undesirable characteristics of natural starch, such as high pasting temperature, poor emulsification, easy retrogradation, and high glycemic index, the application of its original form in the food industry is subject to many restrictions. The functional properties of starch can be improved by physical, chemical or enzymatic modification to improve the processing performance of starch. Octenyl succinic anhydride modified starches (OSA starch) is obtained by introducing OSA groups to the starch molecular chain. It exhibits excellent pasting, emulsification, water retention and anti-digestion properties, thus receiving wide attention from the starch further processing industry. At present, in practical applications, the traditional aqueous phase method is usually used to produce octenyl succinate starch esters. However, the semi-rigid structure of starch is difficult to be destroyed, which limits the attack of chemical/enzyme reagents on starch molecular chains. Therefore, traditional aqueous phase method has the limitations of high reagent consumption and high energy consumption. This made an urgent requirement of development of rapid, efficient and economical modification tech-

niques. The pulsed electric fields (PEF) treatment can destroy starch structure and promote the chemical reaction time. At present, the effect of PEF-assisted esterification on starch functional properties has rarely been reported, and its mechanism is still unclear. Based on this, this study took green and renewable starch as the research object, constructed a processing technology based on PEF physical field to enhance the efficient modification of starch. The study explores the mechanism of PEF-assisted esterification in regulating starch functionality under varying electric field intensities: (1) Low-intensity PEF (1.5-3 kV/cm) enhances starch crystallinity, increases OSA grafting degree, and improves enzyme resistance, leading to reduced starch digestibility. The increased ratio of short to long amylopectin branches inhibits starch retrogradation, improving freeze-thaw stability. (2) Medium-intensity PEF (3-4.5 kV/cm) disrupts starch crystal structure, promoting OSA grafting on starch particle surfaces, enhancing interfacial activity and emulsification properties. (3) High-intensity PEF (5-6 kV/cm) severely disrupts or even dissociates starch crystal structure, further weakening intermolecular bonds, improving water accessibility, and lowering gelatinization temperature.This research not only provides a novel methodology for starch modification but also offers insights into the mechanism of PEF-assisted esterification, paving the way for the development of high-performance starch materials in the food and industrial sectors.

PO-29

Detection and differentiation of bacteria permeabilization induced by pulsed electric fields (PEF) using electrochemical admittance spectroscopy (EAS)

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The application of pulsed electric fields (PEF) on bacterial cells in suspension results in increased cell membrane permeability and a change in the admittance or impedance magnitude of the sample, depending on the pulsing parameters and medium properties. The measurement of the electrical impedance of cells suspended in a liquid medium before and after electroporation has been widely used for decades, offering the possibility to perform label-free, rapid, real-time, and non-invasive assessment of electroporation efficacy. However, the methodology for this technique is still considered inadequate in predicting the dynamics of cell membrane permeabilization and requires more research to improve its specificity for broader application in the food and biotechnology industries. This study aimed to improve the assessment of gram-positive and gram-negative bacteria membrane permeabilization induced by pulsed electric fields by employing the electrochemical admittance spectroscopy (EAS) method.

Bacterial cells (E. coli and L. d. bulgaricus) cultivated in the exponential growth phase were suspended in deionized water (1.4μS/cm; pH - 6.5) to achieve a concentration of 10 OD and transferred into a 1 mm standard electroporation cuvette. Sets of 10 electrical pulses with pulse durations of 10 or 100 μs and pulsed electric field strength ranging from 2.0 to 24 kV/cm were applied to the samples of 100 μl. EAS measurements were performed in a separate 1 mm electroporation cuvette to ensure the accuracy of the results 10 seconds after PEF treatment. The preassessment of the release of metal ions from the aluminium electrodes of the electroporation cuvette into the bacterial sample and temperature rise due to the ohmic heating has not revealed any significant effect on the admittance magnitude and phase angle shift on the measurement results.

We found that L. d. bulgaricus cells demonstrated a more significant increase in admittance magnitude and a larger reduction in admittance phase after the application of PEF strengths from 2 to 24 kV/cm compared to E. coli, suggesting a higher susceptibility to PEF. In contrast, results from metabolic activity, colony-forming units, and changes in optical density dynamics showed higher inhibition of bacteria growth rate for E. coli cells than L. d. bulgaricus, regardless of PEF strength. However, we determined that the specificity of EAS in evaluating cell membrane permeabilization degree is significantly higher and correlates with results from

other methods when comparing differences in admittance and phase shift magnitude instead of absolute values.

PO-30

Extraction intensification of caffeoylquinic acids from Forced Chicory Roots by pulsed electrical field

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The aim of this study was to develop a new method for extracting caffeoylquinic acids (CQAs) from forced chicory roots, which are a low-value by-product of Belgian endive cultivation. The extraction process was designed to be both environmentally friendly and cost-effective. The two primary CQAs under investigation are chlorogenic acid (5-CQA) and dicaffeoylquinic acids (diCQAs), known for their valuable biological activities such as antioxidant, antibacterial, anti-inflammatory, and anti-UV properties. However, the challenge lies in the low concentrations of these compounds in plants, making biomass valorization difficult. The primary objective of this study was to produce a highly concentrated solution of 5-CQA and diCQAs for subsequent purification and functionalization of the desired products.

Innovative technologies, such as pulsed electrical field (PEF), known to enhance extraction of polyphenols have been investigated as pretreatments to enhance the extraction of CQAs by forced chicory roots pressing. The electrical-field strength, temperature, and energy consumption were optimized. Subsequently, the effect of PEF application on the extraction yield of 5-CQA and diCQAs, compared to conventional extraction, was conducted. Furthermore, the degradation of CQAs due to oxidation was investigated and successfully prevented using a tailor-made solution. The results demonstrated that optimized pressing with PEF led to a four-fold increase in the CQAs content. Additionally, a recovery rate of 70-80% for the CQAs present in the biomass was attained.

PO-31

Impact of Pulsed Electric Field Pretreatment on the Functional and Structural Characteristics of Rapeseed Protein isolate from Rapeseed Cake

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The by-product of rapeseed after oil extraction, rapeseed meal, contains about 35–40% protein, mainly consisting of storage proteins such as napin and cruciferin. Rapeseed protein isolate (RPI) can be considered for various markets and applications, e.g., for the fortification of dairy products and cereals, for the enrichment of fruit and vegetable juices and salad dressings. Its multifunctionality not only expands product formulations, but also meets the growing demand for plant-based protein alternatives. As consumer interest in a plant-based diet continues to grow, RPI is proving to be a sustainable and environmentally conscious choice and promises to fill a significant niche in the global protein market. Therefore, RPI is poised for broad application across multiple industries to drive innovation and meet the evolving needs of health-conscious consumers worldwide. The aim of this study was to investigate the effects of pulsed electric field (PEF) pretreatment on the extraction yield of rapeseed protein isolates by evaluating their physicochemical and structural properties. Rapeseed cake was treated under three different conditions: PEF1 (1.7 kV/cm and 28 kJ/kg at pH=11.5), PEF2 (1.7 kV/cm and 24 kJ/kg at pH=7.0) and PEF3 (1.7 kV/cm and 40 kJ/kg at pH=11.5). After PEF treatment, protein isolation was performed using an alkaline extraction method (pH 11.5), and subsequent protein recovery was performed by isoelectric precipitation (pH 4.5). The RPI was analyzed for water holding capacity, oil holding capacity, Fourier transform infrared (FT-IR), protein solubility at different pH values, emulsifying activity and emulsifying stability. According to FT-IR analysis, the samples pretreated

with PEF1 and PEF3 showed a very different protein structure, such as alfa-helix, beta-turn, beta-sheet based on the first amide region. In addition, the results of solubility analysis showed that the solubility of the protein at pH 4 improved in the sample pretreated with PEF1. The results of the study suggest that the PEF technology is promising for the reuse of rapeseed waste. Nevertheless, optimization of PEF pretreatment and extraction conditions is essential for generating protein isolates with crucial functional properties for the food industry.

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PO-32

Inactivation of Alicyclobacillus acidoterrestris vegetative cells and spores induced by atmospheric cold plasma: Efficacy and underlying memchanism

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Alicyclobacillus acidoterrestris (A. acidoterrestris), a thermophilic, spore-forming bacterium, poses substantial challenges in the fruit juice industry due to its resilience against pasteurization, leading to product spoilage. The application of dielectric barrier discharge-atmospheric cold plasma (DBD-ACP) as a non-thermal alternative for inactivating vegetative cells and spores of A. acidoterrestris have been investigated. Our research collectively demonstrates the potential of DBD-ACP to significantly reduce microbial load without compromising juice quality, as a complement to the traditional heat-dependent techniques. For example, A. acidoterrestris vegetative cells decreased by 6.63 and 6.47 log under only DBD-ACP treatment of 30 kV for 6 min in saline solution and apple juice, respectively. However, DBD-ACP was less effective against spores, resulting in 2.64 and 2.43 log reductions at the same treatment parameter. Enhanced sporicidal activity was observed when DBD-ACP was combined with mild heat (85°C), resulting in 4.03 and 3.95 log reductions in apple juice and saline solution, respectively. Morphological analyses and intracellular substance release studies indicated severe structural damage to both vegetative cells and spores, with a notable disruption in spore cortex and membrane integrity. Further investigations into the effects of culture temperature on microbial resistance showed that cells cultured at lower temperatures (25°C) exhibited reduced resistance to DBD-ACP. This susceptibility correlated with changes in fatty acid composition, notably a decrease in cyclohexaneundecanoic acid and an increase in more rigid fatty acids like cyclopentaneundecanoic acid, palmitic acid, and stearic acid, as confirmed by Fourier Transform Infrared Spectroscopy and Gas Chromatography-Mass Spectrometry analyses. Proteomic analyses provided deeper insights, revealing that spores cultivated at 25°C had diminished expression of proteins associated with sporulation, energy metabolism, and membrane transport, alongside a significant down-regulation in peptidoglycan and spore coat proteins compared to those at 45°C. These protein expression profiles suggest a structurally weaker spore formation at lower temperatures, enhancing susceptibility to DBD-ACP. These results highlight DBD-ACP's potential as a viable non-thermal technology for reducing A. acidoterrestris in apple juices, with implications for improving food safety protocols and extending product shelf life. Future studies will aim to optimize DBD-ACP parameters for industrial applications and explore its efficacy against other resistant microbial strains.

PO-33

In vitro study of the antifungal activity of chloride species and peroxide hydroxide generated during treatment with pulsed electric field - Potential use as sanitizing equipment and food handling art

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In systems with pulsed electric field (PEF), electrochemical reactions can occur in the treatment chamber. This can lead to partial electrolysis of

the solutes in the solution and subsequently generate long-lived oxygenic reactive species such as hydrogen peroxide (H2O2) and, in the case of solutions containing chloride compounds, lead to electrolytic production. In this context, the antifungal activity of PEF-treated solutions was tested against Penicillium spp, an opportunistic filamentous fungus that has previously been reported to cause infections in immunocompromised patients and to proliferate well in absorbent building materials and able to contaminate and deteriorate various food products, with special attention given to potential mycotoxin producers. Thus, in the present study Penicillium spp. conidial suspensions (10^5 CFU/ml) were treated with PEF at 0.5 kV/cm for 15 seconds and the effects on conidial germination and membrane damage were determined. The results showed that conidia germination was reduced by almost 80 % ($p \leq 0.05$). To better understand the antifungal effect induced by the PEF treatment, the saline solution was characterised after treatment. Chloride species (CS), oxidation-reduction potential (ORP) (mV), pH and hydrogen peroxide (H2O2) were measured immediately (0h), and at 1, 2, 3, 4, and 24 h after the treatment. Surprisingly, a significant increase in the concentration of CS (0.17 % ± 0.02 g/100 ml solution), ORP (245 ± 0.7 mV), and H2O2 (0.5 ug/ml) were found immediately after the PEF treatment and it remained relatively constant during 24 h. These components can cause oxidative stress and, thus, the loss of viability of the conidia. To confirm the electroporation of conidial cells after PEF treatment, DNA release was simultaneously analysed using propidium iodide dyes. After 72 h, DNA leakage in the PEF-treated conidia was found to be 5-fold higher in the saline solution than in the control. To check whether the conidia recovered after PEF treatment, the conidia were incubated for 24 h in a saline solution treated with PEF. The result showed a significant reduction ($p \leq 0.05$) of 2.47 ±0.02 log compared to the control sample. We hypothesized that the above-mentioned damage by electroporation was due to the accumulation of CS and H2O2 leading to the leakage of intracellular fluids through cell damage. CL and H2O2 can accumulate on the surface of the conidia and easily diffuse through the cell membranes, leading to cell death. In this framework, the extracellular release of DNA induced by electroporation can be considered a marker of cell damage. These results provide new guidelines for the use of saline solution treated with PEF as a disinfectant to sanitise food processing equipment and items.

PO-34

Value-added compounds extraction from apple by-products using pulsed electric fields

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Apple production is one of the main economic activities in Argentina's Río Negro and Neuquén provinces. Apples not meeting the specifications for fresh commercialization are mainly used to produce juice and cider, generating significant volumes of waste. This residual biomass, a source of bioactive compounds of industrial and nutritional interest, can be recovered to obtain high-value products. However, these compounds are difficult to isolate being usually associated with other constituents of the food matrix like lignin, pectin, and proteins. The goal of this work was to study different PEF treatments to enhance the extraction of polyphenols and antioxidant activity from apple peels. Samples obtained after sanitizing and peeling Red Delicious apples were immersed in distilled water in a custom-made chamber (parallel plate electrodes with a 2 mm gap) and subjected to different PEF treatments with electric field intensities of 9 and 15 kV/cm, 1 Hz and various treatment times. During the treatments, the electric current was recorded and the total energy per mass unit was computed. Immediately after PEF treatment, the samples were subjected to a water extraction to perform the analytical measurements. The effect of the PEF pretreatment was studied in terms of Total Phenolic Content (TPC) following the Folin-Ciocalteu method and Antioxidant Capacity (AC) by the bleaching method of the radical cation $ABTS^{+\cdot}$. A control sample prepared following the same procedure but without applying the

PEF pretreatment was conducted. Also, since the PEF treatment caused a temperature rise of up to 29 °C, an additional control was performed, subjecting the samples to that temperature. The statistical analyses of the results showed that the temperature rise caused a 35 to 40% increase in AC and TPC. For the treatment at 9 kV/cm, although increments were observed compared to the control, no significant differences with the control at 29 °C were found, suggesting that this electric field intensity was not strong enough to induce electroporation and/or release the compounds of interest from the food matrix. In contrast, treatments of 15 kV/cm yielded important increases in the TPC and AC even when compared to the temperature control. A PEF treatment of 15 kV/cm and 180 J/g led to 35 and 50% increments of TPC and AC respectively, while the biggest improvements were reached with 15 kV/cm and 750 J/g; this enabled doubling the amount of extracted phenols and triplicating the AC. In conclusion, the results of this work show that PEF technology can be applied to recover value-added compounds with antioxidant capacity and potential health functional properties from a food by-product, contributing also to advance towards a more circular economy.

PO-35

Modification of dietary fiber from apple bagasse by combining pulsed electric fields and enzymatic hydrolysis

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Large amounts of bagasse are discarded during apple juice production. These by-products, rich in dietary fiber (DF) and bioactive compounds, hold the potential for incorporation into foods as natural ingredients. However, their direct incorporation is limited by their insoluble DF content. Pre-modification of DF to improve solubility is necessary, although commonly employed chemical methods carry significant environmental implications. This study aimed to investigate the combined effect of enzymatic treatment and pulsed electric fields (PEF) on technological properties and DF content of apple bagasse. Apple (Malus domestica cv Golden Delicious) bagasse was subjected to enzymatic hydrolysis with Viscozyme (0.05-0.5%) for 1-4 h at 50 °C and/or PEF treatment (10 pulses,2 kV cm-1). Water retention capacity (WRC), oil retention capacity (ORC), solubility, soluble uronic acid (UA), and soluble neutral sugar concentrations (NS) of apple bagasse were determined. Overall, all treatment conditions led to increased solubility, soluble UA, and NS content. PEF raised WRC (27.24 +0.86 g/g) and ORC (6.68 + 0.79), regarding untreated bagasse (WRC = 25.97 + 0.63; ORC = 6.10 + 0.30). This may have been a result of an elevated level of exposure of both polar and non-polar groups. The highest content of soluble UA (10312.20 + 144 ppm), soluble NS (25.90 + 3 ppm), and solubility (69.93 + 0.9%) was obtained after applying PEF and 0.5% of Viscozyme during 4 h. The combination of treatments could break down cell walls, as well as different vegetable membranes and its action can cause an increase in soluble polysaccharides and solubility of DF. These results suggested that the combination of Viscozyme and PEF could be a potential strategy to obtain natural ingredients with improved technological properties and high soluble DF content from plant by-products.

PO-36

A study for achieving a higher effectiveness at less irradiation number on sterilization using pulsed plasma for cut vegetables packaging low oxygen atmosphere

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Pulsed plasma can be produced in a bag, even after packaging cut vegetables, by applying pulsed voltage to the package, sterilize the cut vegetables by generated active species, and reduce the risk of bacterial contamination. Currently, when the cut vegetables are packaged in the bag, in many cases, the inside of the bag is kept hypoxic atmosphere by replacing some oxygen gas to nitrogen gas to keep the vegetables freshness. However, under hypoxic atmosphere, both the concentration of generated ozone and direct inactiva-

tion efficacy by pulsed plasma are concurrently reduced. Though increasing the number of pulses improves the inactivation effect, it concurrently amplifies damage to the vegetables.

Therefore, this study is making full use of the active species generated by pulsed plasma to achieve higher inactivation effect of packaged cut vegetables at fewer pulsed plasma irradiation number under the controlled atmosphere (CA) conditions. According to our previous research and analysis, if we can further use the long-lived active species (such as ozone) generated by plasma at post-treatment, there is still space for improvement in inactivation effect. In this presentation, we provide an efficient solution to take the pulse plasma sterilization of packaged sliced vegetables to a new level, especially under the condition of 10? or lower (5%) condition.

By the experiment, we found that applying 50 shots (50 shots × 2) on each side with alternating flips resulted in higher inactivation efficacy than no-flips with same shots. This innovative approach allowed us to achieve superior inactivation results in normal, 10?and 5?conditions. We successfully resolved the issue about low killing rate by reduced ozone generation observed in low oxygen conditions, achieving almost same inactivation efficacy as the normal condition.

PO-37

Application of pulsed electric field (PEF) treatment before ultrasound-assisted convective drying of organic strawberries

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Drying is one of the oldest and most important processing methods in food technology, with the primary objective of reducing moisture content in food items to enhance their shelf life. However, this process is known to be highly energy-consumed and could potentially compromise product quality. Consequently, extensive research has been conducted in this domain, exploring advancements such as alternative heat sources during drying and diverse pre-treatment methodologies. Thus, the study aimed to investigate the impact of pulsed electric field (PEF) treatment and ultrasound-assisted convective drying on the course of the drying process and the properties of dried organic strawberries.

PEF energy with the values of 0.065, 0.433, and 0.800 kJ/kg was used as a pretreatment and the power of ultrasonic waves: 120, 160, 200 W during convective drying (temperature 70 °C, airflow 1.2 m/s) were used to evaluate its effects on drying kinetics and properties of dried organic strawberries of the Roxana variety. The scope of the work included the analysis of drying kinetics, color, and selected chemical properties: total polyphenols, total anthocyanins, vitamin C, sugars content, the ability to inactivate DPPH and ABTS radicals, and ferric-reducing power.

The shortest drying time was recorded for samples PEF0.43_US160 and PEF 0.065 (125 minutes), while the longest drying time was achieved for PEF0.43_US120 (175 minutes). With the use of higher energies and higher US powers, a material with a brighter color, lower color saturation, and a lower redness was obtained, with a smaller color deviation compared to fresh tissue, which was related to better preservation of the original characteristics of the material. Application of specific parameters - PEF with the highest energy and hybrid drying with the use of the lowest power of ultrasound, as well as those pre-treated with the lowest PEF energy and dried using the highest power of ultrasound, were characterized by a higher content of total polyphenols and anti-radical activity. However, the use of non-thermal methods resulted in a decrease in the content of vitamin C. Furthermore, the highest loss of total sugars was observed in the material treated with PEF_0.065, PEF0.043_US160, and PEF0.043_US200.

Using PEF as a pre-treatment and US during the hybrid drying process led to changes in the structure of strawberry tissue and, consequently, with appropriate process parameters, a shortened drying time and better physicochemical properties of organic strawberries can be obtained.

