UNIVERSITY OF LJUBLJANA | ZF Faculty of Health Sciences

13TH 2025 SOCRATIC LECTURES

SOCRATIC LECTURES

13TH INTERNATIONAL SYMPOSIUM, LJUBLJANA, JUNE 6, 2025

PEER REVIEWED PROCEEDINGS

EDITED BY:
VERONIKA KRALJ-IGLIČ, YELENA ISTILEULOVA AND ANNA ROMOLO

FACULTY OF HEALTH SCIENCES, UNIVERSITY OF LJUBLJANA

Z-STEAM

Socratic Lectures
13th International Symposium, Ljubljana, June 6, 2025

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The members of the Organizing Committee of 13th Socratic Lectures: Yelena Istileulova,Veronika Kralj-Iglič

Program of the Symposium Socratic Lectures, June 6, 2025, 10:00 – 17:00 (Ljubljana time)

09.40 Welcome to participants (Veronika Kralj-Iglič, University of Ljubljana)

09.45 - 10.15 Plenary lecture: Marija Ipavec: My above knee prostheses between 1978 and 2025

10.15 - 10.45 Mojca Debeljak: Instrumented rehabilitation

10.45 - 11.00 Kristijan Cafuta: Charging and Discharging a Capacitor: A Case Study in Solving Differential Equations with Separable Variables

11.00 Scientific sections

Section 1: Horizons in Science and Education (Chairs: Veronika Kralj-Iglič)

11.00 - 13.00 Discussion on the results of the preparatory project Nanostructurome and project J3-60063

Section 2 : STEAM (Chair: Yelena Istileulova)

11.00 - 11.15 Yelena Istileulova: Science and Arts in Dialogue: STEAM, Lifelong Learning and Well-being

11.15 - 11.30 Uršula Vratuša: Creative drama techniques for psychophysical well-being

11.30 - 11.45 Laura Yerekesheva: Symbols of healing: Holism in ancient Central Asian art

11.45 - 12.00 Lucia Bianconi: Therapeutic Mandalas: Turning visible to the invisible

12.00 - 12.15 Indra Sagduna: Gender dynamics in Balinese performing arts: the lens of cross-dressing practices

12.15 - 12.30 Edi Dwi Riyanto: Artistic breakthrough to mitigate cultural and natural disruption of Tengger Indigenous people in East Java Indonesia

12.30 - 12.45 Juan David Zuleta, Mišo Mićić: Performing the self: Autofiction and theatrical identity in "Damn Juan", a theatrical rhapsody by composer and actor

12.45 - 13.00 Matjaž Pikalo: Poetry, music and film: personal perception

13.00 - 13.15 Rosakebia Estela Mendoza: Imagination and prompt engineering: Poetry and the art of asking questions

13.30 Closing of the Scientific sessions

Section 3: Educational Session (Chair: Veronika Kralj-Iglič)

13.00 - 15.00 Determination of biomechanical parameters

15.00 Closing of the symposium

Satellite event: Concert at 13th Socratic Lectures June 8, 2025 at 18.00, Trio Lorenz Hall, Academy of Music, University of Ljubljana, Palace Kazina

Program:

Aleona von Sultanova/Aleona von Sultanova: Hymn to science and the arts (first public presentation), piano and voice: Aleona von Sultanova

Claude Debussy: Reverie, piano: Lara Oprešnik

Marjan Kozina (transcribed by Jaka Pucihar): Mojca's song, piano: Marina Žigon

Emil Adamič/Oton Župančič: Da sem jaz ptičica, chorus Studenec Pivka

Gaetano Donizetti/Anon: Me voglio fa 'na casa, bariton: Neven Stipanov, piano: Elena Startseva Somun

Yevgeniy Krylatov/Yuri Entin: Prekrasnoye Daleoko, voice: Arina Gusevskaya, piano: Aleona von Sultanova

Popular, transcribed by Walter Lo Nigro: Dekle je pralo srajčke dvej, chorus Studenec Pivka

Jaka Pucihar: Vrtiljak, piano: Doroteja Poljanec

Popular, transcribed by Jaka Pucihar: La fiesta, piano: Doroteja Poljanec

Francesco Paolo Tosti/Giovanni Alfredo Cesareo: La serenata, bariton: Neven Stipanov, piano: Elena Startseva Somun

Lara Oprešnik: $E = m c^2$ (first public presentation), piano: Lara Oprešnik

Johann Sebastian Bach: Erbarme dich mein Gott from Matthaus Passion, soprano: Marina Igritskaya, flute: Veronika Kralj-Iglič, piano: Aleona von Sultanova

Johann Sebastian Bach: Invention 4 in D minor BWV 775, piano: Matic Bogataj

Popular, transcribed by Hilarij Lavrenčič: Oblaki so rudeči, chorus Studenec Pivka

Zoltan Peter: At lake, piano: Lan and Alenka Stražišar Lamovšek

Gioachino Rossini: La Danza-Tarantella, bariton: Neven Stipanov, piano: Elena Startseva Somun

Heinrich Germer, Etude No 2 in D majorž: piano: Matic Bogataj

Aleona von Sultanova/Alfred Nobel: You say I am a Riddle, piano and voice: Aleona von Sultanova

Vincenzo Bellini/Felice Romani:	Vaga luna, voice: Neven Stipanov, piano: Elena Startseva Somun
Edward Elgar:	Salut d'amour Op. 12, violin: Branko Brezavšček, piano: Elena Startseva Somun
Davorin Zupanič Turković:	Cantique de Baruch Spinoza form Mass in e-minor, piano: Lara Oprešnik and Aleona von Sultanova, voice: Veronika Kralj-Iglič
Camille Saint-Saens:	Pianistes from Carneval des Animaux, piano: Lara Oprešnik and Veronika Kralj-Iglič

Editorial

The 13th Socratic Lectures symposium on June 6, 2025, was a comparatively small one, with a plenary section, 2 scientific sections (Horizons in Science and Education - consisting essentially of the meeting of the Nanostructurome and ARIS J3-60063 project teams, and STEAM – organized and chaired by Yelena Istileulova). Also there was a poster section. However, the excellence of the participants continued and was kept at the global level. In the plenary lectures, Marija Ipavec reflected on her experience with above-the-knee prostheses, followed by Mojca Debeljak, who presented instrumented rehabilitation, and Kristijan Cafuta, who revealed the mathematics behind charging and discharging a capacitor. There were 7 posters presented on the homepage of the Laboratory of Clinical Biophysics.

The accompanying concert was held at the Trio Lorenz hall with two pianos at June 8, 2025. More details can be found in the contribution entitled »Z-STEAM Activities at the 13th Socratic Lectures Symposium Concert at City Hall, and Opera Coronation of Poppaea« in this proceedings. The concert featured two new compositions written for the occasion, which were performed by the authors: Yelena Istileulova and Lara Oprešnik, respectively.

Volume 13 of the Proceedings of Socratic Lectures contains 16 papers and images of 7 posters. Gallery Marguerite de Saint Champs exhibits photos of the nature in Namibia as reflected in the camera of Tomaž Trebše. The front page is by Drago Videmšek, recording Pupa between the two pianos, in singing ecstasy.

The social event took place at the Kazina jazz club. The students of the Biotechnical Educational Centre Ljubljana have created a traditional Emona cake with a modeled piano keyboard.

We have experienced another memorable get-together in the spirit of excellence and creativity.

Welcome to the Z-STEAM activities within the next Socratic lectures, which will take place in the winter 2026; the news will be updated at www.lkbf.si

Veronika Kralj-Iglič, Yelena Istileulova, and Anna Romolo

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TEXTS

POSTERS

Research

Standard Operating Procedure for the Handling and Processing of Canine Blood Samples and Performance of Routine Hematological and Biochemical Analyses within the Nanostructurome Pipeline

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Abstract:
This Standard Operating Procedure (SOP) outlines the standardized collection, handling and processing of canine venous blood samples for analytical purposes using serum, plasma, or whole blood. It includes protocols for the preparation of serum and plasma, as well as the dilution of whole blood and plasma for the measurement and characterization of extracellular vesicles. The SOP also details the procedures for routine hematological and biochemical analyses, which are integral to the comprehensive evaluation of animals enrolled in the Nanostructurome project. By addressing the preanalytical phase and analytical performance, this SOP ensures consistency, minimizes variability, and reduces the risk of preanalytical and analytical errors. Its implementation is essential for generating accurate, reliable laboratory results, thereby supporting both high-quality research and effective clinical decision-making.

Keywords: Biochemistry; Blood samples processing; Hematology; Plasma; Preanalytical errors

Table of contents:

1. Definitions

ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
BASO: Basophil
CBC: Complete Blood Count
Cl: Chloride
DIFF: White blood cell differential count
EDTA: Ethylenediaminetetraacetic acid
EVs: Extracellular vesicles
EWC: European Waste Code
HCT: hematocrit
HGB: Hemoglobin concentration
K: Potassium
Na: Sodium
NaCl: sodium chloride
PEROX: Peroxidase
PLT: Platelet count
PPE: Personal Protective Equipment
QC: Quality Control
RBC: Red Blood Cell count
WBC: White Blood Cell count
SOP: Standard Operating Procedure
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration

2. Background

Providing high-quality care to animal patients is a fundamental goal of veterinary medicine and often relies on clinical laboratory testing that delivers accurate and clinically relevant data (Vap et al., 2012; Arnold et al., 2019). Veterinary clinical laboratories play a critical role not only in diagnosing, classifying, and monitoring pathophysiological conditions, ruling out potential causes of illness, assessing disease progression or response to therapy, and detecting subclinical disorders in apparently healthy animals (Bush, 1991; Stockham & Scott, 2008), but also in supporting high-quality research. Their ability to generate reliable and standardized data makes them essential in research projects involving clinical patients, particularly in translational and comparative studies. In this context, veterinary laboratories serve as a vital link between clinical practice and scientific discovery, ensuring that research findings are grounded in accurate and reproducible laboratory results.
Unlike human clinical laboratories, veterinary laboratories are not subject to the same legislative and licensing requirements (e.g., ISO 15189), although the expectations for result quality are comparable. In fact, achieving high-quality results in veterinary settings can be more challenging due to interspecies variability (Lumsden, 2006; Vap et al., 2012). To address these challenges, the American Society for Veterinary Clinical Pathology (ASVCP) published the Quality Assurance Guidelines in 2019, which promote continuous quality improvement by identifying sources of laboratory error and offering practical tools for implementing comprehensive quality management systems (Arnold et al., 2019).