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PO-38

Unveiling the interplay between gliding arc discharge (GAD) plasma pretreatment and pulsed electric field (PEF) on Chlorella vulgaris microalgae

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The combination of plasma and PEF is of interest to enhance the treatment efficacy of PEF-induced effects. The mechanism is proposed to rely on plasma-induced radicals to induce lipid oxidation, compromising membrane integrity and increasing combined treatment efficacy over plasma or PEF alone. In addition, the combined treatment has been shown to increase intracellular ROS generation, affecting cytotoxicity and cell death pathways. However, how the combined treatment affects microalgal is poorly understood. Therefore, this study was designed to investigate the effects of GAD plasma in combination with PEF on Chlorella vulgaris. To achieve this, the GAD reactor was powered by an AC power supply consisting of a 270 kHz AC generator and a high voltage transformer (1:33) . Specifically, algal biomass was treated with plasma using: air flow of 22.8 l/min, electrode-to-suspension distance of 30 mm, and treatment duration of 300 s. The discharge voltage of the GAD plasma was controlled by adjusting the output voltage of the AC generator (Vout) within the range of 50-250 V. The plasma-treated algal suspension was then subjected to PEF treatment, with parameters including a pulse duration of 7 μs, 1-10 exponential pulses with a frequency of 1 Hz and a resulting electric field strength of 24-25 kV/cm. Changes in suspension and cell characteristics of the untreated and treated algal suspensions were evaluated.

The results obtained show that the induced effects of the combined treatment depend on the plasma voltage parameters. The application of a lower plasma voltage (Vout= 130 V) alongside PEF pulse increased algal cell permeability comparable to that of PEF treatment alone. At 24 h post-treatment, nucleic acid and protein release was observed at levels comparable to ultrasound-treated control. These observations were accompanied by a loss of cell morphology, suggesting that programmed cell death may have been triggered. In contrast, increasing Vout above 170 V resulted in a variable response of algae to the combined treatment. While the combined treatment induced cell permeability, DNA leakage tended to decrease with increasing Vout. This effect was particularly pronounced after exposure to Vout of 210-250 V in combination with PEF, where DNA release was similar to that of untreated algae. A similar trend was observed for protein release, with a significant decrease after exposure to high-voltage plasma and PEF. Furthermore, microscopic examination showed that algae, although metabolically inactive, retained a structure similar to intact cells. In conclusion, different plasma output voltages induce variable changes in algal cells after combined plasma and PEF treatment. These changes affect the efficiency of PEF for protein extraction and alter the mechanism of death of C. vulgaris.

Acknowledgement: This work was supported by the Research Council of Lithuania under Grant P-MIP-22-257.

Poster session

Coffee Break and Poster Session
Sep 17, 15:20 - 16:50

PO-39

Minimally invasive electrochemotherapy for the treatment of hepatocellular carcinoma: single centre study

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Introduction: Electrochemotherapy is a safe and effective method for the treatment of hepatocellular carcinoma (HCC). Initially, electrochemotherapy was used for HCC in the setting of open surgery. However, in recent years, with the development of newer electrodes, the minimally invasive approach (i.e. percutaneous or laparoscopic) of electrochemotherapy has also been described in the literature.

Methods: We retrospectively analyzed the medical records of all patients with HCC treated with electrochemotherapy using a minimally invasive approach at University Medical Centre Ljubljana between January 2018 and March 2024. Thus, we only included patients who were treated with either a percutaneous or laparoscopic approach.

Results: We identified 7 patients with HCC treated with electrochemotherapy via a minimally invasive approach (3 patients via a percutaneous approach and 4 patients via a laparoscopic approach). In total, electrochemotherapy with bleomycin was performed on 8 HCCs (1 patient had two HCCs). Electrochemotherapy was feasible in all 8 lesions and none of the patients experienced adverse effects. During the median follow-up period of 9 months, a complete response to treatment was observed in all treated lesions.

Conclusion: Minimally invasive electrochemotherapy for HCC proved to be safe, feasible and effective in all 7 patients. The percutaneous approach should be considered as the first method as it is the least invasive. However, in cases where percutaneous approach is contraindicated (e.g. close proximity of tumor to vital organs), the laparoscopic approach should be used.

PO-40

Advancing Cancer Treatment: Automated Application of Electric Pulses and Radiation Targeting Stem Cells guides by artificial intelligence (AI) algorithm

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Tumors frequently show resistance to traditional therapies due their extreme heterogeneity. Cancer Stem Cells (CSCs) play a crucial role in tumor maintenance and recurrence. Therefore, alternative approaches, possibly targeting CSCs, are necessary. Among emerging therapies, high intensity ultra-short pulsed electric fields (PEFs) are considered extremely promising. Our previous results demonstrated the ability of a specific electric pulse protocol to selectively affect medulloblastoma CSCs by inducing irreversible electroporation and radio-sensitization while sparing normal cells. In contrast, the same PEF-exposure protocols, in a glioblastoma model, exhibited reversible electroporation, leading to lower levels of cell death and induction of neuronal differentiation, which conferred radio-resistance.

The aim of our project is to develop an automated device for the application of electric pulses and subsequent doses of ionizing radiation suitable for different types of tumors, allowing practical, efficient, and personalized therapy for each type of tumor. Our study aims to automate the application of PEFs exposure and ionizing radiation using an artificial intelligence (AI) algorithm. AI approach will help the operator to select an optimal PEF/radiation exposure protocol to eradicate CSCs of specific tumor in treatment. Different human cancer model will be used to provide data to train the AI, achieve maximum therapeutic benefit and minimize side effects. The “trained” AI can play a key role in automated delivery system, starting by the selection of PEF parameters administer the personalized dose

of ionizing radiation in a precise time lapse for each specific tumor to treat.
Results of this study will provide new opportunities to concretely improve existing therapeutic options and develop more targeted and effective treatments for cancer.

PO-41

Optimal Interphase Delay to Mitigate Cancellation Phenomenon in Bipolar Pulse Electrochemotherapy with Cisplatin

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Nanosecond bipolar pulses trigger the cancellation phenomenon when the second pulse either partially or entirely nullifies the impact of the first pulse, thereby reducing the permeabilization of cells' plasma membrane and the overall efficacy of electroporation. To address this issue, creating a time delay between the positive and negative phases of the bipolar pulses during electroporation procedures has been suggested as a potential solution, although the specific thresholds remain unclear. Our study examines the impact of various interphase delay durations (ranging from 0ms to 95ms) on symmetric bipolar nanosecond electrochemotherapy (300- and 500 ns pulses) with cisplatin, using 10 Hz, 100 Hz, and 1 kHz repetition frequency protocols in vitro. Mouse hepatoma MH-22a cell line is used as a model. We evaluate the dependence of cell plasma membrane permeabilization and viability on different bipolar pulsed electric field protocols and the resultant dependence of cisplatin-based electrochemotherapy efficacy in the context of bipolar cancellation. It was demonstrated that the cancellation phenomenon is triggered when symmetrical 300, 500 ns bipolar pulses of 4–13 kV/cm are employed, which significantly hinders the efficacy of permeabilization. These effects are more profound when there is no delay between separate phases. At the same time, a threshold delay exists when the phenomenon is minimized, enabling treatment efficiency comparable to monophasic pulses with identical parameters.

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PO-42

Analyzing Breast Cancer Cell Electroporation: Perspectives from Scanning Probe Microscopy Methods

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Cancer cells exhibit unique metabolic characteristics, e.g., high ROS levels, including O2-, OH-, and H2O2. These ROS are crucial in cellular physiology; cancer cells display elevated ROS levels compared to their healthy counterparts. This distinction is pivotal for early identification and understanding of cancer cells' physiological and pathological activities at a single-cell level. Scanning electrochemical microscopy (SECM) is useful for investigating biologically-modified surfaces, including living cells [1]. Several modes of the SECM can be applied to investigate redox processes in living cells, such as feedback, generation-collection (GC-SECM), and redox competition (RC-SECM). GC-SECM mode is the most sensitive for detecting redox species. SECM allows the investigation of substrate topography and its local reactivity with high resolution, as well as the registering of electrically active materials secreted by the cells. The redox activity of human myocardium-derived mesenchymal stem cells (hmMSC) was investigated by GC-SECM mode, using 2-methylnaphthalene-1,4-dione (menadione, MD) as a redox mediator [2]. SECM measurements showed that healthy 2D-cultivated hmMSCs had much higher redox potential to reduce the MD compared to that of the 2D-cultivated pathological hmMSC. In addition, investigation of 2D- and 3D-cultivated hmMSCs showed that 3D cell cultivation

conditions positively affected the redox potential of dilated myocardium-derived hmMSCs and improved resistance of healthy hmMSCs to MD. SECM's non-invasive nature allows for measuring membrane permeability, cell respiratory activities, and other intra- and extra-cellular processes in cancer and other types of cells without touching them [3]. The human skeletal muscle-derived mesenchymal stem/stromal cells (SM-MSCs) and their subpopulations by Alternating Current SECM (AC-SECM) were stimulated, and the efficiency of myogenic differentiation was evaluated. CD56(+) showed higher redox activity than CD56(-) cells, their better response to AC electric field-based stimulation. These results encouraged us to apply SECM for electroporation of human breast cancer cells. Several tests were performed, including redox mediators, electric pulses, and SECM modes. Cells' mechanical properties were measured by an atomic force microscope (AFM), and there was a good correlation between SECM and AFM results.

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PO-43

Synergistic Bipolar Irreversible Electroporation (SBIRE): A Novel Approach for Effective Tumor Removal without Inducing Muscle Contractions

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Irreversible electroporation (IRE) has emerged as a promising modality for tumor treatment, leveraging the controlled application of electrical pulses to induce cell death. However, the associated muscle contractions during the procedure pose challenges. This study introduces a novel approach, termed Synergistic Bipolar Irreversible Electroporation (SBIRE), aimed at achieving tumor ablation without the undesirable side effect of muscle contraction. SBIRE involves the simultaneous application of ns (±1600 V between electrodes with a spacing of 0.2 cm or ±8000 V between electrodes with a spacing of 1 cm, ±500 ns, "+" to "-" delay 1 μs, "-" to "+" delay 200 μs, 5 cycles, 1 Hz) and μs (±300 V between electrodes with a spacing of 0.2 cm or ±1500 V between electrodes with a spacing of 1 cm, ±2 μs, "+" to "-" delay 2 μs, "-" to "+" delay 1000 μs, 25 cycles, 1 Hz) bipolar electrical pulses, strategically designed to synergistically target tumor cells while minimizing impact on adjacent muscle tissue. The experimental setup includes in vitro and in vivo studies utilizing tumor cells and animal models to assess the efficacy of SBIRE. Preliminary results demonstrate the effectiveness of SBIRE in inducing irreversible electroporation within the tumor microenvironment, leading to cell death, and the ablation effect is better than other parameter forms (24.41±0.23 mm3 vs 19.54±0.25 mm3, $p<0.0001$). Importantly, muscle contraction is significantly reduced compared to traditional IRE procedures, highlighting the potential of SBIRE to enhance patient comfort and procedural success. To examine the immune response elicited by SBIRE and its potential in mitigating the advancement of post-ablation melanoma, a subcutaneous B16-OVA melanoma model was employed in C57BL/6J mice. The administration of IRE effectively suppressed the progression of melanoma, resulting in tumor-free mice following a

subsequent injection of the therapy. Furthermore, an increased number of CD8+ T and dendritic cells, but not CD4+ T, B, or NK cells, invaded the region surrounding the ablation site on the seventh day following IRE. Tumorigenesis was inhibited in the SBIRE-treated group of mice through secondary inoculation at the distal end, indicating the existence of a protective immune response. Ultimately, IRE has great potential as a technique to stimulate CD8+ T cell immunity and hinder the advancement of melanoma following ablation. The development of SBIRE represents a significant advancement in the field of tumor ablation, addressing a key limitation associated with muscle contraction during IRE. This technique not only offers a more targeted and precise approach to tumor treatment but also holds promise for minimizing procedural side effects.

PO-44

Cisplatin and bleomycin increase cell mortality during partial irreversible electroporation on hepatocellular carcinoma spheroids model

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Irreversible electroporation (IRE) is a non-thermal method of tissue ablation in which high field strength electrical pulses are applied to unwanted tissue, such as cancerous tissue, to cause cell death through irreversible membrane permeabilization. This technique has the advantage of being selective because, unlike other thermal ablation methods, malignant tissue is targeted with the electrodes without affecting the surrounding tissue and vessels. In the field of oncology, numerous clinical trials have been conducted with irreversible electroporation, giving encouraging results in tumors and cancers of the liver, pancreas, kidney and prostate. In most cases, however, the treatment appears to be effective only for small tumors and at an early stage. Relapses are observed in particular because certain areas of the tumors are only reversibly permeabilized, which means that the distribution of the electric field is not homogeneous and leads to partial ablation. To overcome this limitation, we propose to couple the application of an irreversible electric field with low concentrations of cytotoxic drugs that are internalized in the reversibly permeabilized cells and cause their death.
Specifically, cells from murine hepatocarcinomas in suspension and in spheroids were treated according to the irreversible electroporation protocol with 80 pulses of 100 µs at 1 Hz with field strengths of 1000, 1500, 2000 and 2500 V/cm with or without cisplatin or bleomycin. After comparing cell viability with or without cytotoxic drugs, our preliminary results showed that the addition of drugs increased cell mortality when ablation is partial, i.e. at 1000 and 1500 V/cm. In the 2000 and 2500 V/cm protocols, the addition of drugs had no effect, as ablation is already complete without drugs. Similar results were observed in cells suspension and spheroid models. In addition, studies of the cell death process have shown that the addition of drugs does not allow an increased release of ATP or increase the percentage of apoptotic cells. Thus, coupling IRE as an ablation method with small amounts of cytotoxic drugs could enable the eradication of cells in tumor areas where irreversible electropermeabilization occurs only partially.

PO-45

Microplastic particles (MPs) delivery by electroporation (EP) and their effects on the development of breast cancer cells

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Microplastic particles (MPs) (diameter from 0.1 μm to 5 mm) pose a huge threat to the natural environment. Polystyrene (PS) and polyvinyl chloride (PVC) are commonly present in microplastic particles. They get into the body through food, inhalation and skin. They can cause cytotoxicity, oxidative stress, metabolism disorders, DNA damage, inflammation, immunological reactions, and even carcinogenesis. Breast cancer is the most common form of cancer and the second leading

cause of death due to cancer in women. The link between microplastic particles and breast cancer is a topic that requires further scientific research.

Our study aimed to determine the cytotoxicity of microplastic particles delivered by electroporation on viability of breast cancer cells, and the level of stress oxidative markers.

For the study, we used human breast cell lines: MDA-MB-453 and MCF-7. A solution with polystyrene was used as microplastic particles for testing. Electroporation conditions in the target delivery of MPs, were selected based on the protocols used in electrotransfer and drug carrier delivery. Single milli- and microsecond pulses with low electric field strength were applied. The impact of MPs on cell viability after applying different reversible electroporation parameters was assessed using two independent tests (MTT and SRB). The phenomenon of oxidative stress was determined by examining the degree of lipid peroxidation. Immunocytochemical and -fluorescent methods were used to assess the expression of antioxidant defense markers SOD1-3, GLRX and NOS1-3.

The results showed that microplastic particles (MPs) cause cytotoxicity to both cancer and normal cell lines. A significant decrease in cell viability was observed, especially in the case of cancer cell lines. The synergistic effect of electroporation and MPs was a significant decrease in cell viability, especially in cancer cell lines. The additional use of EP increased the expression of oxidative stress markers compared to the use of MPs alone during incubation in solution with polystyrene. After the use of EP and MPs, an increase in the degree of lipid peroxidation was observed in breast cancer cell lines.

The investigations showed that MPs can induce inflammation and oxidative stress in tissues, which may be associated with an increased risk of developing cancer, including breast cancer. In our research, the best anticancer effect was observed after applying EP with MPs. The cell survival rate was lower than that of the method using only one (EP or incubation in MPs solution). The proposed research enabled us to determine the impact of microparticles on human cells in normal and cancerous breasts. The obtained results may contribute to the development of protocols for further in vivo studies and, in the future, the application of new therapeutic methods of breast cancer treatment.

Financing: SUBZ.D260.24.076

PO-46

Enhanced Visualization and Control of Drug Distribution in Electrochemotherapy Using Indocyanine Green with Bleomycin in a Murine 4T1 Mammary Tumor Model

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Electrochemotherapy (ECT) is a promising treatment modality in veterinary that combines short electric pulses with chemotherapeutic agents to enhance drug uptake and antitumor efficacy. Our study introduces a novel application of ECT using a combination of indocyanine green (ICG) and bleomycin (BLM), validated in the 4T1 murine mammary tumor model. This approach is based on the established efficacy of ECT with bleomycin in treating various cancers and explores the use of ICG to monitor and control drug distribution during treatment.

We employed the 4T1 cell line to evaluate the viability post-ECT using the MTT assay at 24 and 72 hours. The experimental setup included the administration of drugs both as free agents and encapsulated within bilosomes, under the conditions of the ESOPE protocol. Additionally, fluorescent microscopy was utilized to visualize the uptake dynamics of ICG and the ICG-BLM combination facilitated by electroporation.

Our preliminary findings indicate that ICG does not

adversely affect the ECT protocols in vitro, maintaining cell viability without significant detriment. Importantly, our in vivo trials demonstrated that ICG can effectively aid in the real-time visualization of drug distribution, ensuring precise delivery and aiding in the identification of sentinel lymph nodes during surgical interventions.

This study not only supports the safety and efficacy of ICG-enhanced ECT in vitro and in vivo but also underscores the potential of this method in improving the precision of drug delivery in cancer therapy, particularly in breast cancer models. Our results advocate for further investigation into the clinical applicability of this combined approach in managing mammary tumors with the treatment combining ECT with fluorescent guided surgery.

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PO-47

Exploring Immune Stimulation for Cancer Treatment

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Background: Immunooncology, an emerging field bridging medicine and cell biology, aims to activate the immune system (IS) against cancer cells. Despite the immune system's ability to recognize and eliminate cancerous cells, they often evade detection by mimicking normal cells. This study focuses on utilizing electroporation (EP), which involves short electrical impulses to alter cell membrane structure and enhance IS response against cancer.

Objectives: The hypothesis posits that EP can activate surface molecules on IS cells and augment the secretion of factors, thereby improving the elimination of cancer cells by the immune system.

Materials and Methods: Murine colon cancer cells (Ct26Wt) and murine macrophages (P388) were employed in the study. Nanosecond electrical pulses (5-9 kV/cm, 10ns, 200; +/- Ca2+) were used to stimulate P388 cells. Viability both cell lines were checked by MTT assay. Interactions between various cells were observed using 3D Cell Explorer Fluo holotomographic microscope and a confocal microscope. Immunofluorescence was utilized to determine activation markers of the IS (IL-10, INF-ɤ).

Results: Analysis revealed changes in markers expression on P388 cells after exposure to EP or CaEP. Initial observations hinted at increased cancer cells (Ct26Wt) cytotoxicity post-CaEP compared to P388 cells. Microscopic visualization revealed interactions between macrophages and tumor cells, as well as the death of Ct26Wt cells. However, further exploration and wider parameter testing are required.

Conclusions: Immunooncology, though nascent, displays promising potential in cancer therapy. Continued research and technological advancements hold the promise of refining treatment strategies. Immunooncology might emerge as a pivotal approach in combating not only cancer but also various other diseases in the future.

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PO-48

Electrochemoterapy in personalized medicine.A predictive in vitro model for electrochemotherapy in metastatic melanoma

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Personalized medicine is the new frontier in the treatment of cancer. This study aims at setting up a predictive in vitro model of the clinical response of cancer patients to recently introduced treatments (target therapy, electrochemotherapy) and to new potential therapies.

The necessity of personalized therapy tailored on the necessities of the patients is of paramount im-

portance; to obtain such a degree of personalization is fundamental to use an experimental model that resemble the characteristics of the tumor in vivo such as tridimensionality and cell-cell and cell-matrix interactions. One of the most common and lethal tumor type is metastatic melanoma. This work aims at setting up a platform for the screening of novel drugs against metastatic melanoma (targeted therapy) in combination with electrochemotherapy in melanoma spheroids. During the first phase of the study cells from a human melanoma cell line (A375) will be used to generate spheroids, a 3D culture model, or will be seeded in hyaluronic acid scaffolds and/or self-aggregating peptide matrices, to resemble the microenvironment of the tumor and to allow the tumor cells to generate their own extracellular matrix. Once the in vitro 3D model of tumor tissue is characterized, these scaffolds will be used to test different drugs and their dosages in matrices seeded with cells derived from melanoma patients. Patients with in-transit melanoma metastases will be the source of cancer cells. After informed consent, cells from human in-transit nodules will be separated, sorted, and cultured. Electrochemotherapy will be then applied to 3D cell cultures to increase the uptake of drug molecules by the cancer cells, decreasing the drug dosage. The effects of the treatment will be assessed by evaluating the spheroid growth and morphology, extracellular matrix production, gene expression, expression of membrane receptors (e.g. MC1R) and secondary messengers (cAMP).