Laboratory testing is divided into three phases: preanalytical, analytical, and postanalytical. Errors can occur at any stage, but advances in instrumentation, automation, and quality control have significantly reduced analytical errors. Today, preanalytical errors are recognized as the leading cause of unreliable laboratory results in both human and veterinary medicine (Lippi et al., 2006; Hooijberg et al., 2012; Braun et al., 2015; Whipple et al., 2020). The ISO 15189 standard defines the preanalytical phase as encompassing all processes from the clinician's test request through sample collection, identification, and transport, up to the start of the analytical examination (Braun et al., 2015). Studies show that preanalytical errors account for the majority of laboratory mistakes, up to 75.3% according to Whipple et al. (2020), with most occurring outside the laboratory itself. Common issues include incorrect sample-to-anticoagulant ratios, clotted samples, in vitro hemolysis, mislabeling, and unlabeled specimens (Narayanan, 2003; Lippi et al., 2006; Zandecki et al., 2007a, 2007b; Lippi et al., 2008; Lippi et al., 2012; Upreti et al., 2013; Arnold et al., 2019; De la Salle, 2019; Whipple et al., 2020).

Advanced testing technologies cannot compensate for poor-quality samples. Therefore, proper collection, handling, and processing of blood samples are crucial to ensure the accuracy of laboratory results, prevent misinterpretation, and maintain high-quality outcomes in both patient care and research. This includes attention to sample integrity, appropriate tube selection, timely processing, and documentation of any preanalytical variables such as hemolysis, lipemia, or clotting. Adherence to standardized protocols minimizes variability and enhances reproducibility of results (Bush, 1991; NCCLS, 2003; Lippi et al., 2006; Hooijberg et al., 2012; Flatland & Vap, 2012; Vap et al., 2012; Arnold et al., 2019; Whipple et al., 2020).

3. Purpose, Scope and Applicability

The purpose of this SOP is to describe the procedures for the collection, handling, and processing of canine venous blood samples, as well as the performance of routine hematological and biochemical analyses. These procedures are part of the comprehensive evaluation of animals included in the Nanostructurome project.

The scope of this SOP includes step-by-step instructions for the collection of blood samples, preparation of serum and plasma samples, as well as the preparation of diluted ethylenediaminetetraacetic acid (EDTA) whole blood and EDTA plasma samples for the measurement and characterization of EVs. It also covers the determination of complete blood count (CBC) and white blood cell differential (DIFF), including parameters such as red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hemoglobin concentration (HGB), hematocrit (HCT), and other related indices. In addition, the SOP outlines routine biochemical analyses for serum analytes including glucose, urea, creatinine, total protein, albumin, sodium (Na), potassium (K), chloride (Cl), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

This SOP is applicable to a wide range of basic and clinical research settings where blood-based analyses are performed. Its implementation is essential for minimizing preanalytical and analytical errors, thereby ensuring the generation of accurate, reliable, and reproducible laboratory results.

4. Health and safety warning

All personnel must adhere to established health and safety protocols when handling biological samples and operating laboratory equipment. Appropriate personal protective equipment (PPE), including laboratory coats, safety shoes, gloves, and, where necessary, masks and safety glasses, must be worn at all times. Since it is often impossible to determine whether a sample is infectious, all blood samples must be treated as potentially infectious and handled according to standard biosafety precautions.

Laboratory conditions must support safe and efficient work, including adequate lighting, ventilation, and ergonomic accommodations to minimize the risk of repetitive strain, prolonged sitting or standing, and other occupational injuries. Personal protective equipment must be appropriate for the specific procedures and equipment used in each laboratory area. Furthermore, veterinarians and veterinary technicians involved in the collection of

blood samples must adhere to established safety protocols and utilize PPE suitable for field conditions, ensuring the safety and integrity of the sampling process.
Notices, specialized labeling, and safety procedures for the handling, storage, and disposal of all samples, waste, and other supplies must be appropriate for the type of material. Personnel must receive comprehensive safety and biohazard training, including protocols for exposure to hazardous chemicals or infectious agents, prevention of bacterial contamination, awareness of zoonotic diseases, and emergency response procedures (e.g., fire or contamination events). All training must be documented, and staff must be aware of their responsibilities and the location of safety equipment (Laposata & Dighe, 2007; Arnold et al., 2019). In addition, all personnel are required to undergo a mandatory occupational safety examination every two years, in accordance with institutional and regulatory guidelines.

5. Cautions

Laboratory personnel must be informed of the hazards associated with individual reagents (as described in the original manufacturer instructions) and the potential risk of infection prior to beginning any work. The use of PPE is mandatory in the laboratory. Consumption of food and beverages is strictly prohibited. Direct contact of reagents with skin and eyes must be avoided, and reagents must never be ingested. All materials used must be disposed of in accordance with applicable regulations and established good laboratory and research practices. Written operating instructions for all equipment are available to users on site and must be followed at all times.
Veterinarians and veterinary technicians involved in the collection of blood samples must also be aware of biosafety risks and follow appropriate safety protocols. Personal protective equipment suitable for fieldwork, such as gloves, protective clothing, safety shoes, and sharps containers, must be used consistently to prevent exposure to infectious agents and ensure the safe handling and transport of biological samples.

6. Personnel Qualifications / Responsibilities

Personnel involved in this SOP include the following:
Experienced veterinarians or veterinary technicians: Responsible for the collection of blood samples, strictly adhering to established veterinary medical principles and guidelines.
Veterinarians or veterinary technicians: Responsible for the safe and timely transport of blood samples to the laboratory, ensuring sample integrity is maintained during transit.
Laboratory Personnel (including chemistry and veterinary technicians, and chemical engineers): Responsible for the handling and processing of blood samples, as well as the performance of routine hematological and biochemical analyses. All personnel must have completed appropriate training in analytical procedures, instrument operation and calibration, and data documentation.
Quality Control (QC) Officer (ISO/IEC 17025 accredited), also serving as the Head of Laboratory: Responsible for ensuring compliance with quality standards, reviewing analytical results, validating calibration and method performance (including control sample analysis), and identifying any deviations requiring corrective actions.
Laboratory Supervisor (Head of Laboratory): Responsible for overall adherence to procedures, including laboratory safety, regulatory compliance, equipment maintenance, and troubleshooting.
All personnel involved in the handling and processing of canine blood samples, as well as in the performance of hematological and biochemical analyses, must be adequately trained and regularly updated on laboratory procedures, with particular emphasis on minimizing preanalytical and analytical errors.
Laboratory staff are responsible for maintaining a clean and organized work environment, including proper care and routine cleaning of equipment and work surfaces. Any deviations from standard procedures must be clearly documented, preferably in both written and electronic formats (electronic information system - EasyVet), to ensure traceability and support quality assurance.

In addition, personnel must document any factors that could influence the interpretation of analytical results in **Appendix 1** (Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization) of this SOP. These include, but are not limited to, visible lipemia, hemolysis, icterus, the presence of blood clots, or insufficient sample volume. Accurate recording of such observations is essential for proper data interpretation and for maintaining the integrity of the analytical process.

All data must be securely stored and backed up in accordance with institutional data management policies (ISO/IEC 17025). Equipment must be operated and maintained according to the manufacturer's instructions, internal laboratory protocols and according to internal ISO/IEC 17025-based guidance document, V 77 – Equipment management. Regular maintenance and calibration are essential to ensure consistent performance and analytical reliability.

7. Materials, Equipment and Supplies

7.1. Materials and equipment required for venous blood samples collection, handling and processing, including the dilution of EDTA whole blood and EDTA plasma samples for the measurement and characterisation of EVs

2-mL tubes containing anticoagulant EDTA; 0.5-mL EDTA-containing microtainer tubes; 4-mL serum separator tubes; sterile needles or intravenous catheters, used in combination with syringe; appropriate PPE, such as lab coats, gloves, and safety shoes; an alcohol-based antiseptic; sterile gauze pads; adhesive tape or bandages to prevent bleeding; disposable protective gloves; a sharps disposal container; proper labeling materials, such as waterproof markers and pre-printed labels; Eppendorf tubes; pipette tips; waste disposal bins; plastic tubes; plastic cryovials for freezing serum and plasma samples at –80°C for long-term storage; dilution medium (ultraclean water, marine water, physiological saline (0.9% sodium chloride (NaCl) solution), or phosphate-buffered saline, depending on the sample); single-channel calibrated pipettes; and a centrifuge (Heraeus Megafuge 8R, Thermo Fisher Scientific).

7.2. Materials and equipment required for hematological analyses

the ADVIA 120 (SIEMENS) automated laser based hematology analyzer equipped with species-specific software; original, manufacturer-supplied reagents specific to the analyzer, including hemoglobin, basophil (BASO), RBC/PLT, and PEROX reagents, as well as diluents, lysing agents, and cleaning solutions; quality control whole blood materials at low, normal, and high levels; EDTA-anticoagulated whole blood samples; and appropriate PPE (lab coats, gloves, safety shoes).

7.3. Materials and equipment needed for biochemical analyses, including electrolytes

the RX Daytona (RANDOX) automated biochemical analyzer; RANDOX-specific, pre-prepared reagents for the determination of glucose, total protein, albumin, urea, and creatinine concentrations, as well as the enzymatic activities of ALP and ALT; RANDOX calibrators and quality control materials; RANDOX cleaning solutions and acid wash solution, distilled water and physiological saline solution for routine maintenance and prevention of contamination; a Roche electrolyte analyzer (9180, ROCHE) for the measurement of Na, K, and Cl concentrations; ROCHE-specific reagent and waste container (SnapPak cartridge) for electrolyte analyzer; other analyzer-specific solutions and control material: cleaning solution, sodium conditioning solution and ISETROL quality control solutions (three levels); dedicated plastic sample tubes used for transferring serum from the original collection tubes; and appropriate PPE (lab coats, gloves, safety shoes).

7.4. Extra equipment and consumables:

reagent reservoirs, waste disposal bins, 80°C freezer and refrigerator for sample and material storage, plastic tubes, distilled water/physiological saline (0.9 % NaCl) to dilute reagents and samples, especially when the measured values (concentrations, activities) are above the linear range of the assay.

8. Computer Hardware and Software

Hardware: desktop computers; software: **Hematological analysis**: Multispecies system software 6.9.0-MS (ADVIA 120 Hematology System; Siemens Healthcare Diagnostics, Munich, Germany); **Biochemical Analysis**: RX Daytona (RANDOX) analyzer-specific software with a built-in user interface for operation and data management.