PO-49

Modulating Electrochemotherapy Efficacy in Ovarian Carcinoma with Bipolar nsPEFs: Insights into Cell Membrane Permeabilization and Reactive Oxygen Species Levels

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Drug delivery (i.e., cisplatin; CDDP or bleomycin, BLM) through nanosecond pulsed electric fields (nsPEFs) represents an emerging area within electroporation (EP)-based therapies, with the potential to replace conventional European protocols for electrochemotherapy (ECT). Shortening pulses' duration reduces the frequency of painful muscle contractions, minimizes oxidation damage, and enhances treatment homogeneity by incorporating higher-frequency elements. Moreover, the last decade brought the discovery of the bipolar cancellation (BPC) phenomenon. It was defined as the ability of electrical pulses with negative polarity (↓) to reduce or even eliminate the effects of the preceding positive polarity pulses (↑) treatment. However, the exact molecular mechanism remains unknown.

The aim of our study was to analyse the influence of the bipolar (BP) PEFs on the effectiveness of BLM-based ECT of ovarian carcinoma (OC) cell lines (MDAH-2774 and SKOV-3). Cells were exposed to pulses delivered in bursts but as uni- or bipolar, symmetrical, or asymmetrical sequences with a duration of 500 ns (14 kV/cm) or 50 µs (4 kV/cm). The effectiveness of cell membrane permeabilization was investigated using Yo-ProTM-1 uptake analysis. Cell viability was determined by MTT assay. Moreover, changes in ROS levels were observed through DCFDA assay and IF staining.

The experiments that were performed revealed reduced membrane permeabilization after cells' exposure to bipolar nsPEF and increased cell survival. Those effects were enhanced for symmetrical bipolar nsPEF protocol. Interestingly, using asymmetrical BP protocol caused increased ROS levels compared to symmetrical PEF.

In conclusion, the results indicate that modulation of the BPC phenomenon and ROS level might be possible, depending on the symmetry of the used BP PEF protocol.

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PO-50

Curcumin-Electroporation downregulates key heat shock and heat stable proteins in Curcumin supplementation rats

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Breast cancer is a complex and heterogeneous disease. In 2024, 310,720 women will be diagnosed with breast cancer in the U.S. It is alarming as every two minutes, a woman is diagnosed with breast disease in the U.S. alone. Various factors have been identified as potential contributors to the development and progression of breast cancer, including genetic mutations, hormonal changes, exposure to chemicals and drugs, as well as lifestyle factors such as diet. One important aspect of lifestyle that has been implicated in breast cancer is the Western diet, which is characterized by a high intake of processed foods, saturated fats, and sugar.

The Western diet has been shown to increase the risk of breast cancer by 14%. Furthermore, conventional therapies, including medications, surgeries, and radiation treatments, have high side effects and toxicity. However, studies have demonstrated that curcumin (Cur) possesses anti-inflammatory, antioxidant, and anticancer properties, which are inexpensive natural compounds. Similarly, our previous in vitro studies have demonstrated that the electrical pulse (EP) application can further enhance the effectiveness of curcumin against breast cancer cells in electrochemotherapy (ECT). However, the working mechanism is not well known. Towards this, we present a high-throughput, label-free quantitative proteomic study of the anticancer effects of EP with intratumoral curcumin administration (EP+100µL Cur) on induced mammary tumors in female rats. The rats were fed either a Western diet (W) or a Western diet supplemented with 1% curcumin (W+Cur). Here, multiple EP (1000V/cm,100µs,8 pulses at 100ms intervals between pulses) were administered using needle array electrodes.

We identified over 1000 proteins with differential expressions in the W diet and W+Cur diet. Further, a distinct downregulation of CARHSP1-calcium-regulated heat stable protein 1 and HSPB1, also known as heat shock protein 27. The protein-protein interaction shows that proteins like CARHSP1 that respond to calcium levels typically have roles in various cellular processes. Calcium is a crucial second messenger in signaling pathways, and proteins that are regulated by calcium are often involved in processes such as cell growth, apoptosis, and the response to cellular stress.

Likewise, Hsp27 has been shown to modulate signaling pathways that contribute to cell growth, survival, and resistance to chemotherapy. It can promote cancer cell survival against chemotherapeutic agents by upregulating the Akt/mTOR signaling cascade and inactivating p53, thus inhibiting chemotherapy-induced apoptosis. Overexpression of Hsp27 in cancer cell lines is associated with poor prognosis and treatment resistance. However, EP+Cur treatment shows downregulation and is favorable to the treatment of breast cancer. Overall, the EP+Cur treatment approach and alteration of protein/gene can be explored as potential targets for breast cancer and improving patient outcomes.

PO-51

Gene electrotransfer of tumor and muscle tissue with clinically used electric pulse parameters

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Gene electrotransfer, a technology for delivering genetic material into cells and tissues, strongly relies on electric pulse parameters to achieve optimal transfection efficiency. Various parameters such as pulse duration, amplitude, and number of pulses play crucial roles in determining the efficiency. Therefore, in the study we first tested different electric pulse protocols in vitro in murine B16F10 melanoma, L929 fibroblast and C2C12 myoblast cells. One million cells were mixed with 10 µg of plasmid encoding GFP and placed between electrodes with 2.5 mm gap. Electric pulse protocols, delivered by Genedrive electropor-

ator (IGEA, Carpi, Italy) were as follows; GET1: 250 V, 100 µs, 8 pulses, 1 Hz; GET2: 300 V, 100 µs, 8 pulses, 1 Hz; GET3: 300 V, 100 µs bipolar pulse, 3 pulses, 1 Hz and GET4: 300 V, 100 µs, 8 pulses, 5 kHz. Cells were seeded into 96-well plate and placed into Cytation1 microplate reader, where fraction of transfected cells and median fluorescent intensity was monitored every 2 h for 24 h. Further, two most clinically relevant electric pulse protocols (GET2 and GET4) were used for transfection of 20 µg of plasmid with Cliniporator (IGEA) in B16F10 murine melanoma tumors and muscle using electrodes with 6 mm gap. After 48 h tissue was excised, frozen sections were stained with Hoechst, images were captured with microscope and analyzed. In vitro fraction of transfected cells was higher in B16F10 (≈35%), when using GET1 or GET2 protocol, compared to L929 or C2C12 cells (≈15-20%). When using GET3 (≈5-10%) or GET4 (≈20%), the fraction of transfected cells was similar regardless of cell type. However, median fluorescence intensity was similar for all cell lines when using GET1, 2 or 4 protocols and significantly lower (compared to other pulse protocols) in C2C12 and B16F10 cells, but not in L929 cells when using GET3 protocol. In tumor tissue both, GET2 and GET4, protocols resulted in similar percent of transfected cells in B16F10 melanoma tumors, approximately 2%, which is in line with previously published research. However, only the GET 4 pulse protocol was successful in transfecting murine muscle. The findings of the study are important, since GET4 pulses, which are routinely used in clinical practice for electrochemotherapy, successfully delivered genetic material into the tumors and muscle. This indicates that concomitant electrochemotherapy and gene electrotransfer could be feasible, using only one electric pulse protocol.

PO-52

Optimisation and validation of electroporation protocols in 3D bioprinted tumour models of colorectal cancer

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Background: Electroporation offers the possibility for gene transfer or enhanced chemotherapy delivery. While an ultimate goal of this method is its wider application in the clinic and improvement of therapeutic strategies in oncology, the in vitro experimental settings are not necessarily representative of in vivo electroporation. Therefore, new preclinical model systems should be established. Three-dimensional (3D) bioprinting allows for much better mimicking of the tumour architecture and microenvironment compared to standard monolayer cell culturing techniques. However, 3D biofabrication strategies have not been implemented in vitro electroporation experiments yet. Therefore, the aim of this pilot study was to establish protocols for the successful transfer of chemicals into 3D biorprinted tumour models of colorectal cancer (CRC).

Materials and methods: A Clinivet electroporator (IGEA, Italy) and a BioX extrusion-based bioprinter (Cellink, Sweden) were used to carry out the initial experiments. Caco-2 CRC cells were bioprinted at density of 30 million cells/ml in a collagen-based bioink (MatriChem, Bulgaria). Electroporation protocols were optimized for 1) the buffer composition and 2) the duration, strength and number of pulses. Assessment of the percentage electroporated cells was carried out with propidium iodide (PI) and Calcein AM staining of the 3D bioprints.

Results: We have managed to successfully deliver PI inside 3D bioprinted CRC tumor models with efficiency of approaching 80%. Cell viability was not significantly affected by the protocol, allowing the assessment of the effect of chemotherapeutics.

Conclusion: This is the first study using electroporation in a 3D bioprinted tumour model of CRC. This opens the possibility for more predictive pre-clinical studies related both to chemoelectroporation and gene (or miRNA) transfer.

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PO-53

The bystander effect after electroporation with microsecond and nanosecond pulses

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Anti-cancer treatment by electrochemotherapy is based on the phenomenon of electroporation, where tumor tissue cells are exposed to high-intensity, short external electric fields. When the transmembrane potential exceeds a critical value, cytotoxic drugs (such as bleomycin) can be introduced into the cell cytosol during electrochemotherapy or calcium electroporation. Cell electroporation can be performed using microsecond and nanosecond-duration electrical pulses. Conventional microsecond electroporation typically requires about 100 µs (intensity 1–1.6 kV/cm), nanosecond electroporation pulse duration decreases to 100–300 ns, and the amplitude of used pulses increases to 10–19 kV/cm. It is believed that the effect on the cell induced by nanosecond electroporation may significantly differ from that of conventional microsecond electroporation.

Recently, we demonstrated that a bystander effect is observed after microsecond electric field-induced electrochemotherapy. Cells indirectly affected by electroporation and anticancer agents influence neighboring cells (Ruzgys et al., 2021). In this study, we aimed to investigate the bystander effect after nanosecond electroporation and compare it with microsecond electroporation.

Three cell lines are used in the study: CHO-K1, A-549, and 4T1. The efficiency of electrotransfer is evaluated by changing the pulse amplitude, while cell viability and bystander effect after exposure are assessed using BLM and calcium ion electrotransfer. During electroporation using nanosecond pulses, experiments were conducted using a nanoelectroporator (developed by Prof. Vitalij Novickij of VilniusTech), and microsecond pulses were applied using the Amber Charge electroporator (Amber Charge, Lithuania). The bystander effect was induced by transferring cell growth medium onto untreated cells 48 hours after exposure to electric fields. Cell viability was determined 30 minutes, 48 hours (flow cytometry), and 6 days (clonogenic assay) after transferring the bystander medium.

A significant difference in the use of nano- and micro-pulses with CHO-K1, 4T1, and A-549 cell cultures was observed when performing the clonogenic assay. It was found that the strongest bystander effect was observed in A-549 cells. Similarly to microsecond pulses, nanosecond pulses contributed to the induction of the bystander effect. Contrary to that, the bystander effect was observed in CHO-K1 and 4T1 cell cultures after microsecond electroporation but not after nanosecond electroporation.

PO-54

Calcium-mediated Inactivation of Drug-resistant Microorganisms Using Pulsed Electric Fields

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Ca2+ ions are universal signal mediators that regulate many cellular functions, however, recently it was discovered that when calcium is combined with pulsed electric fields it shows high applicability as anticancer treatment. During the treatment pulsed electric fields are used to permeabilize cell membrane (cell electroporation) allowing high concentrations of Ca2+ ions enter the cell resulting in ATP depletion and rapid cell death. In this work, we show that calcium electroporation can be successfully used to kill even drug-resistant or gram-negative bacteria, while other microorganisms are expected to have even higher sensitivity to the treatment due to thinner cell wall and higher susceptibility to PEF. We have characterized the effects of 7.5–20 kV/cm microsecond (1–100 μs) and nanosecond pulse sequences (500 ns, n = 200–2000) for inactivation of S. aureus, methicillin-resistant S. aureus (MRSA), P. aeruginosa and

have shown that it is possible to ensure reversible electroporation, but to use Ca2+ ions (2, 5 mM) for induction of a significant cytotoxic effect similar to the Ca2+ electrochemotherapy, which is used to treat cancer. It is also shown that microsecond pulses are more effective than bursts of nanosecond pulses even when the energy input is equivalent. Importantly, that the both the drug-resistant or gram-negative bacteria are susceptible to the treatment, which potentially shows good applicability both in food processing related context and for wound sterilization procedures. It may allow to partially counter the arising problem of antibiotic resistance.

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PO-55

The Effects of Bipolar Cancellation Phenomenon on Nano-Electrochemotherapy of Melanoma Tumors

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The phenomenon known as the cancellation is observed when bipolar nanosecond electric field pulses are used, which results in reduced electroporation efficiency when compared to unipolar pulses of the same parameters. Basically, the negative phase of the bipolar pulse diminishes the effect of the positive phase. Our study aimed to investigate how bipolar cancellation affects Ca2+ electrochemotherapy and cellular response under varying electric field intensities and pulse durations (3–7 kV/cm, 100, 300, and 500 ns bipolar 1 MHz repetition frequency pulse bursts, n = 100). As a reference, standard microsecond range parametric protocols were used (100 µs x 8 pulses). We have shown that the cancellation effect is extremely strong when the pulses are closely spaced (1 MHz frequency), which results in lack of cell membrane permeabilization and consequent failure of electrochemotherapy in vitro. Finally, we have performed a pilot in vivo study, where we compared the efficacy of monophasic (5 kV/cm, 500 ns, n=100) and biphasic sequences (5 kV/cm, 500 ns + 500 ns, n = 100) in the context of Ca2+ electrochemotherapy (B16 F10 cell line, C57BL/6 mice, n = 28). The data indicated, that mice treated with bipolar pulses did not exhibit prolonged survival when compared to untreated control (tumor-bearing mice), therefore, the bipolar cancellation phenomenon was also occurrent in vivo significantly impairing electrochemotherapy. At the same time, monophasic nanosecond pulses were as effective as the ESOPE sequence resulting in tumor reduction following the treatment and prolonged survival of the animals.

PO-56

Reversible and irreversible electroporation mechanisms: an in vitro study on two pancreatic cancer cell models

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Electroporation (EP) is the increase of permeability of plasma membranes to ions and macromolecules, by intense and short pulsed electric fields (PEF), that induce an over-threshold increase of the physiological transmembrane voltage. Below a certain electric field threshold, the cell can restore the plasma membrane integrity (reversible EP, REP). Above the threshold, irreversible EP (IRE) occurs, leading to cell death due to extensive, non-repairable damage. The advantages of REP, and IRE are widely recognized in biotechnology and medicine. Both REP and IRE applications rely on pulses with duration in the microsecond to millisecond time scale, whereas the main differences are in the number of applied pulses, and/or in the electric field strength. Despite the established applications, physical and biological mechanisms of EP have not been fully elucidated, which implies a poor control of PEF parameters, and definition of pulsing protocols by trials and errors. Indeed,

a better insight into the role of pulse parameters in determining membrane permeabilization or cell death is crucial to control and optimize therapeutic protocols.

Here we report on the preliminary results of the activities carried out in the framework of a national project (Digging into rEversible and irreversible ElectroPoration: in vitro and in silico multiphysical analyses on cEll modelS for cancer Treatment – DEEPEST, PRIN 2022, Italian Ministry of University and Research) which aims to develop and standardize a robust and sensitive methodology for a multi-physics (electromagnetic, thermal and biological), multi-level (single cells, 2D and 3D cell systems) analysis of EP mechanisms, under REP and IRE pulsing conditions, that can be tailored to different cell models, and used to improve outcome and optimization of pulsing protocols.

First experiments were carried out on PANC-1 and MIA PaCa-2 pancreatic cancer cell lines to identify REP and IRE conditions to be further investigated for molecular characterization of the cellular stress mechanisms leading to damage and death. Cells in a low-conductivity pulsing buffer were exposed in 4 mm EP cuvettes by means of an ELECTROcell-B15 high voltage pulse generator (Leroy Biotech). Long (100 μs) voltage pulses, 1 Hz repetition rate, were applied with variable pulse number (1-50) and voltage-to-distance ratio (1 kV/cm – 2.5 kV/cm). A flow-cytometric method based on double-staining of samples with the fluorescent dyes calcein acetoxymethyl ester (CAM) and propidium iodide (PI) was used to quantify EP efficiency and cell death, and to identify REP and IRE pulsing conditions. Cell viability was assessed by the ability of cells to grow over a 24h and 48h period by means of MTT assay. The results of the ongoing activities will be presented at the conference.

PO-57

Delivery of Anticancer Drugs with Protein-Based Nanocarriers Using Nanosecond Pulsed Electric Fields and Shock Waves

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Protein-based nano-carriers show promise in adjusting drug pharmacokinetics, addressing challenges like low solubility and non-specific distribution of anti-cancer drugs. They offer a potential solution for precise targeting in drug delivery, crucial for reducing side effects and enhancing effectiveness. However, further research is needed due to limited understanding and development of these carriers, highlighting the ongoing need for innovative, safe, and cost-effective drug delivery systems. This research focuses on developing an environmentally friendly, biodegradable protein-based nanoparticle using a cost-effective method. The nanoparticles are served as carriers for drugs and natural antioxidants. Nanosecond pulsed electric fields (nsPEFs) and their induced shock waves are chosen for their effectiveness in cell transfection to facilitate the cellular uptake of the nanoparticle/drug complexes, efficiently delivering substances ranging from small molecules to large proteins. The physical and chemical properties of the nanoparticles are analyzed using techniques such as scanning electron microscopy (SEM), ultraviolet (UV)-visible Spectroscopy, dynamic light scattering (DLS), and x-ray diffraction (XRD). The study investigates the delivery of the nanoparticles by physical drug delivery methods, including nsPEFs, shock waves, and ultrasound. The effectiveness of these delivery methods, along with their impact on cellular models when subjected to physical stimulation, are thoroughly assessed. The findings of this study have the potential to pave the way for a safer and more straightforward drug administration system, proving beneficial for healthcare practitioners and the pharmaceutical sector.

PO-58

The effect of pulse duration on electrostimulation and electroporation of excitable S-HEK cells

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Electroporation-based treatments have been shown to affect the function of excitable cells such as muscle cells and neurons. Electroporation and

excitability both depend on induced transmembrane voltage (TMV). Electric pulses used in rapidly developing cardiac pulsed field ablation as well as other electroporation-based treatments of excitable tissues are of different parameters (duration, amplitude, number and repetition rate). Therefore, in the context of the treatments, it is important to understand how pulses of different parameters affect permeability, excitability and function of excitable cells.

We studied the effect of electric pulses of different durations (0.5, 1, 10 and 100 µs) on the TMV and intracellular calcium of genetically engineered excitable S-HEK cells in vitro. We have demonstrated in our previous study that S-HEK are a valuable minimal in vitro experimental system for studying electroporation in excitable cells. Changes in TMV (also action potentials) were monitored optically with potentiometric dye ElectroFluor630 and fluorescence microscopy, the signal of the whole field of view was analysed. We also monitored changes in intracellular calcium using a fluorescent calcium indicator Fura-2. With pulses of all selected durations, one or more action potentials were triggered in S-HEK cells when the electric field strength exceeded a certain threshold. Interestingly, we found that the characteristics of the action potential (shape, time of occurrence) triggered by the threshold electric field are influenced by the pulse duration. By increasing the electric field above the threshold, we achieved prolonged depolarization, which indicates electroporation. All the pulses used in the study also provoked a complex response in intracellular calcium. These results contribute to understanding of basic mechanisms of how the interplay between electroporation and excitation depends on the pulse parameters.

PO-61

Protective Effects of Iron Compounds on Controlled Membrane Damage Induced by Varied Pulsed Electric Field Durations in Cardiac and Skeletal Myocytes

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Cardiovascular diseases are one of the leading causes of human death worldwide. Iron deficiency is prevalent among patients with cardiovascular disease and is associated with poorer clinical outcomes. Therefore, our study aims to investigate whether iron compounds, specifically iron citrate (III) and sodium EDTA iron (III) salt, exhibit protective effects following simulated damage to rat cardiac muscle cells (H9C2) and skeletal muscle cells (L6). The varied sequences of pulsed electric field (PEF) were employed to induce different degrees of damage to the cells (3 pulses of 0.5, 1 and 1.5 kV/cm field intensity, from 0.1 - 10 ms duration, 1 Hz).