9. Step by Step Procedures

9.1. Blood samples collection, handling and processing

9.1.1. Blood samples collection

- Blood samples must be collected prior to any therapeutic or diagnostic procedures (e.g., surgery, infusion, transfusion, biopsy, endoscopy).
- Collect venous blood from the jugular vein using sterile needles or intravenous catheters with syringes.
- Ensure animals are fasted before collection for at least 12 hours.
- Shave the area around the intended puncture site to ensure clear access and reduce contamination risk.
- Disinfect the puncture site with an alcohol-based antiseptic.
- After blood collection, apply sterile gauze to the puncture site and secure with adhesive tape or a bandage.
- Wear disposable gloves throughout the procedure and dispose of used needles in an approved sharps container.
- Use waterproof markers or pre-printed labels for accurate sample identification.
- Confirm that the sample is collected from the correct animal, as indicated on the request form.
- When collecting multiple tubes during a single venipuncture, follow the recommended order of draw:
  - Blood culture tube
  - Coagulation tube (blue stopper)
  - Serum tube (red or yellow-red stopper)
  - Heparin tube (green stopper)
  - EDTA tube (lavender stopper)
  - Glycolytic inhibitor tube (grey stopper)

9.1.2. Sample transport and initial handling

- Transport samples to the in-house laboratory at room temperature as quickly as possible.
- Upon arrival:
  - EDTA whole blood samples (0.5-mL tubs for hematology): place them in a blood mixer for at least 30 minutes before analysis. Analysis should be performed within 1 hour of collection.
  - EDTA whole blood samples for obtaining EDTA plasma (2-mL tubes for preparation of diluted samples for EVs analysis): prepare diluted EDTA whole blood samples and then centrifuge immediately at 1500 × g for 15 minutes at room temperature. Transfer plasma to Eppendorf tubes or cryovials for further processing and storage, respectively.
  - Serum separator tubes for obtaining serum (4-mL serum separator tubes for biochemistry): allow to clot at room temperature (22–25°C) for 20–30 minutes. Keep tubes upright to promote clotting and minimize hemolysis. After clotting, centrifuge serum tubes at 1300 × g for 10 minutes at room temperature. Perform biochemical analyses within 2 hours of blood collection.

9.1.3. Preparation of diluted blood samples for EVs measurement and characterization

- Dilution of EDTA whole blood:
  - Gently invert the EDTA blood collection tube 8 times to ensure homogeneity before pipetting. Then, prepare 1:10 dilution by mixing 100 µL of EDTA whole blood with 900 µL of physiological saline solution.

- Dilution of EDTA plasma:
  - Mix 4 µL of EDTA plasma with 196 µL of physiological saline solution (1:50 dilution).
- Keep prepared diluted blood samples at room temperature until transported for EVs analysis. To preserve the integrity of extracellular vesicles, the diluted samples must remain undisturbed; avoid vigorous shaking or agitation. Samples should be kept in a stable, upright position to minimize mechanical stress and prevent vesicle disruption.

9.2. Hematological analyses using automated hematological analyzer ADVIA 120

9.2.1. Preparation

- Before starting the analyzer, check that all required reagents are available in sufficient quantities and are within their expiration dates.
- Ensure that the waste container is empty or has enough capacity for the upcoming analysis.
- Power on the ADVIA 120 analyzer and allow it to complete its initialization and self-check, then run the quality control (QC) samples.
- Document all required information in the Appendix 11 ('Record of hematological analyses') of the Quality Manual (QM 15) of the Small Animal Clinic. This includes consecutive analysis number, date of analysis, owner's name, species, animal's name, animal's file number and type of hematological analysis required.

9.2.2. Quality Control

- Run QC samples using manufacturer-provided blood controls (low, normal, and high; all from SIEMENS) in accordance with the manufacturer instructions.
- Review QC results to confirm they fall within acceptable ranges.
- If QC fails, troubleshoot according to the analyzer's user manual before proceeding with patient samples.

9.2.3. Sample Analysis

- Prior to analysis, the sample must be placed on a blood mixer for approximately 15–20 minutes to ensure proper homogenization.
- Immediately before aspiration, gently invert the tube 8 times.
- Initiate the analysis through the veterinary software interface connected to the ADVIA 120 hematology analyzer. Select the appropriate test parameters (CBC and DIFF) and ensure the correct animal species (canine mode) is selected.
- Run the EDTA whole blood sample using the manual aspiration port. The analyzer will automatically perform the CBC and DIFF analysis.
- Wait for the analysis to complete and review the results on display.

9.2.4. Result Verification and Documentation

- Verify that all parameters are within expected physiological ranges.
- Flag any abnormal results for repeat analysis or further investigation.
- Save and export results to the electronic information system (EasyVet) and print them for manual documentation.
- Store the analyzed EDTA whole blood samples for 24 hours at 2–8°C to allow for possible re-analysis if needed.

9.2.5. Post-Analysis

- At the end of each analysis session, perform routine maintenance as prompted by the analyzer.
- Routine daily cleaning cycles are automated and pre-programmed within the ADVIA 120 system.
- Perform weekly and monthly manual cleaning procedures according to the manufacturer's maintenance schedule and guidelines.
- Maintenance activities are not recorded in a physical instrument logbook; instead, the analyzer's internal system tracks routine operations. In addition, weekly maintenance of the ADVIA 120 hematology analyzer is documented in Appendix 13 ('Weekly maintenance of the hematological analyzer ADVIA 120')

of the QM 15, while monthly maintenance is documented in Appendix 14 ('Monthly maintenance of the hematological analyzer ADVIA 120') of the QM 15.

- At the end of the day, initiate the "End of Day" procedure within the ADVIA 120 software to store all results related to routine samples and control sample analyses.

9.3. Biochemical analyses, excluding electrolyte, using automated biochemical analyzer RX-Daytona

9.3.1. Preparation

- The biochemical analyzer is programmed to automatically exit sleep mode and begin its initialization process at 5:30 a.m. each day. Allow the system to complete its full startup sequence, including any scheduled pre-analytical maintenance procedures. These typically include: cuvette wash (2 cycles) and priming (2 cycles).
- Verify that all reagents, calibrators, and QC materials are available and within their expiration dates.
- Load the reagents onto the reagent carousel and scan their barcodes to ensure proper identification and traceability.
- Gently mix serum or plasma samples to ensure homogeneity. Visually inspect and document any pre-analytical factors such as lipemia, hemolysis, or icterus.

9.3.2. Quality Control

- Run QC samples (low or normal or high) according to manufacturer instructions.
- If QC results fall outside acceptable ranges:
  - Re-run QC to confirm the deviation.
  - If still out of range, run Randox calibrators to re-establish the calibration curve.
  - We can proceed with patient samples when QC results are within acceptable limits.

9.3.3. Sample Analysis

- Use the RX Daytona software interface to select the appropriate biochemical tests (e.g., glucose, urea, creatinine, total protein, albumin, ALT, ALP) based on the sample type and the veterinarian's diagnostic request.
- Assign the selected tests to the corresponding sample positions on the sample carousel.
- Load the prepared serum (plasma or urine samples) into the designated positions on the sample carousel.
- Initiate the analysis. The RX Daytona will automatically perform the selected biochemical tests, including reagent handling, sample aspiration, mixing, incubation, and photometric reading.
- Store remaining samples according to the type of analysis:
- Routine diagnostic samples are stored in a refrigerator (2–8°C) for up to 2 weeks.
- Research samples are stored in a -80°C deep freezer, in accordance with the specific research protocol requirements.

9.3.4. Result Handling

- Review all analytical results for accuracy, ensuring they fall within expected physiological or reference ranges. Flag any abnormal or unexpected values for repeat analysis or further investigation.
- Validated results are exported to electronic information system EasyVet. A hard copy is not printed; instead, results are manually transcribed onto:
  - the 'Blood sample request form' (Appendix 21 of the QM 15), and
  - the 'Documentation of the Results of Biochemical Tests' form, which is Appendix 16 of QM 15.

9.3.5. Post-Analysis

- Perform routine maintenance and cleaning procedures as prompted by the analyzer, including tasks such as probe washing, waste disposal, and reagent cooling checks.
- At the end of the working day, store all reagents in the refrigerator according to manufacturer guidelines to maintain stability and performance.
- Document all maintenance and calibration activities electronically within the analyzer's internal system or designated digital records, as a physical instrument logbook is not used.
- Ensure that the analyzer is placed in sleep mode at the end of the working day. This process includes several end-of-day procedures such as system flushing, probe cleaning, and temperature stabilization, as guided by the analyzer's software prompts.

9.4. Electrolyte analyses using 9180 ROCHE electrolyte analyzer

9.4.1. Preparation

- Exit Standby mode and ensure the Roche 9180 electrolyte analyzer has completed initialization
- Verify that the SnapPak, cleaning solution, sodium conditioning solution, and ISETROL three-level control materials are available and within expiration dates.
- Perform cleaning, sodium electrode conditioning and run calibration. Ensure the analyzer has completed its daily self-check and is ready for operation.
- Gently mix serum or plasma samples to ensure homogeneity. Visually inspect for hemolysis, lipemia, or icterus and document any abnormalities.

9.4.2. Quality Control

- Immediately after calibration, run quality control samples (low or normal or high) using ROCHE ISETROL controls, following the manufacturer's instructions.
- If QC results fall outside acceptable limits:
  - Repeat the QC test to confirm the deviation.
  - If results remain out of range, recalibrate the analyzer.
  - If QC results are still outside acceptable limits after recalibration, contact Roche service for further assistance.
  - Proceed with patient sample analysis only when QC results are within acceptable limits.

9.4.3. Sample Analysis

- Use the analyzer's touch-screen interface to select the appropriate sample type and analysis (Na, K, Cl).
- Aspirate the serum (or plasma or urine) sample manually using the sample probe. Wipe the probe immediately after sample aspiration.
- The analyzer will automatically perform the measurement and display the results.
- Repeat the analysis if prompted by the system due to sample or measurement error.

9.4.4. Result Handling

- Review the results on the analyzer display and verify they fall within expected physiological or reference ranges. Flag any abnormal or unexpected values for repeat analysis or further investigation.
- Transcribe validated results manually onto:
  - the 'Blood sample request form' (Appendix 21 of QM 15), and
  - the 'Documentation of the results of biochemical tests' form, which is Appendix 16 of QM 15.

- Results are printed but not exported electronically to EasyVet, so enter the results manually into the EasyVet system.

9.4.5. Post-Analysis

- The analyzer performs automatic cleaning after each analysis according to preprogrammed cleaning cycles.
- Manual cleaning is not required, except for wiping the probe immediately after aspiration, which is already included in the analysis procedure.
- Calibration is preprogrammed to occur three times per day, so no manual calibration is needed at the end of the working day.
- At the end of the working day, place the analyzer in standby mode.

9.5. Data acquisition

9.5.1. Hematological analysis using automated hematological analyzer ADVIA 120

The ADVIA 120 hematology analyzer performs CBC, DIFF, and reticulocyte measurements using flow cytometry, laser-based optical detection, and cytochemical staining. Results are generated electronically and include numerical values, histograms, and cytograms (scatter plots), which provide visual representations of cell populations and distributions. Numerical values are automatically transferred to the electronic information system EasyVet for archiving, clinical evaluation, and research purposes. In addition, results are printed for documentation and a backup of all outputs is stored on the hard disk of the ADVIA 120, ensuring data integrity, traceability, and compliance with regulatory requirements.

Red blood cell counts and PLT are analyzed using multi-angle light scatter technology to assess cell size and internal complexity. Hemoglobin concentration is determined photometrically following the lysis of red blood cells. Additional parameters, including HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are calculated based on direct measurements.

White blood cells are stained with cytochemical reagents that highlight specific intracellular components (e.g., peroxidase). The stained cells are passed through a flow cell and analyzed using laser-based optical detection. The system measures:

- axial light loss (cell size),
- side scatter (internal complexity),
- absorbance (cytochemical staining intensity).

The ADVIA 120 is connected to the electronic information system EasyVet, to which it transmits numerical results electronically via a standardized communication protocol. This process constitutes electronic archiving of hematological data, ensuring secure storage and traceability in compliance with quality and regulatory standards.

9.5.2. Biochemical analysis using automated biochemical analyzer RX-Daytona and elec

trolyte analyzer 9180 ROCHE

The RX-Daytona performs biochemical analyses using photometric and turbidimetric methods. Results are generated digitally and include numerical values and reaction curves. Quantification of analytes is based on calibration curves, which are established by measuring the absorbance of known calibrator concentrations. The analyzer uses these calibration curves to determine the unknown concentration or activity of analytes in patient samples by comparing the measured absorbance of the test sample to the absorbance values of known calibrators plotted on the standard curve.

Concentrations of electrolytes (Na, K, Cl) are measured using the 9180 Roche electrolyte analyzer, which operates on the principle of direct potentiometry with ion-selective electrodes (ISE). The results are printed and manually transcribed into the EasyVet electronic information system and onto the Blood Sample Request Form (Appendix 21 to QM 15) for documentation and clinical interpretation.

The results of biochemical analyses (numerical values of concentrations and activities) are generated in digital format and are electronically transmitted to the EasyVet information system, where they are archived for clinical and research use.

A backup of the results is also retained on the RX-Daytona biochemical analyzer. In the case of analyses performed for patients included in research studies, results are additionally manually transcribed into the following documentation:

- The Blood Sample Request Form (Appendix 21 of QM 15), for internal tracking and clinical correlation.
- The Documentation of the Results of Biochemical Tests form (Appendix 16 of QM 15), for inclusion in patient records and to ensure audit readiness.

The results of biochemical analyses (numerical values) are available in three formats: electronically via EasyVet and as a backup on the biochemical analyzer, on the 'Blood sample request form' (Appendix 21 of QM 15), and on the 'Documentation of the results of biochemical tests form' (Appendix 16 of QM 15).

9.6. Troubleshooting

9.6.1. Collection, handling and processing of blood samples

Collection of blood into 0.5-mL EDTA tubes:

- Insufficient volume: may lead to clotting or inaccurate cell counts.
- Clot formation: due to delayed mixing or underfilling.
- Hemolysis: caused by rough handling or small needle gauge.

Troubleshooting recommendations:

- Ensure proper vein selection and use of appropriate needle size (ideally 21–23G).
- Gently invert the tube 8–10 times immediately after collection.
- If volume is low, prioritize this tube for CBC as it requires minimal blood.

Collection of blood into 2-mL EDTA tubes:

- Underfilling: affects plasma yield and EVs analysis.
- Delayed processing: can alter EVs profile and plasma quality.
- Clotting: due to improper mixing.

Troubleshooting recommendations:

- Collect this tube second to avoid platelet activation from earlier draws.
- Mix gently and process within the recommended time frame (usually <1 hour).
- If volume is insufficient, prioritize EVs or plasma based on study protocol.

Collection of blood into 4-mL serum separator tubes:

- Non-compliance with fasting: affects lipemia and analyte levels.
- Hemolysis, lipemia, icterus: interfere with biochemical assays.
- Insufficient clotting time: leads to fibrin strands in serum.
- Low volume: inadequate for both routine and storage needs.

Troubleshooting recommendations:

- Ensure 12 hour fasting prior to collection.
- Allow full clotting (20–30 min) before centrifugation.
- Use clean venipuncture techniques to avoid hemolysis.
- If volume is low, prioritize routine biochemistry and freeze fewer aliquots.

The checklists below provide troubleshooting guidance for the preparation of diluted EDTA whole blood (Table 1) and plasma samples (Table 2), as well as sample handling and transportation (Table 3) of samples for the EVs analysis. Checklists address common issues, their possible causes, and recommended solutions.

Table 1. Dilution of EDTA Whole Blood (1:10)

Issue	Possible Cause	Troubleshooting
Inhomogeneous sample	Inadequate mixing of EDTA tube	Gently invert the blood tube exactly 8 times before pipetting. Avoid vortexing.
Incorrect dilution ratio	Pipetting error	Use calibrated pipettes and verify volumes. Perform dilution in a clean, labeled microtube.
Clotting in sample	Delayed mixing after collection or underfilling of EDTA tube	Ensure immediate and proper mixing post-collection. Discard clotted samples.
Hemolysis	Rough pipetting or small needle during collection	Use gentle pipetting and proper venipuncture technique. Discard visibly hemolyzed samples.

Table 2. Dilution of EDTA Plasma (1:50)

Issue	Possible Cause	Troubleshooting
Pipetting inaccuracy	Very small volume (4 µL)	Use precision pipettes (e.g., P10) and low-retention tips. Pre-wet tips to improve accuracy.
Contamination	Unclean pipette tips or tubes	Use sterile, nuclease-free consumables. Work in a clean environment.
Plasma degradation	Delay in processing or improper storage	Process plasma promptly and freeze the remained plasma as soon as possible at – 80°C.

Table 3. Sample Handling and Transport for EVs Analysis

Issue	Possible Cause	Troubleshooting
Vesicle disruption	Shaking, agitation, or vortexing	Handle tubes gently. Use racks to keep samples upright and stable.
Temperature fluctuations	Exposure to heat or cold	Maintain room temperature (18–25°C). Avoid placing near heat sources or in direct sunlight.
Sample mix-up	Inadequate labeling	Label tubes immediately after preparation with sample ID, dilution, and time. Double-check before transport.

9.6.2. Hematological analyses

Detailed troubleshooting procedures for hematological analyses using the ADVIA 120 analyzer are available directly within the analyzer's software interface and in the printed user manual provided by the manufacturer; due to their complexity and length, they are not included in this SOP.

9.6.3. Biochemical analyses

Detailed troubleshooting procedures for biochemical and electrolyte analyses using the RX-Daytona and 9180 Roche analyzers, respectively, are available within the respective analyzer software interfaces (RX Daytona) and in the printed user manuals provided by the manufacturers (RANDOX, ROCHE); due to their complexity, they are not included in this SOP.

10. Data and records management

Data and records management related to blood sample collection, handling, and the preparation of diluted blood samples for EVs measurement and characterization are detailed in Appendix 1 ('Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization') of this SOP.

Upon completion of hematological analysis, the results are immediately and automatically saved in multiple formats to ensure accessibility, traceability, and compliance with documentation standards (ISO/IEC 17025).

- Numerical values are electronically transmitted to the EasyVet information system, where they are archived for clinical interpretation and long-term storage.
- Numerical values, histograms, and cytograms are simultaneously stored on the hard disk of the hematology analyzer as a local backup.
- Hard copies of the results are always printed for archiving and documentation purposes.

In the case of research, the printed hard copy of the results is stapled to the 'Blood sample request form' (Appendix 21 of QM 15), which contains all necessary information regarding the patient and the patient's owner. This form also serves as a record for documenting any deviations related to blood sample integrity, such as the presence of clots, insufficient volume, or other anomalies. This ensures complete traceability and proper linkage of analytical results with subject-specific data.
All forms of saved results—digital and printed—are used for:

- Archiving and documentation (e.g., for audits, regulatory compliance, or data backup),
- Internal tracking and clinical correlation, and
- Availability to clinicians for timely interpretation and decision-making.

Upon completion of biochemical analysis, results are automatically saved in electronic form in two locations:

- On the RX-Daytona biochemical analyzer, where data includes:
  - Concentrations and activities of analytes,
  - Quality control measurements, and
  - Calibration curve data.
- In the EasyVet electronic information system, where only the numerical results of concentrations and activities are archived. These results are not only stored for long-term record-keeping but are also immediately available to clinicians for interpretation and clinical decision-making.

Although results are electronically archived, all test results are also manually transcribed into the following documentation forms:

- The 'Documentation of the results of biochemical tests form' (Appendix 16 of QM 15), for inclusion in patient records and to ensure audit readiness.
- The 'Blood sample request form' (Appendix 21 of QM 15), for internal tracking and clinical correlation in the case of analyses performed for patients included in research studies. This form also serves to document any deviations related to serum sample integrity, such as hemolysis, lipemia, icterus, or insufficient sample volume, thereby supporting traceability and quality assurance.

This multi-format, dual-location data management system ensures that hematological and biochemical results are securely stored, traceable, and readily accessible—both digitally and in print—for clinical interpretation, regulatory compliance, internal tracking, and research documentation
In accordance with ISO/IEC 17025 accreditation requirements, all hematological and biochemical results, whether stored digitally in the EasyVet system and on analyzers or retained as printed hard copies, are archived for a minimum of six (6) years, ensuring long-term traceability, data integrity, and full compliance with applicable regulatory standards.

11. Waste management

The waste includes disposed gloves, Eppendorf tubes where the samples are diluted, plastic pipette tips, used paper napkins for cleaning the observation chamber and potential sample spill and remnants of the samples. Disposal material should be divided into ap-

propriate waste fractions, according to applicable laws and good research laboratory practice. Potentially hazardous materials should be placed in special containers and delivered to the relevant acquisition units.

All waste generated during blood collection, handling, and processing—as well as from hematological and biochemical analyses—is managed in strict accordance with the veterinary waste classification scheme and applicable national and EU regulations.

- Infectious and potentially infectious waste is classified under EWC (European Waste Code) code 18 02 02* and includes materials such as used blood collection tubes, contaminated gloves, and other disposables that may pose a biological hazard.
- Sharp materials (e.g., needles, lancets, scalpels, and other sharp instruments not contaminated with hazardous substances) are classified under EWC code 18 02 01 and are disposed of in puncture-resistant sharps containers.
- Liquid chemical waste, including used reagents and cleaning solutions from biochemical and hematological analyzers, is classified under EWC code 18 02 05.

All waste is carefully sorted at the point of generation and stored in designated, clearly labeled containers. Waste disposal is handled by a certified external waste management company. Full documentation is maintained for each waste collection, including:

- Tracking numbers
- Mass of each waste category
- Collection dates
- Certificates of disposal

This documentation ensures full traceability and compliance with environmental and biosafety regulations.

12. Related protocols or SOPS

This SOP integrates a combination of optimized, well-established procedures for the collection, handling, and processing of canine venous blood samples, as well as the performance of hematological and biochemical analyses. The optimization of these procedures enhances sample quality, improves analytical reliability, and supports consistent laboratory performance. In addition to these procedures, this SOP aligns with several internal ISO/IEC 17025-based guidance documents developed at the Veterinary Faculty, University of Ljubljana, as part of the faculty's accreditation process. These documents are an integral part of the faculty's quality management system and are strictly followed in daily laboratory practice, including research activities involving numerous patients enrolled in various scientific studies.