Our approach involved evaluating the cellular bioeffects affected by PEF and iron compounds, including proliferation (colony formation assay), viability, metabolism, the expression of heat shock proteins, cell death, and oxidative stress. Initial studies allowed us to determine the non-toxic concentrations of iron compounds for further research (10 - 50 µM for iron citrate (III) and 0.5 - 1 µM for sodium EDTA iron (III) salt). Then, the degree of protection provided by the iron compounds following simulated cell damage was evaluated. This stage was conducted in two variants. In the first, cells were subjected to PEF directly in the presence of the iron compounds, while in the second variant, prior to the application of electric pulses, cells were incubated for 72 hours with the iron compounds. It was observed that cells subjected to PEF directly in the presence of the iron compounds showed no differences in proliferation when compared to PEF alone. How-

ever, in the second variant, iron citrate exhibited superior protective action, indicating its potential efficacy in mitigating cellular damage. This study demonstrated that particularly iron citrate (III) substantially enhances cardiac or skeletal muscle functioning, cell damage-dependent pattern. In summary, our findings suggest that pre-incubating cells with iron compounds, particularly iron citrate, may enhance their resistance to damage induced by pulsed electric fields, thereby holding promise for protective or regenerative therapy for cardiac or skeletal muscle injuries.

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PO-62

A comparison of small molecule intracellular electrotransfer in spheroids and cell suspension

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Although electroporation has been a known technique for quite some time, ongoing research has raised new unanswered questions. One such question is the transfer of established electroporation techniques from in vitro to in vivo settings. One of the main challenges in this transition is the increased complexity of tissues that are composed of various cells, and consequently, the responses of the particular cell in the tissue to the applied electric field are different compared to in vitro settings. This gap, to some extent, can be filled by employing cells in 3D cultures. These 3D cell cultures can be derived from adherent cells (2D cultures) by simply altering the surface of the plate while utilizing the same growth medium. There are a small number of publications utilizing spheroids for small molecule electrotransfer; therefore, comparison of the efficiency of electrotransfer of small molecules into cells in suspension and in 3D settings is still missing. The primary aim of this study was to compare small molecule electrotransfer to cells in spheroids versus those in suspension.

2D cultures (adherent cells) of three different cell types were selected for the experiments. Various parameters of electric fields were applied to examine the electrotransfer differences. Bleomycin and cisplatin were chosen as anticancer drugs for the evaluation of intracellular electrotransfer efficiency. The size of electroporated spheroids was evaluated daily. Additionally, propidium iodide electrotransfer was performed on cells in spheroids and cells in suspension. To evaluate electrotransfer efficiency into cells in spheroids, a methodology was developed to disaggregate spheroids into individual cells, enabling microscopic and flow cytometry analysis.

The results showed that the electrotransfer efficiency of small molecules into cells in a suspension or 3D setting was significantly different. The electroporation threshold of the applied electric field required to achieve electroporation was also different. Spheroids require a higher electric field to achieve electroporation compared to cell suspension. Experiments using different cell lines showed that when anticancer drugs were used, the same cytotoxic effect, using the same electroporation conditions, was achieved when the concentration of the drugs increased at least 10-fold in 3D settings. Also, as the size of the spheroids increased, stronger electric fields and higher anticancer drug concentrations were needed to achieve the same cytotoxic effect.

PO-63

Investigation of the state of cell death by applying pulsed electric field under ROS suppression

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It has been believed that the application of pulsed electric field with frequency components of a few MHz or lower to cells makes pores in a cell membrane by dielectric breakdown and cell death through shedding of internal tissues. However, when we sterilized green juice with anti-oxidation using Pulsed Electric Field (PEF), the sterilization

ratio did not improve even when the application time of the pulses was extended. From this result, we have considered that the electrical membrane breakdown may generate plasma, and it generate Reactive Oxygen Species (ROS), and consequently, ROS involved in the cell death. Therefore, we suppressed ROS generated by applying the pulsed electric field using antioxidants and investigated the sterilization ratio. In this study, we compared survival rates and ROS generation degree in Saccharomyces cerevisiae with and without adding dimethyl sulfoxide (DMSO) which is a scavenger for OH radical when a PEF with a field strength of 7.5 kV/cm was applied. As a result, there were few killed cells on applying pulsed electric field under suppressing generated ROS by adding DMSO.

PO-64

Electroporation-generated extracellular vesicles in tumor and normal cells interactions

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While electroporation (EP) has long been utilized in electrochemotherapy (ECT), the dynamics and composition of extracellular vesicles (EVs) released through reversible electroporation remain largely unexplored. Understanding whether and how EP parameters influence the profile of released EVs is crucial. This study delves into the impact of various EP parameters on EV release from human melanoma cells and their subsequent effects on normal fibroblasts. Specifically, markers indicative of EV-mediated transformation of fibroblasts into tumor-associated fibroblasts were examined, including the expression levels of vascular cell adhesion molecule-1 (VCAM-1), changes in phosphor-histone H3 expression, cell viability, and migration capacity. EVs isolated from two malignant melanoma cell lines subjected to reversible EP were exposed to human primary fibroblasts (HPFs). Results revealed differences in HPF viability, migration capacity, VCAM-1, phospho-histone H3, focal adhesion kinase, and vezatin expression, depending on EP parameters and melanoma cell grade. These findings underscore the significance of further investigations into the effects of reversible EP on tumor cell-derived EV properties.

PO-65

Electroporation induced protein elution out to extracellular media and cytoplasmic membrane blebbing

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Electroporation, a method that uses electrical pulses to temporarily permeabilize cell membranes, has emerged as a useful tool in cell biology and biotechnology. Beyond its primary applications in gene delivery and cell manipulation, electroporation facilitates interesting cellular phenomena, such as membrane blebbing. Such stress response, alongside the electroporation-assisted release of intracellular compounds like proteins and other molecules that together produce Damage-Associated Molecular Patterns (DAMPs), offers a window into the complex interactions within the cellular and extracellular environment. Specifically, the release of DAMPs into the tumour microenvironment through electroporation might have profound implications for understanding immune responses and their impact on cancer progression.

The primary aim of this study is to explore the dynamics of electroporation-induced membrane blebbing, protein release and possible effects in modulating the tumour microenvironment.

Experiments were conducted with Chinese Hamster Ovary cell lines (CHO-K1 and stable eGFP transfected CHO-K1). For electroporation 1HV, 5HV and 9HV 100µs length pulses were used with electric field strengths from 0 kV/cm to 3.6 kV/cm in 0.6 kV/cm increments. Total protein quantitative and qualitative measurements were performed by colorimetric Pierce™ BCA Protein Assay Kit and SDS-Page electrophoresis. Intracellular eGFP fluorescence measurements were made by flow-

cytometry and fluorescent microscopy.
Our study on electroporation-induced protein release reveals that protein yield depends on pulse number and intensity. A comparison showed a single high-voltage (HV) pulse was less effective than multiple (5 or 9) pulses, with no yield difference between the latter, indicating a yield plateau beyond certain parameters. At 3.0 kV/cm for 5 and 9 pulses, protein elution reached 60% of total cellular protein, suggesting a maximum release limit. SDS-PAGE analysis showed no qualitative differences in protein bands between treated and control samples, suggesting cell lysis isn't a significant factor. We also measured intracellular eGFP fluorescence over 32 minutes following the electroporation, which revealed complex elution dynamics contrary to expected rapid action. Additionally, electroporation caused cellular blebbing, with eGFP fluorescence diminishing in the cell body but remaining constant in blebs, indicating unique protein retention dynamics. These findings highlight the nuanced effects of electroporation parameters on protein elution and distribution.

PO-66

Synthetic Cell Models to Understand the Impact of the Actin Cortex on Membrane Electroporation

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Advances in genome editing technologies require a comprehensive understanding of the biophysical mechanisms underlying the effective intracellular delivery of molecular cargo, such as nucleic acids or proteins. While electroporation has proven to be an effective delivery approach, the underlying biophysical mechanisms are poorly understood [T. Kotnik, L. Rems, M. Tarek, and D. Miklavčič, Annu Rev Biophys, 2019]. This research aims to bridge this gap in our knowledge.
The actin cortex, a dense network of actin filaments located directly beneath the plasma membrane, plays a crucial role in maintaining the cell's shape and mechanical stability. Emerging evidence suggests that this actin cortex is a critical, yet often overlooked, factor in the biophysical processes involved in electroporation. Specifically, the actin cortex has been shown to influence membrane permeability, pore size, and pore resealing time after applying electric pulses [D. L. Perrier et al., Scientific Reports, 2019].
Our aim is to develop synthetic cell model systems based on tunable actin cortices within giant unilamellar vesicles (GUVs) [L. Baldauf et al., Biophysical Journal, 2023] to elucidate the mechanobiological mechanisms by which the actin cortex modulates electroporation. We have successfully developed GUVs with a branched actin cortex, and our imaging data shows a strong impact of the cortex on electroporation. By creating a more realistic model system that incorporates the actin cytoskeleton, we hope to gain deeper insights compared to the inherent complexities of live cells or the oversimplified GUV models typically used in previous studies.

PO-111

Deciphering the resealing of membranes after a pulse using impedance measurements by numerical modelling

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Despite numerous experimental evidences for the increase of membrane permeability after electrical pulses, there is still a lack of knowledge about the electrical properties of the membrane in the minutes after the pulse. Bioimpedancemetry is a common technique to observe these changes. In this work, we focus on data obtained with a fast electrical impedance spectroscopy setup for rapid measurement of cell properties after electroporation [1]. It consists of a disk with a diameter of 15 mm and six microelectrodes arranged in parallel and spiralling from the center. The microelectrodes are placed on a cell layer of C2C12 myoblasts, avoiding direct contact with the cells thanks to microseparators. The cell monolayer is submitted to one biphasic microsecond pulse with two different voltages. The measurements consist

of impedance data at different frequencies and different buffer concentrations before the pulse and 5 minutes after.

Our goal is to develop numerical strategies to decipher the dynamics of cell membranes using these experiments. We first developed a robust calibration strategy to deal with the system distortion and to generate true sample impedances. Then, we have developed two methods to analyze the data.

The first is based on an equivalent circuit model and a well-designed numerical optimization procedure. It provided first very encouraging results and allowed to quantify the short-term behaviour – within a few seconds after the pulse – of the membrane conductance. In particular, we were able to distinguish between the release of [KCl] and the dynamics of the membrane resistance [2]. However, the equivalent circuit does not explain some long-term behaviours of the membrane, such as the incomplete recovery of the membrane resistance within five minutes. For this reason, we developed a second approach based on the simulation of the entire experimental setup using a partial differential equation (PDE) model. The whole 3D geometry (a disk with a diameter of 15 mm, a thickness of 15 microns and six spiral-shaped microelectrodes on the top surface) is considered. Our modelling takes properly into account the passive electrodes. When cells are present in the buffer, transmission conditions on the electric potentials between intracellular and extracellular domains were described. Our strategy was validated first using impedance measurements without cells. Preliminary results with cells are then compared with impedance measurements confirming the accuracy of our model.

[1] T. García-Sánchez et al. A new spiral microelectrode assembly for electroporation and impedance measurements of adherent cell monolayers. Biomedical microdevices, 2014.

[2] A. Collin et al. Deciphering immediate post-pulse membrane resealing from 4–electrode impedance measurements by numerical modeling. Bioelectricity, 2023.

PO-67

Electroporation in vesicles under ms-pulsed electric field

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Under electric field, vesicles may exhibit electroporation, a fascinating process that augments the permeability of the vesicle membrane. While the experimental studies have demonstrated tremendous controllability over electroporation under various electric pulses, reported theoretical models largely fail to provide mechanistic insights into the experimental results. In the present study, we propose an improved numerical formalism that can predict pore evolution and consequent pore area under millisecond-long electric pulses. The novelty of our study lies in modeling the growth of large pores and the current passing through them. Implementing the above, we illustrate that the nature of electric pulse and its strength play a vital role in governing the pore evolution. The polar regions are observed to admit maximum pore formation, whereas the large pores form near the angles of minimum pore formation. The contribution of large pores towards the total pore area is significantly more. The effect of charging time is also analyzed, showing that it can largely affect pore numbers and their size. Importantly, a higher charging time can cease pore growth, and, in some cases, even their formation. The transients in surface area variation, captured by our model corroborate well with earlier experiment findings. The present work serves as a precursor for modeling efficient medical procedures in electrochemotherapy.

PO-68

Unveiling Fusion Pore Dynamics: Integrating Fluorescence and Electrochemical Imaging on Supported Bilayers

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The release of neurotransmitters and hormones

occurs from the interior of secretory vesicles storing these molecules, to the outside of the cell via formation of a fusion pore, which traverses two different membranes, the vesicle membrane and the plasma membrane. The process of fusion pore formation constitutes a fundamental step in various biological phenomena, including not only vesicular release, but also viral entry, and intracellular membrane trafficking.

Syntaxin 1 (Stx1) and SNAP25, together with Synaptobrevin 2 (Syb2), form the neuronal SNARE (Soluble NSF Attachment Receptor) complex, which drives membrane fusion events such as synaptic or neuroendocrine dense core vesicle exocytosis. Stx1 and SNAP25 are plasma membrane-associated proteins, while Syb2 is a vesicle membrane-associated protein (aka VAMP2). SNARE proteins are recognized as the primary constituents of the molecular fusion machine, they play a crucial role in bringing the vesicle and the target membranes close together, and in the formation of fusion pores mediating the release of vesicle contents, such as catecholamines.

To understand the relation between molecular transitions measured by fluorescence imaging and catecholamine release measured by electrochemical detection of individual release events, we form a supported bilayer on top of a four-electrode microfabricated electrochemical detector (ECD) array patterned on a microscope coverslip. This bilayer includes purified recombinant SNAP25 and Stx1 proteins, incorporated into the bilayer from proteoliposomes, simulating in this way the cell plasma membrane composition. Natural chromaffin granules (CGs) prepared from bovine adrenal glands carrying endogenous Syb2 and labeled with a fluorescent lipid are applied to the supported bilayer in the space between the ECD electrodes, leading to fusion events. In this way, the vesicle fluorescence and its changes can be imaged in total internal reflection fluorescence (TIRF) excitation mode while simultaneously the release of catecholamine molecules occurs. The released catecholamine molecules diffuse to the different ECD electrodes, generating amperometric currents that depend on the time and location of the individual fusion events. The amperometric spikes indicate the time of fusion with <1ms precision and the location of the release event with ~300 nm precision.

We present all the steps of the approach and preliminary experiments and results of CG-supported membrane fusion events, observed under the TIRF microscope. The approach will help to reveal the relation between molecular events of proteins and lipids with the phases of fusion pore formation and dilation.

PO-69

Efficient Lipid Extraction with Underwater Pulsed Electric Discharge Shock Waves

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Due to the many hazards and inconveniences of fossil fuels, the ever-growing search for a suitable alternative has been on the rise for nearly a century. Plant-based bio-fuels taken from microalga offer numerous promising advantages which include, but are not limited to: renewability, a neutral carbon footprint, less consumption of land, and less flammability. Chlorella vulgaris is a green species of single-celled microalgae of the Chlorophyta division, which is commonly used as a food supplement, and due to its fast growth rate is industrially produced for lipids. The application of pulsed electric discharge is proven to be an effective way to create a breach in extra-cellular matrices of microalgae, which causes the lipid cells to float freely, which in turn enables more lipids to be extracted as a result. This study investigates the effects of the application of nanosecond pulsed electric underwater discharge on its potential to extract lipids from the C. vulgaris. For the purpose of increasing the shock effect created by the discharge, an underwater discharge apparatus was designed and constructed. Various numbers of pulses ranging from 100 to 500 pulses with different frequencies were applied to the algae samples. Subsequently, the lipids were extracted from the samples using a variation of the Bligh-Dyer method, and the results were analyzed and compared with the control

sample, which indicated a near 50% increase in the quantity of the extracted lipids of treated samples. The results clearly prove the method to be effective, profitable, energy-saving, and applicable for large-scale usage.

PO-70

A novel approach for modelling membrane electroporation dynamics

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Electroporation of biological cells using pulse electric field, in the last few decades, brought significant attention towards itself owing to its growing applications in healthcare, food technology and environment. While technological advancements in the hardware is catering to its growing applications, a parallel effort is also there at unmasking the mechanism behind electroporation when exposed with such ultrashort electric pulses. Modelling attempts including full-blown simulations were being performed in the past for cells assuming spherical geometry, it is well-known that cells are hardly spherical. Biological cells tends to explore different morphological space under different stimuli, highlighting their plasticity and the limitations of such spherical studies. This study aims at decoupling geometrical considerations from mechanistic understanding of electroporation. A simple patch of a cell where bi-layers at both end is coupled via cytoplasmic resistance is studied when microsecond pulse electric field is applied. When incorporated with realistic values of resting membrane potential, the bilayers at both ends shows different early-phase and long-term electroporation dynamics. Number of pores formed at the hyper-polarised end is almost three times than the pores formed at the depolarised end at longer times. It has implications on the respective conductance values and other parameters. Physical insights are also presented at the early-phase dynamics of electroporation at both the ends. This study holds relevance when cells with more realistic features will be studied having ion channels and other protein structures.

PO-71

Effect of electroporation in combination with inorganic particles used in tattoo inks

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Electroporation is a technique that exploits electrical pulses to increase cell membrane permeability. In order to kill tumor cells, electroporation is combined with low systemic or local concentrations of anticancer agents, which thus massively penetrate into permeabilized tumor cells. This modality is also known as electrochemotherapy (ECT). ECT can be used to treat several types of cancers, including deep-seated and superficial tumors, such as melanoma (1). Multiple modalities are available to treat melanoma, including surgery, immunotherapy, targeted therapy, radiotherapy, but ECT appears as the best treatment, with an overall response rate of about 80%.

In parallel, an increasing number of individuals are opting to wear one or more tattoos for aesthetic or reparative purposes. Tattoo inks contain micro- or nano- particles, such as titanium dioxide and iron oxides, which are used as colorants. In case of ECT treatment, the presence of these particles in the skin could alter or amplify the effects of electroporation (2), if a tattoo is present in the zone that is being electroporated.

As to the best of our knowledge, there are little if any reports addressing the outcomes of electroporation in combination with tattoo-ink-derived particles and no ESOPE guidelines, our study focuses on the evaluation of the biological effects of such co-exposure. One of possible outcomes can be an increased local tissue heating in vicinity of tattoo inks, local generation of reactive oxygen species (ROS) or local tissue damage due to irreversible electroporation. Cellular models, which involve the use of skin-derived cells: malignant melanoma (A375 cell line) and normal dermal fibroblasts, isolated from a healthy skin biopsy, are used to obtain 2D and 3D models (multicellular spheroids and dermal sheets). Different conditions are compared: presence or absence of metallic/metal oxide particles derived from tattoo ink in combin-

ation with different electrochemotherapy-like electroporation parameters (8 pulses, 100 µs duration, 1 Hz pulse repetition rate), applied at varying electric field intensity).
In the present study, we mainly focus on local temperature measurement upon electroporation in absence/presence of different tattoo inks, as well as cell permeabilization and cell viability assessment following propidium iodide uptake and generalized oxidative stress assessment, which is determined with cell oxidation detection kit. In addition, morphological alterations of the tissues are assessed with different microscopy techniques (optical, electron and second harmonic generation microscopies).
Taken together our study will allow to determine if tattoo inks do or do not have a harmful role upon electroporation.

PO-72

A mechanistic numerical model of cell membrane electroporation that links electroporation and electro-permeabilization

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Electroporation can be defined as the biophysical phenomenon in which cell membrane permeability increases non-selectively to ions and molecules due to exposure to an electric field. The most widely accepted electroporation mechanism is the formation of pores, from which the term “electro-poration” stems. Experiments on artificial membranes and molecular dynamics (MD) simulations indeed corroborate the creation of pores in bilipid membranes when a transmembrane voltage is stablished. However, the experimentally and computationally observed pores are short-lived and collapse immediately after cessation of the transmembrane voltage induced by the applied field. This disagrees with the fact that the state of increased permeability can last for seconds or minutes after field exposure. In addition, numerical models of cell membrane electroporation that only assume the formation of pores fail to accurately reproduce the uptake of small molecules in diverse scenarios (Bioelectrochemistry, 2022, 147:108216), and do not reproduce substantial electroporation features such as the increased permeabilization obtained by splitting in time the electric field exposure. Ten years ago, it was proposed a semi-mechanistic numerical model of cell membrane electroporation in which are distinguished a conducting state and a permeable state (J Theor Biol, 2014, 360:83-94). The conducting state was attributed to the formation of pores (“electroporation”) and, although the authors did not explicitly indicate the mechanism behind the permeable state, later communications by them, and in particular by Prof. Lluís M. Mir, suggest that the permeable state would be caused by a chemical alteration in the composition of the membrane lipids, a peroxidation, which causes a higher permeability (“electro-permeabilization”). Remarkably, a recent study on artificial membranes characterizes the impact of the membrane hydroperoxidation degree on the frequency of pore opening (Proc Natl Acad Sci USA, 2023, 120(11):e2213112120). Departing from these studies, we have conceived and a new mechanistic numerical model of cell membrane electroporation that considers electro-poration and electro-permeabilization as distinguished but linked mechanisms. The model assumes that short lifetime pores are created at a rate determined by the transmembrane voltage and the oxidative state of the membrane. In turn, the oxidative state of the membrane increases in the presence of pores. During the delivery of the electric field, both the formation of pores (electro-poration) and the peroxidation (electro-permeabilization) contribute to the permeabilization of the membrane. After the delivery of the electric field, only oxidation contributes to the permeabilization of the membrane. However, oxidated areas in the membrane laterally diffuse and this maximizes pores creation in subsequent field exposures. Eventually, the oxidative damage is repaired, and the physiological permeability is reestablished.