The following internal guidance documents are referenced in the implementation of this SOP. They form an integral part of the Veterinary Faculty's quality management system and are routinely followed in daily laboratory operations, including research activities involving patient participation:

- V 67 – Document and data control related to the quality management system
- V 77 – Equipment management
- V 79 – Selection, onboarding, and training of personnel
- V 82 – Handling of temperature-controlled equipment
- V 83 – Preparation of test reports
- V 86 – Procurement procedures
- V 87 – Handling complaints and monitoring client feedback
- V 96 – Pipette verification
- V 102 – Preparation of control charts
- V 104 – Thermometer calibration
- V 110 – Validation and verification of chemical analytical methods
- V 162 – Management of nonconformities, risks, opportunities, and corrective actions
- V 163 – Preparation and review of testing offers and contracts
- V 178 – Reception, identification, handling of patients, and traceability
- V 180 – Pipette calibration

Confidentiality Notice:
These internal documents are confidential and intended solely for use within the Veterinary Faculty. They must not be shared, distributed, or reproduced outside the institution without proper authorization.

13. Quality control and quality assurance section

13.1. Instrument calibration and quality control

All instruments (analyzers) used in this SOP are regularly maintained and calibrated by trained service technicians and laboratory personnel. The analyzers are serviced annually by qualified technicians from the supplier, and all calibrations are performed by trained laboratory staff to ensure accuracy and reliability.
Daily quality control is performed on all analyzers using analyzer-specific control materials prior to the analysis of patient samples (see quality control procedures described in the step-by-step section).

13.2. Critical processes parameters and checkpoints

A critical step in this SOP is the correct collection of blood samples, as previously described. The quality of analytical results is directly dependent on the quality of the collected samples; poor sample quality often leads to inaccurate results and misinterpretation. Accurate pipetting is essential for the preparation of diluted EDTA whole blood and plasma samples used for EVs measurement and characterization. Therefore, only precisely calibrated pipettes should be used.
Sample handling and storage conditions are also critical. Prepared samples must be handled gently, as shaking or agitation can disrupt the vesicles. Additionally, diluted samples should not be refrigerated, as low temperatures may compromise vesicle integrity.
Reagent storage and preparation are further critical factors. Although these fall within the analytical phase, they are controlled through regular calibration and the analysis of quality control samples, which help prevent significant analytical errors.

14. Data on procedures and samples

Data on procedures and samples are given in **Table 4**.

Table 4. Data on procedures and samples

Description of the outcome	Prepared diluted EDTA whole blood and EDTA plasma samples. Results include hematological analysis (CBC + DIFF) and biochemical analysis (Na, K, Cl, urea, creatinine, glucose, ALT, ALP, total protein, and albumin).
Time required to obtain the results	15–20 minutes per sample for collection, depending on patient compliance; 10–15 minutes for preparation of diluted blood samples (excluding centrifugation); 5–10 minutes for hematological analysis; and 10–15 minutes for biochemical analysis.
Volume of the sample needed	At least 100 µL of EDTA whole blood and 4 µL of EDTA plasma are required for the preparation of diluted samples (EVs); at least 0.5 mL of EDTA whole blood is needed for hematological analysis; at least 300 µL of serum is required for biochemical analysis, including electrolytes (Na, K, Cl); and at least 300 µL of EDTA plasma and 3 × 300 µL of serum samples are needed for further analyses (to be stored at –80 °C).
Estimated cost without manpower	60 EUR/sample, primarily covering the cost of reagents, analyzer maintenance, and consumables.
Contact person	Alenka Nemec Svete, alenka.nemecsvete@vf.uni-lj.si

Conclusions

This SOP provides a comprehensive framework for the standardized collection, handling, and processing of canine venous blood samples, ensuring the integrity and reliability of laboratory analyses. By addressing both preanalytical and analytical phases, the SOP minimizes variability and reduces the risk of errors that could compromise data quality.

The detailed protocols for preparing serum, plasma, and diluted EDTA whole blood and EDTA plasma samples, along with procedures for hematological and biochemical analyses, support the generation of accurate and reproducible results. These outcomes are essential for the clinical evaluation of animal patients and for advancing research objectives within the Nanostructurome project.

Through consistent implementation by trained personnel, this SOP enhances laboratory performance, supports high-quality veterinary care, and contributes to the success of translational and comparative research. It reinforces the critical role of veterinary laboratories in bridging clinical practice and scientific discovery.

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Appendix 1: Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization

Patient and Owner Information

- Attach patient and owner identification sticker.

I. Blood samples collection (Total volume: 6.5 mL)

- 1x 0.5-mL EDTA tube: CBC + DIFF
- 1x 2-mL EDTA tube:
  - EVs - preparation of diluted EDTA whole blood samples and preparation of diluted EDTA plasma samples
  - 1 x 300 µL of EDTA plasma for further analyses (stored at – 80°C)
- 1x 4-mL serum separator tube without inert gel:
  - 300 µL of serum for biochemical analysis (urea, creatinine, total proteins, albumins, ALT, ALP, Na, K, Cl)
  - 3 x 300 µL for further analyses (stored at – 80°C)

II. Sample handling, processing and preparation of diluted samples for EVs measurement and characterization

Please complete the table below to document sample status and any relevant observations.

Sample	Obtained (YES/NO)	Comments (HIL, small samples volume, other)
0.5-mL EDTA tube		
2-mL EDTA tube		
4-mL serum separator tube		
Diluted EDTA whole blood		
Diluted EDTA plasma		
EDTA plasma		
Serum		

HIL, hemolysis, icterus, lipemia

Research

Extracellular Vesicles in Bovine Colostrum and Milk

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Abstract:
Cow (bovine) colostrum (milk on the day after the calf birth) and milk (milked on the days 3 and 6 after the parturition) extracellular particles (EPs) were investigated by interferometric light microscopy (ILM). Colostrum and milk samples were taken from a Limousin cow on days 1, 3 and 6 after the parturition. Number density n and hydrodynamic diameter D_h of EPs were measured. Higher n was found in skimmed samples compared to the whole samples on days 1, 3 and 6 after the parturition, however the differences were statistically significant only on days 1 and 3 after the parturition. The sizes of the particles (D_h) were within the error of the method, and the differences between the skimmed and whole samples were statistically significant only on the day 3.

Keywords: Extracellular vesicles; Light scattering; Vesicle characterization; Cow; Colostrum; Milk; Nanovesicles

1. Introduction

1.1. About cow udder, lactation, colostrum and milk

Colostrum (first milk) and milk are being produced in the mammary glands of female mammals. Colostrum is the milk produced in the cow's udder during the first days after the birth (Playford et al., 2021). Colostrum is rich in nutrients, immunoglobulins, peptides with antimicrobial activity and growth factors.

There are four quarters of the cow udder. In each quarter, there is a separate mammary gland complex. Each of the four separate mammary complexes has its own separate teat cistern and teat canal. Every one of four teats therefore has one opening (Nickerson et al., 2020).

Lactation is energetically very demanding for a cow. The transition from a dry cow to lactation requires a big amount of energy from the dairy cow (Santos et al., 2012). Standard lactation in diary cattle lasts for approximately 305 days. The lactation curve increases rapidly after calving, peaks a few weeks later and gradually declines until the animal gets dry in approximately 10 months (Wood, 1967).

Both colostrum and milk are necessary for the calf to survive. The first milk secretion after the parturition is known as colostrum. The early growth, development and immune function in the calf depend on colostrum. It is essential that maternal colostrum is fed to the newborn calf during the first hours of life. Colostrum provides the calf with essential nutrients as proteins, lipids and carbohydrates. There are essential vitamins and minerals present in bovine colostrum, bioactive proteins and immunological factors (Lopez et al., 2022).

The transfer of passive immunity occurs via colostrum which is critical for the proper development of the immune system in the calf. After the parturition, the immune system of the calf is very immature because the bovine placenta does not enable the permeability to proteins like immunoglobulins. Calves are born without immunoglobulins in circulation. Therefore, immunoglobulins from the colostrum are critical for the protection of the neonate against the pathogens from the environment and therefore help the development of the immune system (Sutter et al., 2023).

The colostrum turns to milk in approximately 2 or 3 days after the parturition, but the transition from colostrum to mature milk is gradual and takes several weeks. (Mcgrath et al., 2016.)

Figure 1. Limousin cow with the calf (Image from: https://cattleinternationalseries.weebly.com/limousin.html

2. Material and Methods

2.1. Milk Sampling

For interferometric light microscopy, the samples were milked by hand from a Limousin (breed) cow (**Figure 1**) on 1st, 3rd and 6th day of lactation. Milk was collected into tubes VACUETTE® TUBE 3 ml Z No Additive 13x75 white cap-black ring, non-ridged (Greiner AG, Kremsmünster, Austria). To obtain skimmed milk, whole milk was centrifuged at 300g for 15 min. This procedure was repeated twice. The cream was removed from the top using a pipette with the tip shortened for 2 mm by scissors. The tubes with colostrum and milk are shown in **Figure 2**.

Figure 2. From left to right: cow colostrum milked on the day of parturition, cow milk from the third day after the parturition and cow milk milked from the seventh day after the parturition after the skimming procedure. On the top, the cream layer can be distinguished. Colostrum has a yellow hue unlike the white milk.

2.2. Interferometric Light Microscopy (ILM)

The average hydrodynamic diameter (D_h) and the number density of small particles n were determined by ILM using Videodrop (Myriade, Paris, France) as described previously (Romolo et al., 2022). Before measurement, the milk was diluted 100 × by saline for injections (Braun, Melsungen, Germany) to obtain a measurable dilution. Signals of the saline were under the detection limit. The threshold value 4.2 was used. 7 µL of sample was placed between cover glasses and illuminated by 2W blue LED light. The light scattered on the particle was imaged by a bright-field microscope objective and allowed to interfere with the incoming light. The image was recorded by a complementary metal–oxide–semiconductor high resolution speed camera. The obtained pattern that includes contrasting black and white spots was recognized as a particle and its position in the sample was assessed. Number density of the particles is the number of the detected particles within the detected volume (e.g. 15 pL). D_h was determined by tracking the position of the imaged particle within the recorded movie. It was assumed that particles undergo Brownian motion due to collisions with surrounding particles. The diffusion coefficient D of the motion of the particle is taken to be proportional to the mean square displacement d of the particle between two consecutive frames taken in the time interval Δt, $\langle d^2(\Delta t)\rangle = \langle 4D\,\Delta t\rangle$ while D_h was estimated by assuming that the particles were spherical and using the Stokes-Einstein relation $D_h = kT/3\pi\eta D$. Each particle that was included in the analysis was tracked and

processed individually and the respective incident light signal was subtracted from each image. Processing of the images and of the movies was performed by using the associated software QVIR 2.6.0 (Myriade, Paris, France).

2.5. *Statistical analysis*

All measurements were performed in triplicates and presented by the average values and standard deviations. The differences were evaluated by the t-test using the Excel sofware. The value p = 0.05 was taken as a threshold for statistical significance.