PO-73

Electrical conductivity effect on Anisakis spp inactivation by PEF and impact on fish quality

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Larvae of the nematode family Anisakidae can cause parasitic infections in humans through the consumption of fishery products. These larvae are widely distributed geographically, with rates of parasitism close to 100% in certain fish species. Current legislation mandates freezing fish products that may contain Anisakis spp. if they are to be eaten raw or undercooked. However, this method affects fish quality. Recently, Pulsed Electric Fields (PEF) technology has been shown to effectively inactivate Anisakis. The electrical conductivity of the treatment medium significantly influences its lethality, but there is limited information on its effect on Anisakis inactivation.

The objective of this work was to evaluate the influence of the electrical conductivity of the treatment medium on Anisakis inactivation in hake bellies and blue whiting, and to assess its effect on hake microbiota quality during shelf life.

The lethality of Anisakis spp. to PEF technology (field strength: 1.2 to 3 kV/cm, pulse width: 7 µs, specific energy: 20 to 40 kJ/kg, and conductivity: 0.4 to 8 mS/cm) in hake (Merluccius merluccius) and blue whiting (Micromesistius poutassou) was determined by the mobility test described by EFSA. Fish microbiota of control and PEF (3 kV/cm; 7 µs; 20 kJ/kg; 0.4 and 8 mS/cm) samples of hake fillets were evaluated during shelf life, both without MAP and with MAP (50% CO2 - 50% N2) at 4ºC. Additionally, an expert panel from a fish company (Scanfisk Seafood S.L., Zaragoza, Spain) conducted a preliminary organoleptic analysis to detect differences between control and PEF samples.

Inactivation of Anisakis increased when PEF treatments were applied at higher electrical conductivities in both hake and blue whiting. A PEF treatment of 3 kV/cm, 20 kJ/kg, and 8 mS/cm inactivated 85-95% of the Anisakis larvae in both species, whereas at 0.4 mS/cm, a field strength of 5 kV/cm and 20 kJ/kg were required for the same level of inactivation.

Fish microbiota was not affected by electrical conductivity. Control and PEF samples showed no difference in microbiological counts; it was the effect of MAP that controlled microorganism growth. Preliminary organoleptic analysis detected no differences in quality between control and PEF samples, regardless of conductivity.

The lethality of Anisakis spp. in hake belly and blue whiting increased with higher electrical conductivity of the treatment medium. PEF treatments at higher electrical conductivity did not affect the microbiological quality of hake, and no differences in quality between control and PEF samples were observed.

PO-74

The dynamics of synergetic bacteriocidic effect of pulsed electric fields and antibiotics

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Currently, antibiotics are the primary defense against various bacteria. However, their effectiveness in killing bacteria has decreased due to the development of antibiotic resistance. To address this issue, new antibiotics are being developed, and efforts are underway to reduce antibiotic consumption. Overuse of antibiotics in agriculture and medicine contributes significantly to antibiotic resistance. Consequently, reducing antibiotic usage in both fields is crucial. As a result, alternative methods with the potential to substitute for antibiotics are being heavily researched. One such method is electroporation, which involves applying pulsed electric fields (PEF) to bacterial suspensions. Electroporation effectively deactivates bacteria, reducing their numbers by 4-5 logs and making it one of the most efficient tools for bacterial inactivation. However, PEF alone can only temporarily decrease bacterial numbers, as bacteria tend to regain their numbers after a certain period following the PEF treatment. In contrast, antibiotics work more slowly but have a longer-lasting effect as long as they remain in the bacterial suspension. Combining PEF

with antibiotics offers the possibility of rapid initial bacterial reduction by PEF treatment, followed by sustained inhibition using. Therefore, we investigated the simultaneous use of PEF and antibiotics on both gram-positive and gram-negative bacteria to prolong the inhibitory effect of PEF.

Two types of bacteria were used in the experiment: the gram-positive Lactobacillus bulgaricus delbrueckii and the gram-negative Escherichia coli DH5α strain. PEF was applied using a BTX T820 pulse generator with amplitudes up to 24000 V/cm and pulse durations between 10 and 100 µs, delivering up to 10 pulses at 1 Hz. Cells at the concentration corresponding to 10 OD were electroporated in deionized water with a conductivity of 1 µS/m. Ampicillin, neomycin, and kanamycin were used as antibiotics at their respective IC50 concentrations. Cell deactivation was assessed using metabolic activity measurements at various time points up to 24 hours post-PEF application. Changes in OD were monitored every 10 minutes up to 10 hours post-PEF. In addition, treatment efficiency was evaluated using a colony formation assay.

The results demonstrate that PEF is a rapid and effective method for bacterial inactivation. However, bacterial regrowth typically begins approximately 3 hours post-electroporation. In contrast, the full effect of antibiotics is observed approximately 10 hours after incubation. Our findings suggest that simultaneous application of PEF and antibiotics results in rapid bacterial killing by PEF, with antibiotics preventing bacterial regrowth.

PO-75

Comparative Study of the Effects of Nanosecond and Microsecond Pulsed Electric Fields on Saccharomyces cerevisiae

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Pulsed Electric Field (PEF) technology has a wide range of applications in food processing and biotechnology due to its ability to modify the permeability of cell membranes through electroporation. PEF provides an efficient non-thermal method for microbial inactivation, enhances the extraction of valuable intracellular compounds, increases fermentation rates and yields by improving substrate uptake and metabolic activity, and induces cell autolysis. While some applications require irreversible electroporation of the cell membrane, others benefit from a reversible effect. The reversibility or irreversibility of electroporation depends on processing parameters such as pulse and treatment duration, and electric field intensity. Most current research on microbial electroporation has been conducted using pulses with durations in the microsecond (µsPEF) range. However, the effects of nanosecond pulses (nsPEF), characterized by extremely short durations coupled with high field strengths, remain less understood.

This study addresses this gap by comparing the effects of nsPEF using a novel high-performance, solid-state multilevel inverter, against µsPEF treatments, employing Saccharomyces cerevisiae as a model microorganism.

Saccharomyces cerevisiae SafAle™ S-04 cells were collected in the stationary growth phase and suspended in McIlvaine buffer (pH 7, 1 mS/cm). The yeast suspensions were subjected to PEF treatments at a constant electric field strength of 30 kV/cm. Unipolar pulses varied in pulse width: nanoseconds (500 ns, 100 ns, 50 ns, 10 ns) and microseconds (1 µs), and in treatment durations (1 µs, 5 µs, 50 µs). Reversible and irreversible electroporation, as well as yeast inactivation, were evaluated after 30 minutes and 24 hours of incubation.

Pulses applied in the range from 1 µs to 100 ns demonstrated similar performance. Treatments with a total duration of 50 µs irreversibly electroporated more than 50% of the population. When the total treatment duration decreased to 5 µs, reversible electroporation predominated, being observed in up to 50% of the population. For a total treatment time of 1 µs, only reversible electroporation was observed, ranging from 10% of the cells (100 ns) to 40% (1 µs). Moreover, under these treatments applied with pulses of 100 ns or higher, irreversible electroporation aligns with microbial inactivation.

In the case of shorter pulses, irreversible electroporation was not detected with 50 ns long pulses for treatment durations of 1 and 5 µs, and with

pulses of 10 ns for all the treatment durations tested. However, these treatments inactivated up to 40% of the population, suggesting a possible intracellular effect of PEF without affecting cytoplasmic membrane permeability.

The findings underscore the critical role of pulse width in PEF treatments, indicating a need for further research to fully understand its effects on electroporation efficiency and cell viability. This study lays the groundwork for optimizing PEF parameters for various applications in the food and biotechnological industries.

PO-76

Stimulation of Saccharomyces cerevisiae metabolism and growth using pulsed electric fields

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The yeast Saccharomyces cerevisiae is a widely used microorganism in biotechnology and food industry e.g. for production of ethanol, biopharmaceuticals or as a leavening agent for baked goods. Sublethal treatment of Saccharomyces cerevisiae with Pulsed Electric Fields (PEF) has been reported in literature as stimulating the growth and metabolism of Saccharomyces cerevisiae. During my master thesis the inoculum for a fermentation experiment was treated by PEF with an electric field strength (E) of 0.5 – 5 kV/cm and a specific energy input (Wspec) of 0.5 – 25 kJ/kg. The effect of the PEF treatment on the CO2 production (measured as percent CO2 in the fermentation vessel headspace) and the microbial growth rate (measured as colony forming units per mL) during the following fermentation was investigated. It was possible to show an increase of the rate of CO2 production by a factor of 1.08 for three treatments with E = 0.5 kV/cm and Wspec = 0.5/2.5/25 kJ/kg respectively and by a factor of 1.05 for a one treatment with E = 5 kV/cm and Wspec = 25 kJ/kg. The microbial growth rate was increased by a factor of up to 1.14, although the results for microbial growth rate were not statistically significant. These results show the potential of PEF treatment of Saccharomyces cerevisiae in a fermentation inoculum and can be viewed as basis for further studies. An adapted method for potential further studies, focusing on microbial growth, glucose depletion and ethanol production was also devised.

PO-117

Modification of corn starch using pulsed electric fields: effects on composition, structure, and techno-functionality

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Background: Starch, a ubiquitous carbohydrate is widely used in the food industry due to its film-forming, gelling and viscosity properties. Nevertheless, native starches suffer from limitations such as slow gelatinization kinetics and low digestion rates. To overcome these limitations, structural modifications are required, but traditional methods such as heating and chemical modifications may compromise its nutritional quality. Non-thermal techniques such as pulsed electric fields (PEF) offer promising advantages, improving energy efficiency, and preserving nutritional quality compared to conventional methods. This work aimed to evaluate the effect of PEF on the physicochemical properties of corn starch through the evaluation of alterations to granule structure, molecular organization, and composition.

Materials and Methods: Corn starch was suspended in distilled water (5% w/v) and subjected to bipolar 20-μs pulses at 40 Hz with electric fields strengths ranging from 1.25 to 8 kV/cm and treatment times varying from 200 to 2000 pulses. Following the PEF treatments, corn starch suspensions were freeze-dried and sieved. The starch powder was characterized considering microstructure (scanning electron microscopy) and techno-functional properties, including swelling power, water absorption capacity (WAC), and oil absorption capacity (OAC). The amylose/amylopectin content and syneresis of the starch gel were also evaluated.

Results: All PEF treatments disrupted the native granular structure and organization in a dose-dependent manner. The SEM images proved

that PEF induced pores in the starch granules by electroporation, which resulted in the leaching of amylose from the granules. Generally, the amylose content of PEF-treated starch was reduced from 25.81% (control) to as low as 21.88% applying 1.25 kV/cm and 2000 pulses. PEF treatment significantly increased the swelling power (from 4.38 to 4.76 g water/g starch) however, the oil retention was reduced from 1.87 to 1.77 g oil/g starch. Moreover, the PEF treatment of starch appears to lower the syneresis of starch gels, plausibly by inducing more H-bonding in the amylose/amylopectin network.

Conclusions: PEF treatments led to significant modulation of corn starch physicochemical characteristics, notably enhancing WAC while decreasing OAC. These changes are attributed to structural modifications, including increased porosity and microscopic pore development within starch granules, along with increased H-bonding and suggest the potential of PEF to modify starch for various applications, thereby contributing to advancements in development of novel starch-based ingredients.

PO-59

The impact of electroporation on the therapeutic efficiency of colorectal cancer 3d printed cells under hypoxia

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Medical University of Plvovdiv, Bulgaria

Colorectal cancer (CRC) is the third leading malignant pathology in the world. The poor prognosis is due not only to the high percentage of late diagnosed cases, but also to the resistance acquired by tumor cells to chemo- and radiotherapy. Therefore, new therapeutic approaches are urgently required. The reduced amount of oxygen in the tissue microenvironment leads to autophagy, increases the self-renewal of cells, and CRC progression. Hypoxia is a major constituent of the tumor microenvironment and is also a proven driver of chemotherapy resistance. 3D bioprinted tumors closely resemble primary carcinomas morphologically and biochemically, with shown higher resistance to standard chemotherapeutics. The current study aimed to investigate and evaluate the electroporation efficiency of the therapeutic scheme - standard chemotherapeutic (5-FU) and autophagy inhibitor (chloroquine), alone and in combination, on 3D bioprinted HCT-116 3D cells under normoxic and hypoxic conditions. The study showed that when used in IC50 values in combination, CQ enhances the cell growth inhibitory effect of 5-FU in normal oxygen levels. However, this additive effect is lost when cancer cell 3D-printed aggregates are treated in an extremely hypoxic state. Interestingly, electroporation reverses the drug delivery efficiency and even enhances cell death under hypoxia. In conclusion, our data demonstrate better drug delivery with electroporation and suggest that electrochemotherapy could lead to more optimal treatment of human CRC cancer 3D cell models. This evidence indicates their potential implementation as a more realistic platform for studying the effect of therapeutics and subsequent application in personalized medicine.

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Poster session

Coffee Break and Poster Session

Sep 18, 15:20 - 16:50

PO-77

Surgery and electrochemotherapy: An option for a feline with recurring infiltrating sarcoma

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Soft tissue sarcomas are 7-15% of skin and subcutaneous tumors in cats. Sarcomas have a common mesenchymal origin and similar behavior, but their differentiation is difficult diagnostic. They can present a pseudo capsule or be very infiltrat-

ing and manifest a very aggressive local growth, although those of high grade can also present metastasis (5-15% in felines). Surgery is the main therapeutic tool, but if the surgery is performed with a narrow margin, the chances of recurrence increase significantly. So other alternatives must be implemented: new surgery, chemotherapy, radiotherapy, cryosurgery. In the Electrochemotherapy an electric field is generated with predetermined frequency and time, a chemotherapeutic (Bleomycin, Cisplatin) is applied locally and / or intravenously before. The chemotherapeutic enters the cell in greater percentage and induces the apoptosis.
I possible to apply it on the tumor directly or as an adjuvant after surgery, inside the surgical bed and treat possible cancer cells that could not be treated. It is very useful in those tumors that are very difficult to resects or resolved with little margin or in those cases in the surgical intervention it is very aggressive.
February 2019, a 6-year-old male feline with a recurrent high-grade soft-tissue sarcoma infiltrative with 2 previous surgeries, measuring 5 cm x 6 cm x 4 cm located between the back of the neck and the inter scapular area, showed up at the oncology consultation. Other veterinarians suggested euthanasia for the rapid evolution and recurrence and the location was difficult to resolve surgically with margin. But given the others alternatives; tumor resection, electrochemotherapy and subsequent reconstruction with flap technique are proposed. The procedure was accepted by the owner and due to the high recurrence rate of these types of tumors, the cat was monitored every 15 days for 3 months and then every 1 months for 6 months and every 2 months for 12 months to evaluate the evolution of the surgical suture. So far 48 months have passed since the procedure was performed and today, January 2024 there is no evidence of tumor recurrence and no evidence of metastasis. In conclusion, the procedure performed was very satisfactory, without recurrences or metastasis.

PO-78

Electrochemotherapy in combination with surgery and radiotherapy. The role of translational medicine

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Introduction. Electrochemotherapy is a new treatment modality for skin tumors. It consists of the administration of intravenous Bleomycin in a low dose followed by the application of an electric field that forms pores in the cell membrane. This allows the cytotoxic potency to be increased more than 1,000 times, with very few adverse effects. It produces tumor destruction regardless of the histology. It is used both in human and veterinary medicine with very good results.
Objective. To describe the role of electrochemotherapy as a new step for treating cancer patients combined with surgery or radiotherapy, comparing results in human and veterinary medicine.
Methodology. In this work, we propose the indications for electrochemotherapy in combination with surgery or radiotherapy. The experience both in human and veterinary patients is compared and presented. Ten canine and ten human patients with skin cancer in similar locations were treated (5 with surgery and ECT and 5 with radiotherapy and ECT). The treatments in vet medicine were performed using the EPV-200 (BIOTEX SRL, Argentina) electroporator, and in human medicine the OncoPore (BIOTEX SRL, Argentina) electroporator. In both cases intravenous bleomycin was used, and the procedures were conducted in the same manner.
Results. Electrochemotherapy is a very useful treatment modality that can be used in combination with surgery, as a cytoreductive procedure to allow a less aggressive surgery. It can be used as a recue procedure when a relapse in the scar is seen, avoiding a new resection. When combining

with radiotherapy, it can be used as a rescue tool in irradiated areas. However, depending on the extension of the relapse, a great tissue defect can be produced, and this must be considered when planning the treatment.
Conclusion. Electrochemotherapy is a therapeutic alternative with a high local response rate and few adverse effects for those patients who are not candidates for surgery or present a relapse after surgery or radiotherapy. Human and veterinary treatment outcomes are similar, and experience from both can be summed to increase the knowledge in the field.

PO-79

Impact of Reversible Electroporation on Melanoma Cell Viability and Extracellular Vesicle Function

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Our study investigates the impact of reversible electroporation on melanoma cell viability and growth, along with the functional properties of isolated extracellular vesicles (EVs). Preliminary experiments established an electrical field strength of 800-1600 V/cm for 100 µs at 1 Hz frequency and 8 pulses (ESOPE) as an optimal range that minimizes cell death, thereby validating the reversible electroporation approach. EVs from untreated melanoma cells were observed to promote growth; however, their stimulatory effect was compromised when melanoma cells were treated with EVs from electroporated cells, impeding tumor development. Significantly, this study also recovers and evaluates medium and large EVs—often overlooked in isolation protocols—for their potential as carriers of biomolecules essential for EV biogenesis and function. Techniques such as impedance measurement along with wound healing and cell adhesion molecule analysis revealed a 'bystander effect' following electroporation, which resulted in decreased proliferation and migration, particularly evident in the A375 melanoma cell line. Furthermore, the application of EVs influenced intracellular junctions and the adherence of melanoma cells, potentially altering intercellular communication pathways and adhesion dynamics. These insights suggest a promising role for reversible electroporation in the modulation of EV-mediated cell communication, with significant implications for the development of targeted cancer therapies.

PO-80

Potential of Ultrashort Pulsed Electric Fields to Empower Traditional Cancer Treatment by Breaking Solid Tumor Barriers

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Although cancer immunotherapy is deemed to shed light on the long-lasting fight against cancers, its efficiency on solid tumors is barely satisfactory due to the compactness blocking antibodies. Pulsed electric fields has been a powerful tool for ablating solid tumors, and narrowing pulse duration can improve the field homogeneity penetrating into the tumor. In this study, we compromised the integrity of multicellular tumor spheroids (MTSs), downgrading their compactness with ultrashort pulsed electric fields. The 3D multicellular tumor spheroids were cultured in 96-well microplates coated with 1.5% (w/v) agarose. After 3-5 days of culture, the consistency of size of C6 and U-87 MG spheroids was confirmed. After being exposed to ultrashort pulsed electric fields, the viability of spheroids was measured with Calcein-AM/PI fluorescent images and CellTitler luminescent assay. Immunofluorescence analysis confirmed that ultrashort pulsed electric fields can suppress the proliferation capacity with lower Ki-67 signal. Ki-67 protein is highly expressed during he G1, S, G2, M phase of the cell division, while cell in G0 phase cannot express Ki-67. Thus Ki-67 index is positively related to cancer cell division, differentiation, infiltration and metastasis. In addition, the cell-cell junction was broken by ultrashort pulsed electric fields with lower expression of adherens junction protein N-cadherin and tight junction protein ZO-1. It is evidenced that the capability of ultrashort pulsed electric fields to downregulate the intercellular adherence, as well as suppress the epithelial-mesenchymal transition, a key process of metastasis of cancer cells. At last, aqueous fluorescent

nanoparticles were applied to simulate the anticancer drug or therapeutic antibodies. Under the supervision of fluorescence microscopy, the degree of nanoparticles penetrating into the spheroids was positively related to the number of ultrashort pulsed electric fields, marked with a higher fluorescent signal from the inner quiescent zone or a necrotic core. In conclusion, we emphasize that ultrashort pulsed electric fields could be promising for downgrading the compactness of solid tumors, being a powerful assisted therapy for the delivery of anticancer drugs and therapeutic antibodies.

PO-81

Novel Bipolar Pulses for Improved Co-transfection Outcomes: Implications for CRISPR Cas 9 Delivery

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CRISPR Cas 9 technology has been instrumental in genetic research since its first implementation. Though the technology continues to show significant promise, there are challenges with clinical implementation. Notably, simultaneously delivering requisite molecular components (donor DNA, guide RNA, and the Cas 9 protein) across the highly selective cell membrane is a challenge in vivo. Electroporation has been proposed as a potential solution to this challenge due to its ability to induce transient pores in the cell membrane enabling simultaneous transport of all three molecular components of CRISPR systems.

For clinical applications, traditional electroporation protocols require substantial optimization to ensure meaningful viability in vivo and issues surrounding intense muscle contractions are associated with monopolar pulses. Bipolar pulses have shown to be less stimulatory than traditional monopolar pulses, indicating that these bipolar pulses may be able to overcome current issues with clinical translation. However, the transfection capabilities of bipolar pulses have yet to be fully investigated.

In this study, transfection outcomes of a novel class of bipolar pulse waveforms were compared to traditional electroporation protocols using 2D suspension models and 3D collagen tissue mimics. These novel pulses introduce several new parameters such as shape, balance, pulse duration, and pulse delay. After identifying an electric field strength which preserved cell viability, a variety of waveforms and delivery rates were used to transfect a green fluorescent protein (GFP)-encoding plasmid. Transfection efficiency and viability were then evaluated using microscopy and flow cytometry. Optimal protocols were then used to assess co-transfection using a red fluorescent protein (RFP)-encoding plasmid in addition to GFP plasmid to ensure the feasibility of simultaneously delivering multiple molecular components (DNA, RNA, and Cas 9) required for CRISPR Cas 9 editing.

Cell viabilities similar to current electroporation protocols, that have been identified as optimal, were achieved by utilizing the novel waveforms at an electric field intensity of 1500V/cm. However, the novel waveforms resulted in significantly higher transfection rates with the best performing treatments resulting in a 94-fold increase compared to transfection rates seen in the traditional protocols. Interestingly, while trends in transfection rates were preserved during co-transfection, the overall transfection rates increased with an average of 79% of transfected cells expressing both plasmids.

The novel bipolar pulses used in this study demonstrate significant improvement over traditional electroporation in relation to transfection and cotransfection efficiency, however, these results and the assumed non-stimulatory nature of these specific bipolar waveforms requires further in vivo validation.

PO-82

Study on the Effect of Microsecond Pulsed Electric Field in Promoting Wound Healing in Diabetic Mice

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Effective treatment of diabetic wounds, which are chronic wounds that are difficult to heal because of microenvironmental factors such as inflammation, immunodeficiency, infection, and hypoxia, remains challenging worldwide. Here, we introduce an emerging physical therapy that chooses

microsecond pulsed electric fields (μsPEFs) to cause reversible electroporation of fibroblasts and selectively kill senescent cells, providing new ideas for diabetic wound healing.Firstly, electroporation experiments were conducted on the more important fibroblasts in wounds, and by regulating the experimental parameters of μsPEF, it was found that μsPEF could regulate the signaling pathways within the fibroblasts, and activate or inhibit the function of the fibroblasts; then, during wound healing in diabetic mice, μsPEF improves the inflammatory microenvironment during the inflammatory phase, promotes cell proliferation and differentiation, facilitates neovascularization and improves wound blood flow during the repair phase, and regulates collagen synthesis ability during the remodeling phase to reduce the formation of scar tissue. In summary, μsPEF can provide favorable conditions for wound healing. This study confirms the mechanism of action of μsPEF in promoting the diabetic wound process and provides a new therapeutic strategy for clinical practice.

PO-83

PEF effect on a 3D in vitro model: a breast cancer case

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Pulsed electric fields are used to perform cell membrane electroporation. They are conveniently adopted in cancer therapy associated with chemotherapy, in order to improve the drug uptake maintaining the cell integrity (i.e. reversible electroporation RE), or alone, in order to destroy cells with an irreversible process (i.e. irreversible electroporation IRE). The RE therapy is currently used in Europe as an effective treatment of several types of superficially metastatic tumors like breast cancer recurrences. IRE is used in USA for cancer treatment.

Recent studies put in evidence that electric stress can activate the immunological response of the cells. The 3D cultures realized by Hyaluronic acid scaffold functionalized with an adhesion sequence, allow the generation of the extracellular matrix, ECM, typical of the cell lines and enhance the cell-cell and cell-ECM interactions. From previous studies, a different response in terms of electroporation intensity of the 3D and 2D cell cultures to the same protocol conditions was evidenced.

Therefore, here we evaluated two breast cancer cell lines (MCF7 and MDA-MB-231) and established and validated their growth as 3D cell culture in Hyaluronic acid scaffolds. In these experimental condition, the cells were able to generate ECM with collagen and demonstrated an electroporation enhancement. In these models, the markers of reversible and irreversible electroporation will be evaluated and the results will be compared with 2D growth conditions. The 3D cultures will be exposed to different electroporation protocols, one typical of RE and IRE, in order to evaluate the stress response through PCR analyses of cytokine and inflammatory pathways.

PO-85

Rare non-malignant, locally aggressive lesions of the head and neck treated by electrochemotherapy

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Certain benign lesions in the head and neck area can be locally aggressive causing serious symptoms. Such non-malignant tumors are fibromatosis and multiple adnexal neoplasia related to Brooke-Spiegler syndrome (BSS). Fibromatosis is a slow-growing and locally infiltrative condition caused by uncontrolled proliferation of fibrous tissue arising from deep musculoaponeurotic structures, while in BSS the numerous cylindromas, trichoepitheliomas, spiradenomas are at a high risk of malignant transformation. In both diseases surgery is usually the choice of treatment. In fibromatosis it is difficult to achieve clear resection margin due to the complex anatomy and frequent entrapment of neurovascular

structures in the head and neck, while in BSS the numerous lesions limit the surgical management.

Between 2015-2022 five BSS patients (3 female, 2 male, mean age of 47 years), and one 37-year-old female patient with severe symptoms caused by compression of a bifocal histologically confirmed fibromatosis in the left upper neck and in the supraclavicular region were treated in our institute with ECT. All BSS patients had multiple adnexal neoplasia located on the head and neck region and had previous surgeries resulting in numerous scars and extensive areas of alopecia. Our patient with fibromatosis after incomplete resections of the tumor, received immunomodulatory therapy (thalidomide) which was suspended because of its side effects. In the case of fibromatosis due to severe symptoms caused by compression from the tumor mass, and in the cases of BSS in order to provide tumor removal and disease control, ECT was decided by our multidisciplinary tumor board.

In all cases ECT was performed according to the ESOPE criteria with standard needle electrodes, and was repeated if necessary (in one case 8, in another case 2 settings of ECT were carried out) Tumor response was evaluated according to a Recist 3.0, and iRecist criteria.

After 6 months all treated BSS lesions flattened, showing partial regression; in the case of fibromatosis follow up was carried out by MRI, which confirmed 3 months after ECT the clinically significant regression: tumor volume was decreased by 77% for the bigger lesion and by 45% for the smaller one. We did not experience any serious side effects.

With the ECT treatment of those nonmalignant tumors our aim, the disease control was achieved. These cases demonstrate, that ECT has its role in the treatment of selected benign lesions, which are surgically not manageable.

PO-86

Bleomycin electrosclerotherapy (BEST) to manage head and neck venous malformations: a new therapeutic option and a case series

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Background & Aim: Venous malformations (VMs) are congenital vascular anomalies with a prevalence of 1%. More than 40% occur in head and neck region. Diagnostic workup consists in clinical assessment of soft, dark-red, or bluish masses accompanied by Doppler Ultrasound and Magnetic Resonance. Only symptomatic or enlarged VMs are treated. Recent application of intralesional Bleomycin with reversible electroporation (electrosclerotherapy-BEST) demonstrates promising results in VMs' regression.
Material & Methods: Eight adults, 7 females and 2 males (median age 39 years, range 21-78) and a total of 12 VMs (5 mobile tongues, 2 oral pelvis, 2 submandibular spaces, 2 cheeks, 1 palatine tonsil) were treated. All patients were submitted to BEST with Cliniporator ™ according to the standard operative procedures of the ESOPE Study.
Results: No major complications (loss of tissue or functional impairment) were observed. Everyone had mild swelling and pain for some days. The 4 tongues, 1 submandibular space and 1 oral pelvis VMs underwent complete response and patients are still free of disease at 11 median months of follow up (range 33-1 months). The patient with complex tongue, cheek and oral pelvis VM and the other one with cheek VM underwent partial response at 3 months after BEST and the other 2 VMs (1 submandibular space and 1 tonsil) remained stable at 15 and 6 months, respectively; but patients no longer had pain or swelling.
Conclusions: BEST increases cell membranes' permeability to Bleomicyn, enhancing its sclerotizing effect.8 It can be applied in adults and children. Results are often acquired in a single session. Multiple sessions are recommended in case of wide lesions or symptomatic recurrences. The treatment is safe, with organ and functional sparing.

PO-87

Nano-Electrochemotherapy (NEC) to enhance head and neck cancer treatment

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Electroporation (EP) is the application of a localized electric field able to increase the permeal-

ization of molecules into cell membranes inducing the temporary depolarization of the voltage-gated channels. Electrochemotherapy (ECT), a combination of EP and chemotherapy, is used as standard operating procedure (SOP) for cutaneous metastases, primary skin and mucosal cancers that are not amenable to surgery. To increase the efficacy of ECT treatment, the combination of EP with nanomedicines is starting to present a valid adjuvant strategy for the treatment of head and neck cancers. The designed nanocarriers (e.g., liposomes-Lps) should be able to guarantee an intracellular drug-controlled release by application of external electric field. Moreover, thanks to the similarity between cellular and liposomal membranes, EP could be used as external trigger to obtain simultaneous and reversible electro-permeabilization that permits: i) easier uptake of the encapsulated drugs by the electroporated cells and ii) enhanced drug release into cytosol. The proof of concept to evaluate the Lps response when subjected to electroporation both from a morphological and functional (drug release) point of view. Lps were produced using microfluidic technique platform (Precision NanoSystems Inc.). DSPC:Chol 50:50 and 70:30 molar ratio in ethanol solutions [10mM] were used for Lps production. The flow rates ratio (FRR) and total flow rate (TFR) were optimized using a Design of Experiment (DOE) approach. Charged lipids DOTAP (cationic lipid) or DOPS (anionic lipid) were added until 5% molar ratio. Electroporator Gendrive IGEA was used. Gentamicin sulfate (GS 1mg/mL in PBS, pH 7.4) was used as model drug for liposomes loading.

Morphological and dimensional characterization was performed. Encapsulation efficiency (EE%) and effect of electroporation on drug release were tested. Preliminary cell uptake on Mesenchymal Stem cells (MSCs) was performed using fluorescent labeled Lps.

The results showed that more suitable Lps composition is DSPC:Chol 70:30 with DOTAP. Dimensional range was lower than 250nm with dispersion values between 0.2-0.3. EP, performed at increasing voltage values (160V, 200V and 250V) did not alter the dimensions of the Lps not causing irreversible poration of the lipidic membranes. Charged Lps, with 70:30 DSPC:Chol molar ratio, showed greater values of EE% (about 30%) compared to 50:50 DSPC:Chol (about 10 %). Single cycle EP (Volt:160V, n° pulse:8, length:10□s, RT) performed on GS loading Lps allowed to achieve a 46% GS release from liposomes. The cellular uptake of Lps undergoing EP, was faster compared to 0V standard conditions.

The preliminary results are encouraging in being able to use Lps nanosystems as drug carriers to obtain better cellular penetration and on-demand drug release by exploiting electroporation stimulation. Trials are ongoing on head and neck squamous cell carcinoma to exploit the nanocarrier-EP platform.

PO-88

Characterising and enhancing immunogenic cell death following reversible ion electroporation

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Calcium electroporation was first proposed as an alternative to electrochemotherapy due to the known safety, lower cost, and ease of administration of calcium ions. The mechanism of CaEP-induced cell death is widely accepted to involve ATP depletion and loss of homeostasis, leading to either apoptosis or necrosis in tumours depending on the severity of ATP depletion (1). However, several studies have shown the ability of CaEP to induce the release of damage-associated molecular patterns (DAMPs) from cancer cells, including ATP and HMGB1 (2,3). It has also been shown that CaEP is equally effective as electrochemotherapy to induce immunogenic cancer cell death and long-term immunological memory in animal models (3) and is equally effective in clinical settings (4). This suggests that calcium may not only act as a homeostatic disruptor but also as an adjuvant to drive anti-cancer responses within electroporated tumours.

This project will investigate the mechanistic pathways by which electroporation of calcium and

other ionic species function to induce tumour cell death. This study aims to elucidate the cellular pathways underpinning cell death induced by ionic electroporation, and to enhance immunogenic cell death and systemic anti-cancer responses by delivering various ionic species.

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PO-89

The Effects of Buffer Composition on Gene Electrotransfer by Nanosecond Electric Field Pulses

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The introduction of foreign genetic material into cells through the pores formed due to electroporation is a technique called gene electrotransfer (GET) or electrotransfection. This non-viral gene delivery method is very promising not only for gene therapy but also as a biotechnological step for the development of long-term transfected cell lines.
In this study, we evaluated the transfection efficiency of reporter genes (green fluorescent protein and luciferase) in Chinese hamster ovary (CHO-K1) and murine breast cancer (4T1) cell lines using different composition of electroporation buffers (a total of 8 buffers). We have compared the microsecond protocols (1.2/1.5 kV/cm × 100 µs, n = 2–8) with high frequency (1 MHz) bursts of nanosecond pulses (4/5 kV/cm × 300 ns, n = 250, 500). It was shown that buffers highly affect transfection efficacy as well as cell viability post-treatment. High-frequency nanosecond protocols ensured a better transfection efficacy than µsPEFs regardless of the plasmid used.
Finally, the most prominent high-frequency submicrosecond range protocol was successfully applied for the long-term electrotransfection of the murine 4T1 cell line with linearized luciferase-pcDNA3 plasmid. Later, as a proof of concept, 4T1-Luc tumors were induced in mice (BALB/C) and tumor luminescence was visualized in vivo. It is concluded that high-frequency nanosecond range protocols are suitable for transient and long-term transfection, while the efficacy of the procedure can be also modulated by buffer composition.

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PO-107

Lentigo Maligna Melanoma and Acral Lentiginous Melanoma Treatment with Electrochemotherapy

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Introduction: lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM) are characterized by their ill-shaped borders and commonly manifest on highly visible areas such as the face or the soles of the patients. The challenge in treating these melanomas lies in preserving function and achieving satisfactory aesthetic outcomes

while ensuring complete surgical excision with an appropriate safety margin. Electrochemotherapy (ECT) can be an efficient treatment for both LMM and ALM.

Case presentation: two patients with histologically confirmed LMM and one patients with ALM presented at the Department of Dermatology and Allergology in Szeged, Hungary. A 79-year old female patient presented with LMM on the left cheek, which was first treated with radiotherapy resulting in partial remission. Following the recommendation of our multidisciplinary tumor board, ECT was performed, leading to a clinically complete response after six months. A 83-year-old female patient had LMM on the forehead and right upper eyelid. After ECT, she was prescribed topical imiquimod and showed partial response four months later. An 82-year-old male patient diagnosed with ALM on the right sole underwent two rounds of ECT. During the second treatment, two biopsies were obtained, neither confirming the presence of melanoma. Only minimal, local side effects were observed. All patients were treated with intravenous bleomycin according to the ESOPE (European Standard Operating Procedures of Electrochemotherapy) criteria.

Conclusions: scientific literature underscores the complexity of managing LMM and ALM due to their propensity for irregular borders and localization on cosmetically sensitive areas. Our cases support the role of ECT as a promising treatment modality for LMM and ALM. Further clinical studies and refinement of treatment protocols are warranted to elucidate the optimal utilization of ECT in the management of these melanomas and to expand its clinical applicability.

PO-90

The synergistic electrotransfection effect of low-amplitude continuous wave application and nanosecond electroporation

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Electroporation is a phenomenon in which the cellular membrane temporarily becomes permeable to various hydrophilic molecules, including pDNA. It has been shown that plasmid DNA, following cell treatment with electric pulses, interacts with the affected membrane and forms membrane-pDNA complexes. Subsequently, the pDNA is internalized into the cell.

Recently, electrotransfection using nanosecond electric pulses was demonstrated. However, the transfection efficiency was not as high as compared to that using microsecond electric pulses. One reason for this is the short duration of the pulses, which induce limited electrophoretic forces to drag pDNA to the affected membranes. Several publications suggest that additional low-voltage pulses can increase the transfection efficiency of microsecond pulses. Here, we employed low-voltage continuous waves after the application of nanosecond pulses to enhance the transfection efficiency of the cells.

The CHO cell line was utilized for the experiments. A concentration of 2 million cells per milliliter was prepared in a laboratory-made electroporation medium with a conductivity of 0.1 S/m, pH of 7.1, and an osmolarity of 270 mOsm. Cell electroporation was performed using a 1 mm electroporation cuvette. Each treated sample contained 50 µl of cell suspension. GFP-coding plasmid DNA was used at various concentrations ranging from 50 to 300 µg/ml. Nanosecond electroporation was conducted using a nanosecond pulse generator developed at VilniusTech University (Lithuania). Various forms of low-voltage waves were generated using a continuous wave generator. Transfection efficiency was assessed using a flow cytometer (BD Accuri C6).

We utilized nanosecond electroporation parameters to achieve approximately 20 percent transfection efficiency. When an additional low-voltage continuous wave was applied to the electroporated cells, the transfection efficiency increased dramatically to 60 to 70%. Furthermore, the cells did not experience cell death, maintaining around 90 percent cell viability. The results will give insights in the effectiveness of the waveform and the duration

of the continuous wave applications alongside the application of nanosecond electric pulses.

PO-91

Electroporation-Enhanced Resveratrol Delivery into 3D-Hyaluronic Acid-Peptide Scaffold Cells for Effective Triple-Negative Breast Cancer Treatments

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Triple-Negative Breast Cancer (TNBC) is the most aggressive sub-set of breast cancer, that is also metastatic. TNBC is characterized by its clinical absence of the three most common receptors, Estrogen, Progesterone, and Human Epidermal Growth Factor Receptor 2 (Her2) and therapeutic modalities, including the prevalent chemotherapeutic agent Cisplatin. It has the worst survival and a lower 5-year survival rate compared to other subtypes, such as luminal A, luminal B, and HER2-positive breast cancers. Thus, there is an unmet need for alternative, effective and affordable therapies that are gentle on both the body and the purse.

Towards this, in this research, we explored the efficacy of Resveratrol (Resv), a natural polyphenolic compound that is widely available in red grapes and other dark colored fruits and vegetables, on MDA-MB-231, human, TNBC cell line. For this purpose, a 3D scaffold matrix, composed of hyaluronic acid (HA) and self-assembling peptides (SAPs; specifically, EAbuK), which are further condensed with a Laminin-derived adhesive motif (IKVAV). The major advantage of 3D cell culture is that it mimics more closely the in vivo model, meeting the 3R principles (Replacement, Reduction, and Refinement). Given the known limitations surrounding the bioavailability of Resveratrol, this study integrates Electroporation (EP) to enhance the uptake of the intracellular translocation of Resv, employing brief electric fields to transiently permeabilize cellular membranes.

MDA-MB-231 human TNBC cell line, derived from a Caucasian woman was seeded within the custom-fabricated HA-EAbuK-IKVAV scaffold that simulates the three-dimensional architecture of biological tissue environments. The experimental protocol involves the administration of Resv with the application of electric pulses with parameters set at 800–1,000 V/cm and 100μs duration, at a frequency of 1Hz, followed by a comprehensive assessment of cell viability and reactive oxygen species (ROS) production. The results of this study are compelling, revealing a cell viability, as low as 24% at 24 hours of treatment with EP and Resv, underscoring the therapeutic potential of this approach. Moreover, the concomitant use of Electroporation and Resveratrol at specified concentrations significantly augmented ROS generation (4x) compared to singular treatments, indicative of a potent synergistic interaction that exacerbates TNBC cell mortality via enhanced ROS-mediated apoptosis. These robust findings underscore the potential utility of Resveratrol as an anti-TNBC agent and highlight the pivotal role of electroporation in augmenting its cellular uptake and therapeutic impact.