3. Results

Higher number density *n* of nano size particles was found in skimmed colostrum or milk when compared to the whole milk on days 1, 3 and 6 after the parturition, however differences were statistically significant only on day 1 and 3 **(Figure 3)**.

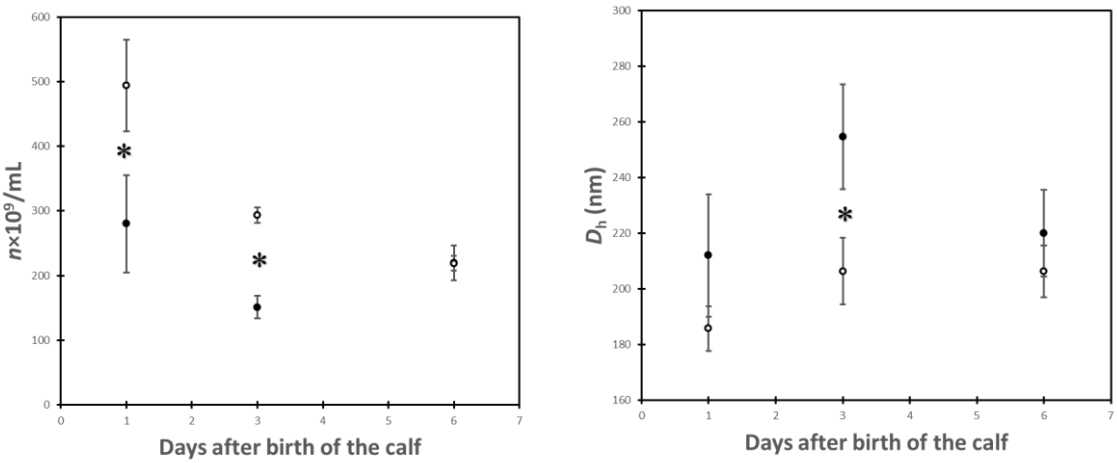

Figure 3. A: average number density *n* and B: average hydrodynamic diameter D_h of EPs in the cow whole samples (full circles) and skimmed (empty circles) samples. The error bars represent standard deviations. Asterisk (*) represents statistically significant difference between the whole and the skimmed samples.

The differences in hydrodynamic diameters of the particles D_h were within the error of the method. Larger particles were found in the whole samples, however differences were statistically significant only on day 3 **(Figure 3)**.

4. Discussion

Higher *n* and lower D_h in skimmed samples might be explained by removal of larger particles with the cream during the preparation of skimed milk. Previously, higher *n* of micro and nano-sized particles was found in skimmed mature equine milk (measured by flow cytometry and interferometric light microscopy, respectively) than in the whole mature equine milk, and the difference was statistically significant (Arko et al. 2024a). Furthermore, higher *n* was found in the skimmed samples than in the whole samples of equine colostrum and milk when measured by interferometric light microscopy on days 1, 3 and 7 after parturition, but the difference was statistically significant only on day 7 after the parturition (Arko et al. 2024b).

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Review

Future of Healthcare Trends in Orthopaedics and Biomaterials

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Abstract:
Personalized medical technologies are increasingly enhancing precision, efficiency, and patient outcomes. Innovations such as virtual surgical planning, robotic assistance, and real-time 3D navigation have revolutionized procedures like total knee arthroplasty and hip replacement, providing unparalleled accuracy and significantly reducing recovery times. The integration of artificial intelligence (AI), advanced imaging, and patient-specific 3D-printed implants further refines surgical precision, minimizes intraoperative complications, and facilitates personalized care. In the realm of sports orthopedics, wearable sensors and motion analysis technologies are transforming diagnostics, injury prevention, and rehabilitation, enabling real-time decision-making and improving patient safety. Additionally, health tracking devices are accelerating recovery and supporting preventive care, ultimately revolutionizing sports performance management. Concurrently, breakthroughs in biologics, biomaterials, and bioprinting are advancing the treatment of cartilage defects, ligament injuries, osteoporosis, and meniscal damage. These innovations are poised to establish new benchmarks in regenerative medicine. The primary aim of this review is to explore cutting-edge medical technologies employed in surgical practices and highlight significant innovations. Furthermore, we seek to underscore the importance of the biocompatibility of modern biomaterials and outline prospective directions for future research in this field.

Keywords: Artificial intelligence in healthcare; Orthopaedic innovation; Personalized medicine; Robotic surgery; Technological innovation; Biomaterials

1. Future Orthopaedics

1.1. Advances in medical engineering, biomechanics, and biomaterials

In orthopedics, research focuses on understanding the human musculoskeletal system, which is defined as an interconnected network of bones, muscles, tendons, cartilage, and nerves that support body mechanics, maintain posture and stability, and provide necessary movement. Clinical orthopedics treats musculoskeletal injuries across a variety of specialties, including sports medicine, rehabilitation, spine care, deformity correction, pediatrics, and hand care. In the past decade, advances have been made that meet the demands of modern surgery, allowing for procedures with fewer complications and greater precision (Tueni & Amirouche, 2025). Advances in medical engineering, biomechanics, and biomaterials are transforming the medical profession, which is essential for the development of personalized medicine and rehabilitation. From nanoscale biosensors to multidisciplinary molecular research, the collaboration of variety clinicians, technicians, and clients is important to achieve optimal healthcare outcomes and manage costs.

1.2. Artificial intelligence (AI) in medicine

Most fields are using AI-based solutions to automate and speed up time-consuming procedures. Specifically, in orthopedics, AI systems use massive amounts of data to explore numerous scenarios, predict surgical outcomes, and select the most appropriate treatment, providing patients with the highest level of personalized care (Federer and Jones, 2021). AI is also advancing in the creation of customized medical devices such as personalized implants created after scanning, specific to the patient, especially in surgeries such as spinal fusion (Shin et al., 2022) and bone-tumor reconstuction orthopedics (Yon et al., 2025). In addition, 3D-printed, patient-specific surgical guides are in use, offering numerous advantages in orthopedics.

1.3 Robotic surgery in orthopaedic surgery

Research consistently indicates that robotic-assisted total knee arthroplasty (rTKA) achieves better mechanical and anatomical alignment than conventional total knee arthroplasty (cTKA) (Alrajeb et al., 2024). A large-scale study results suggests that rTKA is associated with lower rates of aseptic loosening, prosthetic joint infections, revisions, and overall complications at the five-year follow-up (Wainwright et al., 2025).

Moreover, rTKA offers enhanced precision in component alignment, potentially leading to improved long-term implant survival. However, the clinical benefits in terms of functional outcomes are not consistently superior to conventional techniques. The increased surgical time and associated costs are important considerations. Further high-quality, large-scale randomized controlled trials are needed to better understand the long-term benefits and cost-effectiveness of robotic-assisted TKA (Deckey et al., 2021).

1.4 Bioprinting in orthopaedics

Three-dimensional bioprinting (3-D printing) or regenerative medicine allows for local treatment of the area where the anomaly is located in the context of minimally invasive surgery, thus avoiding complications and severe immune rejection.

The challenges in bioprinting remain in the ability to print cells that can survive, proliferate, and build a functioning matrix on a tissue-specific scaffold that provides full support for the cells using growth factors that promote cell development (Lam et al., 2019). Such products include knowledge in the fields of artificial intelligence, robotics and diagnostics, with a focus on the commercialization of technology for life science research and bioprinting (Fonseca et al., 2020), and molecular studies in the replication of human tissues such as cartilage and bone, combining synthetic biology and 3D bioprinting.

In addition to bioprinting, collaboration between physicians and scientists has led to the discovery of noninvasive treatments such as stem cell therapy, which involves directing stem cells placed in patients to replace and repair diseased cells and eventually generate healthy cells (Lee & Hui, 2006; Akpancar et al., 2016; Im, 2017), and exosomes, which contain valuable molecular components of the stem cell, such as proteins and RNA (Wu et al.,

2022). Different administration of exosome treatments to improve cell-to-cell communication are being elaborated.

The advantages of image segmentation and the development of patient-specific 3D models before and during surgery, image segmentation for 3D printing has become a key aspect of orthopedic surgery. Recently, with the advancement of AI technology, organ anomalies can be accurately identified and modeled from CT or MRI images, and 3D objects can be accurately rendered. Patient-specific three-dimensional constructs can be printed using rendered volumes, with the goal of using the patient's cellular components to replace damaged or diseased tissue (Yazdanpanah et al., 2022).

1.5 Virtual surgical planning

The transition from surgery to recovery is marked by the post-operative phase, where advances in rehabilitation tools, wearable technologies, and virtual reality ensure effective healing and faster recovery of mobility. Artificial intelligence technologies will give surgeons capabilities they did not have before. Automation also allows for seamless communication and storage of all data in an easily accessible and organized format, while managing operations by tracking all supplies and ensuring that all components arrive at the hospital on time. Supplies and delivery procedures are another factor that can occasionally cause delays in surgeries. The computational technique such as finite element analysis plays a key role in optimizing implant design that allow the simulation and analysis of implant behavior under various physiological loads and conditions. Using finite element analysis, designers can identify stress distributions, potential failure points, and areas for improvement in the implant structure (Tueni & Amirouche, 2025).

2. Limitations of technological innovation in orthopaedics

Among the technical limitations is the assumption that systems may not accurately capture real-world scenarios, which can lead to performance gaps. In addition, issues with system compatibility and integration with existing healthcare technologies can hinder usability. Technology adoption among users, especially older adults who may not be comfortable with virtual reality or telehealth systems, may be another limitation related to participation rates and overall satisfaction with the treatment approach.

Data security and privacy are significant limitations to modern AI-assisted treatment approaches. As more and more patient data is shared across digital platforms, the risk of data breaches increases. Ensuring the privacy and security of patient data is critical to maintaining trust.

A major limitation is financial; while the long-term benefits can be significant, the initial costs of advanced systems and training are prohibitive for some healthcare facilities. These costs include software licensing, hardware purchases, and maintenance costs. Additionally, regulatory compliance for new devices and therapies can be lengthy and complex, which can delay the adoption of innovative technologies. Additionally, a significant limitation to the use of modern treatment trends is the dependence on technology, as overreliance on virtual platforms can lead to a lack of practical training for healthcare professionals, reduced quality of care in cases where personal approaches to surgery are necessary (Tueni & Amirouche, 2025; Wah, 2025).

Furthermore, it is imperative that AI systems are trained on accurate and up-to-date procedures, particularly within the context of a rapidly evolving medical landscape where surgical techniques and best practices are continuously advancing. Given this dynamic environment, careful and deliberate input into the training process is crucial to ensure that AI systems are equipped to adapt to new research findings and clinical discoveries.

2.1 Biomaterials and biocompatibility

Nanobiomaterials science requires a deep clinical understanding of the cellular and molecular basis that governs the function of nanostructures and cells. The challenges of developing optimized structures with properties that mimic those of natural bone or cartilage and respond to loading and stress without premature failure are still being explored.

The use of nanotechnology in combination with bioprinting is emerging as a novel approach in orthopedic research due to its potential to improve current orthopedic biomateri-

als and facilitate the development of tissue engineering innovations and tissue regeneration solutions for muscle, tendon, bone, and biodegradable implants (Tueni & Amirouche, 2025; Kumar et al., 2025).

2.2 The healthcare trends, opportunities and risks

The growing integration of new technologies is driving a fundamental revolution in the healthcare sector. The development of AI, machine learning, and massive data analytics is slowly transforming the way patients are diagnosed, treated, and cared for. AI-powered solutions are improving the efficiency and accuracy of healthcare delivery, demonstrating exceptional capabilities in personalized medicine, early disease detection, and predictive analytics. Just as telemedicine and remote patient monitoring systems have already overcome geographic limitations to some extent and offered easy and accessible healthcare services, especially in underserved areas, AI in orthopedics is following the trends ((Tueni & Amirouche, 2025).

In medical rehabilitation, wearable technology, and sensor technologies have already enabled individuals to actively participate in tracking and managing their health. Such devices enable real-time data collection, enabling preventive and personalized rehabilitation care. As these technologies continue to develop and integrate into standard healthcare practices, the future of healthcare is likely to be more accessible, efficient, and effective than ever before, but adoption, adaptation, and entry costs will be significant (Thacharodi et al., 2024). Practical experience and findings from different surgical specialties will be essential to ensure a comprehensive understanding of the transformative role of AI. Awareness will also be important for policymakers, healthcare institutions and technology developers to address barriers and promote equitable access to AI-powered surgical solutions. Among the limitations of current technologies is the reliance on recent studies, which requires long-term evaluations of clinical outcomes. Future research should focus on integrating AI with new technologies such as augmented reality, fostering interdisciplinary collaboration as well as addressing socio-ethical dimensions to fully realize the potential of AI in advanced healthcare and biomaterials technology (Wah, 2025).

3. Conclusions

Artificial intelligence and robotics are revolutionizing surgical practices by improving precision, efficiency, and patient outcomes. As global healthcare systems increasingly embrace AI-powered technologies, the integration of robotics into surgery addresses key challenges along the importance of biocompatibility of new biomaterials is raising.

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Research

Instrumented Rehabilitation

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Abstract

Conventional rehabilitation often struggles to deliver high-intensity, personalized, and engaging therapy. In recent years, robotic and sensor-based technologies have emerged as promising tools to enhance rehabilitation outcomes, particularly for individuals with neurological conditions and motor impairments. This review, combined with clinical insights from our rehabilitation institute, explores how robotic devices are transforming motor rehabilitation by enabling precise assessment, individualized training, and greater patient motivation. The paper synthesizes current literature on robotic rehabilitation and incorporates real-world clinical experience. It covers technologies used for upper and lower extremities, balance, and gait, with a focus on objective assessment tools and game-based systems that improve patient engagement. Robotic systems such as the Tyromotion Amadeo and Pablo, Hocoma Armeo and Lokomat, and the Motek C-Mill enable repetitive, task-specific training and collect objective data on strength, range of motion, balance, and gait. Game-based interfaces enhance cognitive engagement and therapy adherence. These technologies support transparent decision-making for clinicians, patients, and payers, while reducing therapist workload and allowing for potential telerehabilitation applications. Robotic technologies offer significant advantages in rehabilitation by combining measurable progress tracking, adaptive training, and patient-centered design. Although challenges remain—such as cost, accessibility, and data privacy—these tools complement the clinician's role and contribute to more effective and transparent therapy planning. Continued integration and research are needed to optimize long-term outcomes and expand access.

Keywords: Neuroplasticity; Motor Recovery; Instrumented Rehabilitation; Robotic Rehabilitation; Exergames; Virtual Reality

1. Introduction

Conventional rehabilitation, while foundational in clinical practice, presents inherent limitations in terms of precision, adaptability, and patient engagement. In contrast, modern rehabilitation technologies, particularly robotic devices, offer new possibilities for targeted assessment and training. These technologies are reshaping the rehabilitation landscape by enabling measurable, high-intensity, and personalized therapy, ultimately improving functional outcomes (Xiong et al., 2025).

Neurological conditions and movement impairments, such as stroke, spinal cord injury, and traumatic brain injury (TBI), frequently lead to motor deficits that require long-term and intensive intervention. The concept of neuroplasticity—the brain's lifelong ability to reorganize itself in response to experience and learning—provides a theoretical and practical foundation for rehabilitation. Recovery of motor function is possible by rerouting motor commands through intact neural pathways, a process significantly influenced by environmental stimuli and task-specific training (Sahrizan et al., 2025).

To support neuroplasticity and optimize recovery, rehabilitation must adhere to several key principles: early intervention, a high number of task-specific repetitions, and controlled movement selection. Moreover, patients' needs evolve throughout the rehabilitation process, requiring adaptable and individualized therapeutic strategies. Robotic technologies, through their capacity for precise and repeatable movement control, offer a valuable tool to meet these dynamic requirements (Nizamis et al., 2021).

Importantly, robotic systems do not only enable objective assessment of motor function but also provide engaging and interactive therapy environments. Integration with virtual reality (VR), for instance, has been shown to enhance patient motivation and engagement, further supporting motor learning. As a result, technology-assisted rehabilitation holds great promise in addressing the core challenges of modern therapy: efficiency, precision, and sustained patient motivation (Wankhede et al., 2025).

In this article, we explore how robotic technologies are transforming rehabilitation by facilitating personalized, adaptive, and goal-oriented interventions for individuals with motor impairments, drawing on both current literature and clinical practice experiences from our University rehabilitation institute Republic of Slovenia Soča.

2. Methods

This article presents a narrative review of the current scientific literature on robotic technologies used for assessment and training in neurological rehabilitation, supplemented by clinical insights from routine practice at University rehabilitation institute Republic of Slovenia Soča. The goal is to provide an integrated perspective that reflects both evidence-based findings and real-world implementation in clinical settings.

2.1. Literature Review

A comprehensive literature search was conducted using databases such as PubMed, Scopus, and Web of Science. The search focused on articles published between 2015 and 2025 and included studies addressing robotic rehabilitation for motor recovery in neurological conditions such as stroke, spinal cord injury, and traumatic brain injury (TBI). Keywords used in the search included: *robotic rehabilitation*, *neuroplasticity*, *motor recovery*, *assessment tools*, *virtual reality*, and *personalized therapy*. Peer-reviewed articles, systematic reviews, randomized controlled trials, and relevant clinical guidelines were considered. Studies were selected based on their relevance to robotic systems used for either assessment or training, particularly those highlighting clinical outcomes, therapy parameters, and patient engagement.

2.2. Clinical Practice Insights

To complement the literature findings, clinical experiences were drawn from therapists and clinicians working at University rehabilitation institute Republic of Slovenia Soča. Information was collected through discussions, therapy observations, and analysis of anonymized case examples. These insights reflect the practical application of robotic devices in daily rehabilitation routines, including their role in assessment, patient motivation, therapy planning, and adaptation over time.

The integration of both sources, scientific evidence and clinical practice, aims to present a holistic overview of the use and impact of robotic technologies in rehabilitation. This approach allows for a critical evaluation of the current state of the field while highlighting practical considerations and challenges encountered in real-world therapeutic environments.

3. Results

3.1. Motivation through Game-Based Rehabilitation

One of the key factors influencing rehabilitation outcomes is patient motivation and adherence to therapy. Both literature and clinical experience confirm that integrating game-like elements into therapy, so-called exergames, significantly increases patient engagement (Fernandes et al., 2025). These systems transform repetitive physical exercises into interactive and enjoyable digital games, thereby reducing the perception of therapy as a chore (Malik et al., 2022).

Somatosensory games, which rely on motion and sensor input, offer real-time feedback and track progress across sessions. In clinical practice, these tools have been associated with improved therapy adherence, increased engagement and enjoyment, and enhanced cognitive involvement. Such benefits are particularly evident in long-term neurological rehabilitation as well as in rehabilitation training for older people with mild cognitive impairment, where sustained motivation is often a challenge (Chang et al., 2022).

3.2. Technologies for Fingers and Upper Extremities

Precision in hand and arm rehabilitation is essential, especially for patients recovering from stroke or traumatic brain injury. Robotic devices allow for controlled, repetitive, and individualized training of both gross and fine motor movements (Adar et al., 2023; Zariffa et al., 2012). Overview of some technologies, used at our institute for upper limb and fingers are presented in **Table 1**.

Table 1. Overview of some technologies at University rehabilitation institute Republic of Slovenia Soča, used for upper limb and finger instrumented rehabilitation.

Device	Functionality	Key Features	Clinical Use
Tyromotion Amadeo	Finger rehabilitation	Strength, ROM, tone, spasticity assessment	Early to chronic phase, detailed evaluation
Hocoma Armeo	Upper limb exoskeleton	Gravity support, 3D movement training	Motor recovery post-stroke/TBI
Tyromotion Pablo	Hand and arm coordination	Sensor-based training, gamified tasks	Engagement, bilateral coordination training

These technologies have enabled therapists to better tailor therapy plans to individual patient needs, while also enhancing the objectivity of motor assessments.

3.2. Technologies for Lower Extremities, Balance, and Gait

Restoring gait, balance, and postural control is a core goal in rehabilitation of neurological and orthopedic patients. Several advanced technologies have shown promising outcomes in helping patients relearn natural movement patterns through structured and intensive repetitive training (Elmas Bodur et al., 2024; de Miguel Fernandez et al., 2023). Overview of some technologies at our institute for lower limb rehabilitation, balance and gait are presented in **Table 2**.

These tools, through consistent and repeatable movement guidance, promote motor relearning and support neuroplasticity while adapting to patient-specific recovery trajectories. Therapists as well as literature have confirmed the positive outcomes in patients with neurological issues in using these tools for gaining better balance and gait functions (Huo et al., 2024).

Table 2. Overview of some technologies at University rehabilitation institute Republic of Slovenia, used for lower limb rehabilitation, balance and gait.

Device	Functionality	Key Features	Clinical Use
Hocoma Erigo	Early mobilization	Tilt table with leg movement, cardiovascular support	ICU/early neurorehabilitation
Hocoma Lokomat	Robot gait training	Body weight support, adjustable guidance	Gait re-education in neurological patients
Biodex Balance	Balance assessment and training	Objective metrics, dynamic training protocols	Fall risk prevention, proprioception retraining
Motek C-Mill	Treadmill-based gait training	Virtual/augmented reality environments	Gait adaptability, obstacle navigation

4. Discussion

The integration of robotic technologies into neurological rehabilitation offers a paradigm shift not only in training but also in assessment (Choi et al., 2024). One of the most valuable contributions of these systems is their capacity to collect objective, quantifiable data throughout the rehabilitation process. By measuring parameters such as strength, range of motion (ROM), balance, and gait characteristics, robotic devices provide reliable and reproducible metrics that enhance the transparency and precision of clinical decision-making (Zhang et al., 2023).