PO-92

The search for an optimal IRE protocol in terms of pulse duration considering damage due to temperature effects

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Searching for an optimal dose response in terms of pulse number or pulse length in an EP-based protocol requires the measurement of the electroporated tissue area and threshold trajectories, a major task. Recently it was shown that these trajectories could be determined by the time gradient of the associated electric field [1]. These results are extended to search for an optimal dose-response for an IRE protocol in terms of pulse duration considering damage due to temperature effects. The methodology introduced here is illustrated with partial data taken from [2]

who studied the effect of local temperatures on ablation size from an IRE protocol using constitutive pulses in the range 1-100 μs. Preliminary results show that for constitutive pulse lengths in the range of 1-100 μs, without considering damage, ablation increases logarithmically with pulse duration, while the threshold decreases exponentially. Moreover, all pulse duration trajectories have common ablation and threshold curves. Damage increases with pulse number and pulse duration. An optimal dose-response in an IRE treatment, such as in [2] but with a fixed pulse number and frequency, is predicted as the critical pulse duration dosage yielding maximum ablated tissue area with minimal damage due to temperature.

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PO-93

Effectiveness of a Novel Basket-Shaped Pulsed Field Ablation Catheter for Intra Pulmonary Vein Ablation

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Background: Pulsed field ablation (PFA) has emerged as an alternative energy source for catheter ablation for atrial fibrillation. The safety and efficacy of this novel energy source have been demonstrated in previous reports. However, aiming for pulmonary vein (PV) isolation may lead to recurrence due to one small arrhythmogenic reconnection. We have previously demonstrated the safety of ablation within the PVs. This study aimed to elucidate the efficacy of a new PFA device for true “single-shot” PV ablation and isolation (PVI) through targeting of the intrapulmonary and ostial in a single catheter position in both the acute and chronic periods.
Methods: Five swine were utilized in this study. The device is an expandable basket-shaped catheter (maximum diameter 20mm), equipped with 27 electrodes with pacing and sensing capability (panel A, Access PoinF-spline PentaRay catheter and Carto system (Biosense Webster, Diamond Bar, CA). Total Joule delivery per vein was 11.25, over 8 deliveries. Ablation was performed in the superior vena cava (SVC), right inferior PV (RIPV), left inferior PV (LIPV), and right superior PV (RSPV); ablation was first performed within the respective vein (distal bipole delivery), then in the ostial aspect (proximal bipole delivery). PVs were identified by fluoroscopic, intracardiac echographic and 3D EAM guidance (panel B). We assessed the local electrogram voltage amplitude and capture thresholds pre and post-ablation.
Results: In total, PFA was delivered to 11 PVs and 5 SVCs. The sharp (near-field) potential was consistently eliminated by PFA application utilizing the distal bipoles, consistent with PV ablation (panel C). Commensurate with this, PV sleeve capture was eliminated; however, ostial atrial potentials and capture threshold were not significantly changed (7.1±3.2mA). Following ostial ablation, ostial signals were eliminated, and capture threshold increased further. 11 veins were evaluated chronically at 30 days – of these, 9 remained without PV potentials (panel D).
Conclusions: This study demonstrates the effectiveness of a prototype PFA device with ability to achieve pulmonary vein sleeve and ostium ablation without need for catheter repositioning and stenosis.

PO-94

Comparison of high-frequency pulse train alternating form on endothelial cell electroporation and permeability

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Brain endothelial cell plays a key role in the structure and function of the blood-brain barrier. When high pulsed electric field is applied to cells, it can induce cell electroporation and increase intracellular pathway permeability. Two electrode pins

(1 mm diameter and 2.3 mm spacing) are used to treat the adherent endothelial cells. The applied electric pulse: the amplitude 1 kV, the positive/negative width 100 ns, 200 pulse burst with total energized time of 2 μs per burst at 4 Hz, and We use change in fluorescence of YO-PRO-1 dye to quantify membrane permeability by electric pulses. We investigate the alternating form the pulse interval between positive and negative the effect of multiple alternating bipolar and single alternating bipolar pulsed electric fields on the permeability of endothelial cells. In multiple alternating bipolar mode, the electroporation effect area both increases first and then decreases with the increase of the period. we first fix the interval at 500 ns (peek occurs when the period is around 5 μs), and then we adjusted the interval to half of the period (peek occurs when the period is around 15 μs). Under the same period, electroporation of the fixed interval one is weaker than that of half of the period. In single alternating bipolar mode, the electroporation effect area decreases with the increase of the period, but always stronger than multiple one.

PO-95

Dynamics of plasma membrane charging and relaxation measured by strobe fluorescence microscopy

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Chinese Hamster Ovary (CHO) loaded with a voltage-sensitive FluoVolt dye were exposed to 1-μs electric pulses at field strengths above and below electropermeabilization threshold. The cells were exposed early after their attachment to the substrate when they still maintained a nearly round shape. The dye was excited by ˜8-ns laser flashes delivered at varied delays with respect to the electric pulse. The delays were changed in 50-ns increments or decrements, to collect cell images at different times during cell charging and relaxation of the induced transmembrane potential (TMP).

Cells were exposed at 0.09-0.48 kV/cm to build the dependence of FluoVolt fluorescence on PM charge and cell diameter. The obtained curve had a sigmoidal shape with linearity in the range of ±45% fluorescence change from the resting level. The data correlated with the theoretical values with a linear range corresponding to ±400 mV TMP change. Beyond this range, FluoVolt signal did not change despite applying a stronger electric field, presumably because the electropermeabilized membrane did not charge further. However, electropermeabilization could not be detected by standard methods (such as electroporative Ca2+ uptake) at field strengths below 1 kV/cm.

The induced TMP was maximum at cell poles facing the electrodes and gradually decreased towards the cell equator. At 70□ angle, TMP change decreased on average by 62%. This dependence matched theoretical values in most cells exposed at 0.09-0.15kV/cm. Large cells exposed at □0.25kV/cm displayed a smaller induced TMP at the poles than predicted. The charging time constant equaled the relaxation time constant for electric field strengths □0.15kV/cm. More intense pulses reduced the charging time constant but did not affect the discharging one, and this difference was more pronounced in larger cells.

Analysis of PM time constants at the different angles to the electric field showed no difference between charging and discharging for the cells exposed at □0.15kV/cm. In cells exposed at □0.25 kV/cm charging and discharging did not differ significantly at the 70□ angle, where minimal polarization was observed. However, the charging time constant decreased with the angle to the electric field, reaching the smallest value at the poles, while the discharging time constant remained nearly unchanged.

Our results suggest that more intense pulses briefly increase the membrane conductance, preventing further charging. This electropermeabilization event is short-lived and apparently beyond the detection limits of common electroporation detection methods such as a dye or Ca2+ uptake.

PO-96

Stream pulsed electric fields integral (sPEFI) and energy properties of tissue ablation on irreversible electroporation

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The pulsed electric fields ablation (PEFA) based on irreversible electroporation (IRE) is a technique that induces cell apoptosis or tissue ablation by creating irreversible pores in cell membranes under pulsed electric field (PEF), and has been widely in tumor therapy. The optimal combination of pulse electric field intensity, pulse length and pulse number can obtain good tissue ablation effect in micro- and nanosecond PEF. In the paper aims are proposed a stream Pulsed Electric Fields Integral (sPEFI) as an index of the combination, and established a functional relationship between sPEFI and electric field energy and tissue ablation by animal experiments, and revealed the laws of PEFA. Methods are to design some experiments of the pulse duration, pulse energy, pulse number and electrode spacing using Sprague-Dawley rat liver, to setup the empirical equation of sPEFI and electric field energy properties of ablation area from experimental data, and no effect and thermal effect threshold line in the Td-E0 map. Results were shown that sPEFI and energy curves exhibit exponential increases and appear saturation at the thresholds, the pulse initial voltage could control the saturation energy, and the no effect threshold of sPEFI is approximately 0.56 (kV/m)2s while the thermal effect is approximately 219.6 (kV/m)2s. Conclusions are that sPEFI depends on the pulse waveform and is a controlled parameter, which can be used as the index of the optimal combination of pulse intensity, width and number. Electric field energy of tissue consumption depends on pulse waveform and tissue resistance and is an uncontrolled parameter.

PO-97

Impact of pulse parameters on the conductivity variations in Biological tissues, treated with electroporation

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High intensity, short duration voltage pulses used for cell electroporation could also be used to determine the conductivity of the biological tissues using different pulse protocols. Given the shape and the size of the sample, such as a parallelepiped, the pulses are applied between two parallel faces, and voltage and are measured, and the resistance of the parallelepiped sample is determined. From the resistance and sample size, the conductivity is computed. In this research, the variation in the conductivity of the tissue, in this case, potato tubers were measured, when the pulse length, pulse number and pulse amplitude were modified. The pulse number was varied from 1 to 16 (1, 4, 8, 16) and the pulse lengths chosen were 10 µs, 100µs and 1 ms. The applied voltage amplitude was varied from 5 to 700 V. Given an electrode gap of 7 mm the electric field intensity varies between 3.5 V/cm to 1000 V/cm. The color change, which indicates the degree of electroporation (electroporated sample becomes dark) was verified after 24 h in order to determine the electroporation threshold corresponding to the protocol. For each protocol at least three measurements were considered and for each set of measurement the corresponding uncertainty was evaluated.

The results indicated that using 6 to 830 V/cm, 100 µs pulses, the conductivity varied from 0.04 to 0.42 S/m for one pulse, while it varied from 0.04-0.7 S/m for 16 pulses. The conductivity varied from 0.04 to 0.4 S/m for one pulse and to 0.04-0.8 S/m for 16 pulses, using 1 ms, 6 to 520 V/cm pulses. These mimic the biological tissue variations, which could be used for further studies.

PO-60

Pulsed field ablation for cardiac arrythmias: parameters prediction via machine learning

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Pulsed-field ablation (PFA) is a promising non-thermal tissue ablation technique that utilizes high-amplitude ultra-short pulsed electrical fields (PEF) to induce cell death through irreversible electroporation (IRE) in tissues. PFA can effectively treat various medical conditions, including cancer and cardiac arrhythmias such as paroxysmal and persistent atrial fibrillation. However, the selection the PEF protocols, the electrode parameters and characteristics to get specific and well defined ablative lesion is a challenging task which hinder IRE cardiac ablation treatment standardization as well as optimal device development, as well as regulatory decision-making for PFA protocols and procedures. In this context, artificial intelligence and machine learning algorithms could potentially improve the accuracy and effectiveness of PFA procedures by identifying the most suitable targets and predicting optimal PEF parameters. Toward such a direction, our study provides, for the first time, a robust and effective prediction and optimization of the IRE parameters for human cardiomyocyte ablation treatment in vitro. To this aim, a novel artificial intelligence model has been developed and validated through esperimental data extracted from digital libraries and literature to estimate the ablation area, electrode configuration, number of pulses, amplitude, period, and repetition rate of the applied signals.

PO-98

Optimization of pulsed electric field (PEF) processing conditions for wheat flour treatment using Response Surface Methodology (RSM)

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Processing wheat flour with PEF technology can potentially modify starches' physicochemical and structural characteristics, which may influence glucose release during gastrointestinal digestion along with detrimental effects on the flour quality characteristics due to starch damage increase.

This work was focused on the optimization of the pulsed electric fields (PEF) processing conditions for wheat flour using response surface methodology (RSM) with the aim to induce digestibility changes avoiding or minimizing the level of damaged starch (DS). DS content was determined by enzymatic analysis and used as a response variable to identify the optimal treatment conditions in terms of flour-water concentration (5, 10, and 20% w/w) and PEF processing conditions namely field strength of 3 and 5 kV/cm for 20-284 pulses, corresponding to a total specific energy input of 3, 5 and 15 kJ/kg using 10 µs rectangular wave monopolar pulse at 50 Hz. The physicochemical properties and digestibility of PEF-treated samples at optimal conditions compared to untreated samples were analyzed by SEM and an in vitro semi-dynamic digestion model, respectively.

Results demonstrated that, the application of PEF at processing conditions of 10% of flour-water concentration (w/w), 34 pulses, and 5 kJ/kg induced a lower level of DS (3.7%), which is within the acceptable limit for high-quality flours (up to 8%) and similar with those obtained for untreated samples. Under optimized conditions, PEF treatments on wheat flour samples caused a significant decrease in the rate of starch digestibility during the gastric and intestinal phase. For instance, a significant decrease (from 24.4% to 18.1%) in the rapidly digestible fraction (RDS) and a significant increase in the resistant starch fraction (from 70.1 to 78%) were observed. SEM micrographs exhibited unaffected size, shape and integrity of starch granules treated by PEF optimal conditions.

This preliminary study suggests that PEF treatments may modulate the starch digestibility of wheat flour by slowing the rate of digestion with minimal flour quality damage. However, further experiments should be performed in order to determine the effects of PEF treatments on the functional and mechanical properties of wheat flour.

PO-99

Flyback Versus Piezo Transformer Based Converter Topologies for Bipolar Pulsed-Power Applications

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Traditionally, magnetics-based power converter topologies are the most prevalent in medical devices, but with the emergence of piezoelectric transformers (PZT), the possibility of magnetics-free solutions presents itself to address issues with electromagnetic interference and compatibility with MRI. Our study presents a comparative analysis between two topologies, flyback transformer (FBT) and piezoelectric transformer (PZT) based converters, for use in high-voltage (HV) bipolar pulse generators for medical applications such as electroporation. With stringent design requirements such as high voltage and current ratings, rapid pulse repetition frequencies, fast rise times, high power densities, reliability, galvanic isolation, flexibility, and controllability, the choice of pulse generation topology is critical. In this study, performance metrics such as efficiency, charging time for capacitors, input power requirements, component count and capacitor configurations are thoroughly compared between the two topologies using LTspice software simulation. Work is ongoing to compare the solutions experimentally.

Both topologies are designed to achieve the same circuit specifications, converting a 24 V input DC voltage, to a 2 kV output DC voltage and up to 40 A output current pulses. The PZT based topology requires four MOSFETs on the inverter side, four diodes on the rectifier side, along with the commercially available piezoelectric transformer. On the other hand, the FBT based solution employs one MOSFET, one diode and a purpose designed electromagnetic transformer.

Both FBT and PZT topologies offer isolation, but they employ different mechanisms to achieve it. In FBT, isolation is primarily achieved through the transformer itself. In PZT topologies, isolation is typically achieved through the physical separation of the piezoelectric elements. The FBT relies on electromagnetic induction in a magnetic core, while PZT utilizes the direct and indirect piezoelectric effect.

Initial simulations show that in addition to removing magnetic components, the PZT solution demonstrates higher efficiency (92%) compared to FBT (80%). Furthermore, the PZT has a smaller size than the FBT electromagnetic transformer. More detailed results of circuits operation over a complete capacitor charging cycle will be provided during the conference, along with the complete circuit designs.

Our findings offer valuable insights for demonstrating the potential of a magnetics-free PZT based solution for high voltage (HV) medical device application requirements, balancing factors such as efficiency, power consumption, and component count.

PO-100

A Smart and Portable Electroporation System for More Rigorous Experiments

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Electroporators are prevalently applied in various scenarios of cell and tissue engineering. An electroporator delivers high intensity electric pulse waves to temporarily puncture reversible microholes in a cell membrane or a bacterial shell. It enables delivering of much larger gene segments across cell membranes and bacterial shells compared to conventional transfection methods. However, electroporators of today are usually heavy and large, in combination with old-fashioned "keypad + LCD display" operation, resulting in a steep learning curve and cumbersome to use. In addition, none of the existing electroporators provide visual feedback to confirm the correct generation of the desired waveform.

We report an ultra-small electroporation system, that includes ultra-small (155 x 155 x 33 mm, 0.7 kg) host station and a set of accessories that facilit-

ates experiments and provides users with scientific research level data and plots to compare between the designed waveform and the actual generated one.

The host station is a battery powered device that can be wirelessly controlled by a smart phone APP, facilitating saving and retrieving of setting history, measurement of resistance at the terminal, user-defined waveform setting, and visualization of the actual waveform to help users ensure correct experimental setup. In detail, the host station is powered by a rechargeable battery with 12 V nominal voltage. Its output is in the form of high voltage pulses, whose parameters can be fine-tuned according to needs. For example, voltage amplitude is tunable from 20 V to 200 V with 1 V step size, voltage sign (+, -) is exchangeable by pre-setting in the APP, center distance between two consecutive pulses is settable from 8 μs to 2 s, the number of pulses to be released is controlled from 1 to 100. All of these parameters are monitored by an internal microprocessor and an analog-to-digital converter (ADC), and displayed on the APP interface together with the set waveform, in order to visualize the difference between set value and the actual value.

In addition to the host device, the authors have developed a series of accessories that are extremely convenient for researchers and their experiments. The first accessory is called Unity Module. It enables connection of one electroporation cuvette to the high voltage pulses generated by the host device. The second is an Octuple Module that enables parallel processing of multiple sample cell suspensions loaded in multiple electroporation cuvettes. Last but not least, a Clamp Module, which directs the high voltage pulses through to a pair of crocodile clips. The clips can then be connected to any two electrodes in contact with cell.

In conclusion, the authors developed an electroporator system with intelligent user interface, versatile pulse generation and scientific level data recording characteristics. The system is dedicated as a next-generation tool for research in cell and tissue engineering and beyond.

PO-101

High-Performance Modular Pulse Generator for Electroporation Applications

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The application of pulsed electric fields (PEF) to different biological targets in so-called electroporation processes causes a series of relevant microscopic and macroscopic effects that are of interest in a broad range of scientific and industrial fields. This has created a wide range of electroporation applications ranging from in vitro cellular-level applications to clinical macroscopic applications, or industrial intensive applications including. In all these applications, pulse generators play a key role generating the desired voltage/current levels with specific features for each application.

In the current context, PEF applications require generators able to generate pulses ranging from few V and mA in in-vitro applications, to tens of kVs and hundreds of As in industrial applications. Frequencies and pulse widths also ranges from kHz and hundreds of µs, to MHz and ns in other applications, pushing towards ps generators. The same applies to the generated waveform, including exponential decay or square waveforms, or even more complex variable waveform. This context requires different technological solutions that leads often to suboptimal implementations and generator redundancies.

In this context, this paper details the design and implementation of a scalable high-performance modular pulse generator intended to be used in a wide range of PEF applications. The proposed converter is based on the use of wide bandgap power devices, i.e. silicon carbide devices, that allows for fast operation achieving rising times of a few ns, i.e., enabling frequencies beyond the MHz range. The proposed generator is implemented following a modular approach, where each module (Fig. 1(a-b)) includes two 750-V full-bridge inverters, allowing 3000 Vpp bipolar output voltage with a current capability exceeding 100 A per module. Finally, the global architecture includes an isolated control and power module that enables building as many par-

allel or series modules as required to achieve the desired voltage and current levels. As a result, a high-performance generator that can generate arbitrary waveforms exceeding tens of kV and hundreds of As in the MHz range is obtained (Fig. 1(c). Moreover, the scalable approach allows to cover a wide range of applications using an efficient implementation. The final version of this paper will include detailed implementation and experimental results, including the main waveforms of the generator already implemented.

PO-103

Simulation study on magnetoporation induced by pulsed magnetic field combined with magnetic nanoparticles based on pore energy

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Compared with traditional therapy, magnetic field combined with magnetic nanoparticles therapy is a promising development direction in tumor treatment due to its non-contact, small side effects and wide adaptability. However, the specific theory and mechanism of this combination therapy have not been fully elucidated, hampering its development and promotion process. Therefore, this paper takes as a clue the dynamic development relationship between pore energy and pore state in cell membranes. Firstly, the magnetic stress of magnetic nanoparticles and tumor cells under magnetic field was determined using the Maxwell stress tensor. Based on the function-energy relationship, the expression of the pore energy under the action of the magnetic field is obtained. Combined with the effects of line tension, surface tension and steric repulsion on the pore energy, the pore energy equation containing the above four contributions is formulated. Furthermore, tumor cells were selected as the focus of investigation, and a coupling model was established in COMSOL to simulate the random distribution of magnetic nanoparticles interacting with a monolayer of cells. The parameters of the pulsed magnetic field were set by the magnetic field module. The pore energy and the perforation characteristic were added to the partial differential equation module. The influence of different pulsed magnetic field parameters on the cell perforation characteristics was analyzed through the calculation results. The results show that when the magnetic induction intensity B≥0.8T, the pore density reaches the order of magnitude of 10^{13} (perforation threshold), leading to the perforation of the cell membrane. Once perforation initiates, the pore radius continues to increase until the pulse concludes. Under the action of square wave, triangular wave and sinusoidal half-wave, the square wave has the greatest influence on the pore radius, resulting in the strongest magnetoporation effect. With the increase of pulsed amplitude and pulsed width, the perforation characteristics such as pore density, pore radius and pore area satisfy the change trend of exponential function. This study reveals the perforation characteristics of magnetic perforation of cell membranes through simulation, offering a theoretical foundation and practical guidance for the combined treatment of pulsed magnetic field with magnetic nanoparticles.

PO-102

Rapid joule heating improves vitrification based cryopreservation

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Biological time can be effectively stopped when living biological systems are successfully cooled and stored at cryogenic temperatures (i.e., cryopreservation). Vitrification, an attractive ice-free alternative to conventional convective slow cooling methods, turns liquid water to a glassy phase by cooling rapidly enough to avoid ice formation and chilling injury. However, rewarming techniques that are rapid and scalable (both in throughput and biosystem size) for low concentrations of cryoprotective agent (CPA) for reduced toxicity are lacking, limiting the potential for translation.

Here, we introduce a joule heating–based platform technology, whereby biosystems are rapidly rewarmed by contact with an electrical conductor that is fed a voltage pulse. A format of rapid rewarming using joule heating can achieve rapid and scalable rewarming with low CPA concentrations for cryopreservation of biosystems at different scales. We

demonstrate successful cryopreservation of three model biosystems with different thicknesses, including adherent cells (~4 μm), Drosophila embryos (~50 μm), and rat kidney slices (~1.2mm), using low CPA concentrations (2-4M). For all 3 applications, a single monopolar voltage pulse was deliver using a voltage pulse generator (ECM 830) for rapid heating. We perform scaling analysis and numerical modeling to rationally design the joule heating to achieve rapid rewarming, followed by experimental validation. In short, the pulse width should be selected based on biosystem heat diffusion length, ranging from 10-100 μs for single cell layer up to 100 ms for thin (~ 1.2 mm) tissue slices. The optimal voltage is calculated by heat capacity, the targeted heating rate (critical warming rate, therefore heating power) and the size, ranging from. For a targeted SAR in the range of 3–600 × 10^{11} W/(m^3) and system between 1-15 cm^2, the voltage between 60-450 V (given internal capacitor of 4000 μF and current limit 500 A) will be needed. Numerical simulation predicts that warming rates from 5 × 10^4 to 6 × 10^8 °C/min can be achieved.

Comparison with conventional convective warming methods demonstrates that joule heating greatly improved viability at lower CPA concentrations for all the model biosystems studied. Our results present a general solution to the cryopreservation of a broad spectrum of cellular, organismal, and tissue-based biosystems.

PO-104

Experimental study on protein denaturation induced by MV/cm class electrical pulses

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The electric field strength on the plasma membrane of a living cell exposed to a pulsed electric field (PEF) of several kV/cm is automatically enhanced and exceeds 1 MV/cm. While electric fields of 1 MV/cm are known to permeabilize phospholipid membranes, molecular dynamics (MD) simulations predict that they affect the function and structure of membrane proteins with diverse structures [1]. There is growing interest in the effects of PEF on proteins, not only from a scientific perspective, but also in terms of its application to the physical control of protein function and structure, and the denaturation of proteins that occurs during the field sterilization of food products. However, it is not easy to identify PEF effects on proteins in living cells because other biological events such as Ca2+ ion influx and subsequent biochemical reactions make it difficult to identify PEF effects on proteins. Previously, we experimentally demonstrated that electrical pulses with 0.2 MV/cm or more denature urease proteins in aqueous solution [2]. Furthermore, we reported that electrical pulses on the order of 1 MV/cm class disassembled transthyretin aggregates [3]. Based on these studies, we have experimentally investigated the effects of 1 MV/cm class electric fields on the hierarchical structures of proteins. Two proteins, egg white ovalbumin (OVA) and bovine serum albumin (BSA), with similar molecular weights and different structures, exposed to 10 times of 1.3 MV/cm, sub-nanosecond pulses, 1 ns, were analyzed and compared in the respective hierarchical structures. Native-PAGE showed that PEFs with 0.5 MV/cm or more altered the tertiary structure of OVA but not of BSE. Native-PAGE showed that PEFs with 0.5 MV/cm or more altered the tertiary structure of OVA but not of BSE. Thiol group (TG) measurements and dynamic light scattering (DLS) indicate that the tertiary structure of OVA was loosen by the cleavage of the sole disulfide bond, whereas no significant change was observed in BSE. Circular dichroism (CD) analysis indicates significant changes in the secondary structure of neither OVA nor BSA. Throughout the experiment, pH and temperature remained unchanged. Hydrogen peroxide concentration was not changed significantly to affect the three-dimensional structure of proteins.

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PO-105

Where exactly do pores form in the complex organization of the plasma membrane? Insights from molecular simulations

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Previous studies have shown that intense electric field promotes formation of pores in a lipid bilayer, where some types of lipids are more and some less prone to pore formation. These studies typically investigated model lipid bilayers comprising up to three different types of lipids. However, the cell plasma membrane is a complex assembly of various types of lipids and membrane proteins. The lipids are furthermore asymmetrically distributed in the two bilayer leaflets and are organized in domains. Thus, it is important to understand how pores form in the complex lipid organization of the plasma membrane. This lecture will present results from molecular dynamics simulations using coarse-grained membranes containing more than 60 different lipid types, mimicking the realistic composition of plasma membranes. Simulations using such membranes demonstrate that pores do not form homogenously along the membrane surface, but colocalize with domains that have specific features, the most important being high density of polyunsaturated lipids. Furthermore, the simulations show that poration does not depend solely on local lipid arrangement, but also on membrane mechanical properties and the polarity of the electric field. Combining results from these coarse-grained simulations with atomistic simulations can help us understand how the rate of poration in lipid membrane domains compares with the rate of electric-field induced damage to membrane proteins such as voltage-gated ion channels. As such, these studies provide new insights into molecular mechanisms of increased membrane permeability associated with electroporation.

PO-106

Effects of microsecond pulsed electric field on tubulin structure and self-assembly

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Tubulins form the essential building blocks of microtubules, the largest type of filaments crucial for various cellular processes. Notably, tubulin proteins possess remarkably high structural electric charge and dipole moment. The functionality and interactions of tubulin proteins are significantly influenced by the electric charge present on atomic groups in their amino acid residues. Consequently, applying an external electric field presents a promising avenue for manipulating the behavior of cytoskeletal proteins like tubulin.
In this study, we illustrate several alterations in tubulin structure and self-assembly polymerization resulting from Pulsed Electric Field (PEF) treatment (using pulses of 100 microseconds width, at 1.5 MV/m, with 20-60 pulses fired at a repetition frequency of 1 Hz). Dynamic Light Scattering analysis revealed an increase in the effective size of tubulin molecule and the amount of aggregates following PEF treatment. Subsequently, we observed a notable decrease in tubulin concentration across all PEF-treated samples, particularly after the removal of the majority of aggregates through ultracentrifugation. Polymerization assays (UV-Vis turbidimetry measurements) demonstrated the loss of self-assembly capability in PEF-treated tubulin, which was further confirmed through phase contrast microscopy. Additionally, fluorescence spectroscopy employing 8-anilinonaphthalene-1-sulfonic acid (ANS) probe indicated an upward trend in fluorescence intensity in treated tubulin samples, suggesting PEF might induce structural

alterations in tubulin, exposing more hydrophobic groups to the molecule surface.
Our findings contribute to the advancement of novel electromagnetic techniques for influencing biomolecular function at the nanoscale. PEF treatment induces structural changes in tubulin molecules while retaining its fundamental self-assembly capability. Furthermore, we identified the PEF parameters threshold for irreversible tubulin changes and observed the aggregating effect of PEF treatment on tubulin.

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PO-108

nsPEF-mediated productivity improvement in bioprocessing – A cross-species evaluation of bacterial and yeast expression platforms in bioreactor cultures

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The technological advancement of pulsed electric field treatments into the nanosecond (nsPEF) time scale has opened an exciting new field of applications in the stimulation of biotechnological cultures. The underlying mechanisms and effects are still largely unknown, but thought to combine into a milder and only temporary cellular response, which however can have long-lasting impact on the culture. In bioprocess engineering, hope is that nsPEF treatment can be exploited as a versatile productivity booster via its generically applicable, physical principle.
Owing to the wide range of structural elements and metabolic pathways in cells that may be susceptible to nsPEF-induced changes, factors related to biomass formation (e.g. cell division rate and substrate utilization efficiency), as well as factors related to the biosynthesis of compounds (e.g. gene expression, intracellular accumulation or secretion) come into play as potentially fruitful optimization targets.
In this study, we report on the in-process application of nsPEF to microbial expression platforms grown under strictly defined conditions in fully PAT-controlled bioreactor setups. Recombinant E. coli and P. pastoris strains, containing GFP-tagged proteins, served as models to investigate the interdependence and cross-species comparability of nsPEF treatment and biological response. A standardized experimental approach was taken, with industry-near batch and fed-batch cultures executed in parallel twin runs interfaced to an nsPEF system in bypass loop configuration. On global culture and individual cell population level, physiological key parameters were followed via real-time monitoring and off-line analyses.
Focussing on growth kinetics, morphological features and protein expression, positive overall impact was observed across species borders, yet tracing back to differential effects depending on culture type and environment. Careful adjustment of nsPEF treatment conditions emerged as a prerequisite for optimal outcome, and caution is due in the selection and interpretation of state-of-art PAT tools. Next to a holistic combination of multiple indicators and data sources, our results recommend a closer look on the individual cells and population dynamics to leverage the full potential nsPEF-based stimulation in biotech production settings.

PO-109

Enhancing starch for 3D food printing: pulsed electric field modification and functional insights

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Modifying starch for 3D printing is necessary to enhance printability, structural integrity, customization of properties, and compatibility with additives, fostering innovation in food manufacturing while meeting regulatory standards. This research delves into the exploration of pulsed electric fields (PEF) as a way of tailoring the physicochemical attributes and processing characteristics of starch. PEF is seen as a promising technique for modifying food materials, such as starch, to enhance their functional properties and suitability for novel applications like 3D bioprinting. The study investigates

the impact of PEF on the physicochemical properties of pregelatinized corn starch (PGCS), and its potential for 3D printing.

PGCS was subjected to PEF treatments at an intensity of 15 kV/cm, a 20 μs pulse width, and operated at a frequency of 20 Hz for a range of 100-400 pulses. The study sought to observe the effect of this treatment on the starch's inherent granular structure, composition, structural order changes, tecno-functional properties, as well as the ability to form 3D printed constructs.

The findings revealed that PEF disrupted the native architecture of the starch granules, leading to changes in the structural organization. The treatment also improved the starch's water and oil absorption capacities, with results showing an increase of up to 56.8% and 104.5%, respectively. While PEF had a minimal impact on the starch's composition, it significantly improved pasting viscosities and the texture of the starch gel. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analyses suggested that the PEF treatment resulted in reduced crystallinity and elevated transition temperatures in the modified PGCS. When used to make hydrogels, the PEF-treated PGCS exhibited improved gel hardness. This characteristic was correlated with the creation of superior 3D printed constructs, which displayed sharper edges compared to those made from less modified counterparts.

Overall, the PEF-induced modifications enhanced the functionalities of the starch, conferring desirable rheological attributes for 3D bioprinting PGCS-based foods. These findings underline the potential of non-thermal technologies, like PEF, in rationally manipulating the physicochemical and processing behavior of starch. Such advancements could open new avenues in food science and technology, particularly in the development of 3D-printed food products.

PO-110

Inhibition of Color Change for Long Term on Meat of Bonito during -18□ Freezing by Applying Pulsed High Electric Field

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The temperature has to keep at -50°C or less by using ultra a low temperature freezer for long-term storage of meats and also at cutting and transporting, because the temperature below -50°C suppresses a color change of the meats. Therefore, meat prices become high. The color change of the meats is caused by that myoglobin converts to metmyoglobin by the decomposition of ATP. The decomposition of ATP, which is a freshness indicator of meats, causes by ATPase that is a decomposition enzyme of ATP. If ATPase could be deactivated, the long-term storage would be possible in a general-purpose refrigerator because the meat color does not change at -18°C. Therefore, we considered the application of pulsed power technology to deactivation of ATPase. Pulsed high electric field has been reported to be able to suppress enzyme activity without thermally impact to targets depending on pulse conditions.

In this presentation, meat of bonito was used for a sample applying the pulsed high electric field, because it undergoes a noticeable color change from fresh red to red brown over a short period of time. Furthermore, since it has been reported that myoglobin is easier to become metmyoglobin at between -5°C and -10°C, pieces of the meat of bonito were stored in a freezer set at -6□ and -18□ after applying the pulsed high electric field. Then, the change in color of the meat of bonito and the rate of conversion from myoglobin to metmyoglobin for the elapsed time were investigated. As a result, it is found that application of the pulse high electric field can suppresses the conversion myoglobin to metmyoglobin in the meat of bonito.

PO-112

Development of pulsed electric field pasteurization system for protein-rich liquid foods

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Pulsed electric field (PEF) is a promising non-thermal pasteurization method for protein-rich liquid foods. There are many reports showing the usefulness of PEF method in batch processing and low flow rate experiments. However, in high-flow rate processing of 100 L/h or more for industrialization, serious problems such as reduced bactericidal effect, spark discharges, and ingredient adhesion to the electrodes occur. These problems are mainly caused by the velocity distribution in the perpendicular to the flow. The flow velocity near the tube walls is much lower than that in the central flow and thus receives more electrical energy from the repetitive high-power pulses, resulting in the local heating. This not only degrades food components, but also promotes coagulation and deposition of components on the wall, triggering spark discharge. We have developed a continuously operating pasteurization system combining a PEF and a moderate thermal energy for protein-rich liquid foods such as milk and liquid whole eggs. This paper describes the ability of our system and the durability of the PEF treatment cell, which is basically co-linear type and can be stacked in series to the flow. The shape and geometry of the cell was designed to optimize in terms of the electric field distribution, the velocity distribution of the flow, the temperature distribution using COMSOL Multiphysics. The flow system was pressurized to 0.5 MPa and the flow rate was adjusted to 10 L/h by a needle valve located at the exit of the flow system. 2 μs-long voltage pulses were repetitively delivered to the electrodes and the pulse repetition rate was set so that the average number of pulses to bacteria for one stage was more than 1. Temperature of the flow was monitored at several points of the system including inlet and outlet of the PEF treatment cell. Long-life milk with Listeria innocua (2 x 107 /ml) was completely sterilized by 26 kV/cm PEF and the thermal energy at 60□. Liquid egg with Enterobacter hormaechei (2 x 107 /ml) was treated by 19 kV/cm PEF and the thermal energy at 55□, resulting in a 2.3 log reduction in bacterial number. Both milk and liquid whole egg were continuously operated for more than 1 hour without trouble under the same conditions as for the pasteurization. No electrode damage or adhesion was observed.

PO-113

Enhancement of bioactive properties of maillard reacted peptides by pulsed electric fields

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Background: Maillard reaction (MR), which refers to a series of complex chemical reactions between amino acids, peptides or proteins with a reducing carbonyl compound, is commonly used in food processing to improve the flavour and bioactivity of hydrolysates and peptides. Emerging processing technologies, such as pulsed electric field (PEF), could be used to improve the extent of the MR, reducing the reaction temperature achieving similar flavour enhancement and bioactivity. This study aimed to investigate the effect of PEF on the progression of the MR and the bioactive and sensory properties of Maillard-reacted peptides (MRPs) derived from salmon peptides.

Methods: Salmon peptides obtained by enzymatic hydrolysis were subjected to wet PEF-assisted Maillard reaction. The PEF treatment was applied in continuous mode at electric fields of 0, 10, 15, and 20 kV/cm to a solution containing 4 mg/mL of salmon peptides (molecular weight <10 kDa), 1 mg/mL of xylose and 0.5 mg/mL of cysteine, at pH 7.1. MR was then immediately performed at 70, 80, and 90 °C for 150 min. The resulting MRPs were analysed for browning index (at 420 nm), generation of intermediates (at 294 nm), antioxidant activity, Angiotensin I-Converting Enzyme (ACE) inhibitory activity, FTIR spectra, and sensory evaluation.

Results: Absorbance at 294 and 420 nm increased with reaction time, indicating the production of MR intermediates and browning compounds, which was supported by FTIR results. At temperatures of 70-80 °C the treatment at 20 kV/cm increased the browning index, but at 90 °C this was achieved by the treatment at 10 kV/cm. PEF treatment also decreased the final content of MR intermediate products. By applying electric fields of 15 kV/cm the antioxidant and ACE Inhibitory activity of MRPs was improved, but higher electric fields had no effect or even decreased the bioactivity of the MRPs, possibly caused by the changes in final and intermediate MR products. All MRPs possessed a meaty flavour.

Conclusion: The use of PEF-assisted MR on salmon peptides was effective to produce tasty MRPs with improved bioactivity.

PO-114

Polyphenolic content and antioxidant activity of pulsed electric field-assisted extracts of green rooibos.

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Antioxidant activity associated with green rooibos infusions is attributed to the activity of polyphenols, particularly aspalathin and nothofagin. This study investigated the polyphenolic content and antioxidant activity of pulsed electric field-assisted extraction of green rooibos. Aqueous green rooibos was treated at a PEF intensity of (0 - 2kv/cm), generating specific energy of (0 – 10kj/kg). After PEF treatment, crude green rooibos extracts (CGRE) were extracted at 40°C for 30 min. The resulting freeze-dried extracts were analysed for total phenolic (TPC) and flavonoid content (TFC), reducing power (RP), 2,2-diphenyl-1-picrylhydrazyl (DPPH-RS) and peroxyl radical scavenging (PRS).

The TPC and TFC increased with an increase in PEF intensity, from 357.59 – 684.04 mg GAE/g and 117.00 – 195.67 mg CE/g, respectively. Extraction of polyphenols occurred as a result of pore formation and disintegration of the cellular material.

Regarding antioxidant activity, no significant differences ($p > 0.05$) were observed in DPPH-RS between the control and PEF-treated sample (1 kv/cm) at 49 and 53%; however, an increase in PEF intensity (2 kv/cm) resulted in an increase in DPPH-RS of 83%. A similar phenomenon was observed where the PRS for the control, 1 and 2 kv/cm were reported as 37, 39 and 42%, respectively. On the other hand, PEF had no effect on the RP of CGRE since no significant differences ($p > 0.05$) were reported between treated and untreated samples. Consequently, the RP as depicted by an increase in absorbance at 700nm ranged from 1.56 – 1.70.

The results of this study demonstrated the effect of PEF as a suitable pre-treatment to enhance the extraction of polyphenols from green rooibos, even at lower temperatures. Furthermore, varying the electric field strength was an important factor in polyphenol extraction.

PO-115

Enhanced Extraction of Cellulose and Lignin from Agro-Industrial Wastes Utilizing Alkali Treatment Assisted by High-Voltage Electrical Discharges (HVED) for Wood Adhesives Application

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This study investigates a novel approach for the extraction of cellulose and lignin from almond and walnut shells, agro-industrial wastes, specifically focusing on its applicability in the production of wood adhesives. In this research, we propose a more sustainable and efficient technique by employing alkali treatment coupled with High-Voltage Electrical Discharges (HVED). The synergistic effect of alkali and electrical discharges enhances the breakdown of biomass components, facilitating the extraction of cellulose and lignin with improved yield and purity. The employment of almond and walnut shells as a raw material not only addresses environmental issues linked to waste management

but also offers an economically viable and sustainable option for extracting cellulose and lignin. The application of HVED in the extraction process significantly enhanced the yield of cellulose and lignin from almond and walnut shells compared to conventional methods. Analysis of the structure was conducted using techniques such as Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Two-Dimensional Heteronuclear Single Quantum Coherence Nuclear Magnetic Resonance (2D HSQC NMR) spectroscopy. These methods unveiled changes in the functional groups, crystalline structure, and chemical composition of the cellulose and lignin that were extracted. Analysis of morphological changes was conducted using Scanning Electron Microscopy (SEM) visualization, which unveiled advancements in morphology. Moreover, Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) demonstrated the enhanced thermal stability of the isolated cellulose and lignin. The findings presented in this study illustrate the effectiveness of HVED-assisted alkali treatment in augmenting the extraction efficiency and enhancing the characteristics of cellulose and lignin obtained from almond and walnut shells. Consequently, this approach shows great potential for the utilization of these materials in wood adhesive applications.

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