These data serve multiple stakeholders. For clinicians, they support evidence-based planning, adaptation, and optimization of therapy. For patients, they offer a tangible understanding of progress, helping to maintain motivation and trust in the therapeutic process. For insurers and healthcare providers, objective documentation enables fair and consistent decisions regarding therapy continuation or conclusion. This contributes to a more transparent and patient-centered model of care, where decisions are no longer based solely on subjective clinical impressions but grounded in measurable outcomes.

In clinical practice, the use of robotic systems also addresses several challenges inherent to conventional rehabilitation. These include:

- The need for high-dose, high-repetition task-specific training to support neuroplasticity;
- The ability to deliver personalized and adaptive therapy that adjusts to the patient's functional level over time;
- The provision of motivating and engaging environments, particularly through game-based and virtual reality interfaces;
- The reduction of therapist workload, allowing for more efficient use of clinical resources.

Furthermore, some systems allow for remote monitoring and telerehabilitation, which can expand access to therapy for patients in remote or underserved areas, an increasingly relevant feature in light of healthcare system pressures and demographic shifts (Desai et al., 2023).

Despite these advantages, several limitations and challenges must be considered. The high cost of robotic systems can limit availability and implementation in smaller or resource-constrained institutions. These systems also remain constrained by the reliance on large, cumbersome equipment that necessitates supervision (Rodriguez-Fernandez et al., 2021). Takebayashi et al. (2022) reported that robotic self-training alone did not yield superior improvements in upper-limb function compared to conventional self-training; however, it may offer additional benefits when integrated with standard therapy in certain patient groups (per-protocol analysis) Data privacy and cybersecurity concerns must be carefully managed, especially when dealing with personal health data and remote access (Monoscalco et al., 2022). However, there are cases that confirm effectiveness and improvement of self-learning with a robot for upper limb rehabilitation training (Klinkwan et al.,

2023). Additionally, these technologies may not be suitable for all patients, particularly those with severe cognitive impairments, where understanding instructions or engaging with digital interfaces may be difficult (Aprile et al., 2021), as also confirmed by our therapists. The safety of robotic devices, especially in unsupervised or home-based contexts, also remains an area requiring continuous attention and development (Gonzalez et al., 2021), although Gil-Agudo et al. (2023) reports no adverse events.

In summary, robotic technologies for rehabilitation provide a comprehensive toolset for assessment, training, and outcome monitoring. While they are not a substitute for human expertise and therapeutic relationships, they significantly enhance precision, engagement, and objectivity in therapy. Addressing barriers to access and expanding evidence on long-term outcomes will be key to their broader integration into routine clinical care.

5. Conclusions

Technological advancements are significantly enhancing the field of rehabilitation by enabling accurate assessment, effective training, and increased patient motivation (Metzger et al., 2014). Robotic devices, sensor-based systems, and game-driven environments are no longer futuristic concepts; they are actively shaping the way rehabilitation is delivered today and will continue to evolve its practice in the future.

These technologies support the fundamental principles of neurorehabilitation, such as early intervention, task-specific training, and the need for high repetition. They bring precision, adaptability, and objectivity into therapy, helping clinicians tailor interventions to individual needs while tracking progress over time. In doing so, they contribute to more patient-centred, transparent, and data-driven care.

Robotic systems go beyond facilitating movement—they also collect meaningful, quantifiable data that improves clinical decision-making, justifies therapy continuation or modification, and supports communication with patients and insurers. This enables more fair and informed decisions while empowering patients with visible, measurable indicators of their progress as also confirmed by our therapists.

While these tools are powerful, it is essential to emphasize that therapists continue to play a major role in the rehabilitation process. Technology does not replace the expertise, judgment, and human connection provided by clinical professionals; it enhances and supports their work. The therapist's ability to interpret data, adapt strategies, and provide motivation and emotional support remains irreplaceable.

Looking ahead, the future of rehabilitation is interactive, measurable, and empowering. With robotics, sensors, and virtual environments, we offer not just therapy, but renewed hope and a clear path forward for people recovering from neurological and musculoskeletal conditions. To fully realize this potential, ongoing efforts must focus on improving accessibility, addressing data security, and further integrating these technologies into everyday clinical workflows.

In summary, robotic technologies are not simply tools for rehabilitation—they are catalysts for transforming it into a more effective, engaging, and evidence-based process, guided by both data and human care.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

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Review

Burkitt Lymphoma and Diffuse Large B-cell Lymphoma in Children

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Abstract:

Every year, around 400,000 children and adolescents are diagnosed with cancer, with leukemia, brain tumors and lymphomas being the most common. Lymphomas in children are divided into Hodgkin's lymphomas and non-Hodgkin's lymphomas, including Burkitt's lymphoma and diffuse large B-cell lymphoma. Burkitt's lymphoma is most common in sub-Saharan Africa and in industrialized countries, while diffuse large B-cell lymphoma is more common in adolescents, with the incidence increasing with age. The diagnosis of Burkitt's lymphoma and diffuse large B-cell lymphoma is based on a lymph node biopsy and histologic analysis, which shows medium-sized cells with a "starry sky" and a high mitotic index. Staging is based on *the International Pediatric Non-Hodgkin's Lymphoma Staging System*, and risk stratification takes into account disease stage, tumor resectability, lactate dehydrogenase level and *TP53* mutations. Most cases of Burkitt's lymphoma and diffuse large B-cell lymphoma are successfully treated with standard chemotherapy, with low-risk patients achieving a survival rate of up to 97%. Patients in higher stages or with elevated lactate dehydrogenase levels benefit from chemotherapy plus rituximab, resulting in an event-free 1-year survival rate of over 90%. However, relapses have a poorer prognosis and despite aggressive treatments such as CAR-T cell therapy and stem cell transplantation, cure rates remain low (27–36%). Advances in molecular diagnostics and targeted therapies offer the opportunity to personalize treatment and reduce toxicity while achieving high cure rates. Future protocols will be more precise, effective and patient-centered, which will improve the survival rate and quality of life of patients with Burkitt's lymphoma. In addition to these advances, it is also important to raise awareness of the disease among the general public and promote interdisciplinary approaches in research and clinical work.

Keywords: Burkitt lymphoma, Diffuse large B-cell lymphoma, Pediatric oncology, Treatment, prognosis, Chemotherapy

1. Introduction

According to data from the World Health Organization (WHO), approximately 400,000 children and adolescents are diagnosed with cancer worldwide each year (World Health Organization, 2005). The most common pediatric malignancies are leukemia and brain tumors, followed by lymphomas, which account for approximately 10–15% of all childhood cancers (Gallamini & Juweid, 2021).

Lymphomas in children are broadly categorized into Hodgkin lymphoma and a heterogeneous group of non-Hodgkin lymphomas (NHL). Among high grade non-Hodgkin lymphomas, two major categories are recognized: lymphoblastic lymphoma and mature B-cell lymphomas. The latter includes subtypes such as Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL), and several less common variants (Egan et al., 2019). In contrast to adults, low-grade lymphomas, such as follicular lymphoma, are very rare in children.

Burkitt lymphoma is the most frequent subtype of mature B-cell NHL in children, representing up to 80% of cases (Egan et al., 2019; Minard-Colin et al., 2015). It is further classified into endemic and sporadic forms. The endemic form, predominantly seen in sub-Saharan Africa, is strongly associated with Epstein–Barr virus (EBV) infection and regions with high malaria prevalence (Quintana et al., 2020). The sporadic form, more common in industrialized countries, occurs with an incidence of approximately 3.5 per million, predominantly in boys, and peaks between the ages of 5 and 14. After this age, its incidence declines (Gallamini & Juweid, 2021; Egan et al., 2019).

In contrast, DLBCL has an incidence of approximately 6.5 per million and is the most common subtype of non-Hodgkin lymphoma in adolescents (Gallamini & Juweid, 2021; Egan et al., 2019). Unlike Burkitt lymphoma, the incidence of DLBCL increases steadily with age (Gallamini & Juweid, 2021; Egan et al., 2019).

2. Clinical Presentation

B-cell lymphomas in children and adolescents can present with a wide range of symptoms, including peripheral lymphadenopathy, abdominal masses, weight loss, head and neck tumors, organomegaly, B-symptoms (fever, night sweats, and unexplained weight loss), abdominal or bone pain, and neurological manifestations (Saatci et al., 2024).

Burkitt lymphoma is characterized by rapid tumor growth, and patients often present with a short duration of symptoms. Clinical presentation largely depends on the tumor's anatomical location. The most common site of involvement is the abdomen, seen in approximately 60% of cases, followed by head and neck masses in 16% of patients. Peripheral lymph node involvement occurs in about 4% of cases, while central nervous system (CNS) symptoms are present in approximately 2% (Saatci et al., 2024). The gastrointestinal mass usually presents with symptoms resembling appendicitis, including pain, vomiting, gastrointestinal (GI) bleeding, and signs of acute abdomen (Egan et al., 2019; Fish et al., 2021). The spread of the disease into bone marrow and central nervous system at the time of diagnosis is confirmed in about 20% of patients (Egan et al., 2019; Goldman and Cairo, 2023).

In DLBCL, peripheral lymphadenopathy, abdominal, or mediastinal mass are usually observed. The CNS and bone marrow are less commonly involved compared to Burkitt lymphoma (Egan et al., 2019).

3. Diagnosis

The diagnosis of Burkitt lymphoma and DLBCL relies on histological examination of a lymph node biopsy, although distinguishing between the two can sometimes be challenging. Histology typically reveals monomorphic, medium-sized cells with round or ovoid nuclei and basophilic cytoplasm, often displaying a characteristic “starry sky” appearance. A high mitotic index is also commonly observed (**Figure 1**).

A hallmark of Burkitt lymphoma is the expression of MYC oncogene, most commonly driven by translocation between chromosome 8 and 14 (*t*(8;14)). Less common translocations involving MYC include *t*(2;8) and *t*(8;22). In addition to MYC rearrangements, other genetic alterations—such as mutations in *TP53*, *ID3*, or *TCF3*—may

also be present (Egan et al., 2019). In contrast, MYC translocations are less frequently observed in DLBCL, occurring in approximately one-third of cases (Poirel et al., 2009).
Disease extent is assessed using ultrasound, chest computed tomography (CT) and abdominal magnetic resonance imaging (MRI). In most cases, due to rapid progression of symptoms, there is not enough time to perform positron emission tomography–computed tomography (PET–CT), which also might be helpful.
Evaluation of cerebrospinal fluid (CSF) by cytological examination is essential, and in cases with suspected central nervous system involvement, brain MRI is indicated. Staging is based on the *International Pediatric Non-Hodgkin Lymphoma Staging System* (Rosolen et al., 2015).

- **Stage I**: A single tumour formation not located in the abdomen or mediastinum.
- **Stage II**: Multiple lymph nodes on one side of the diaphragm, single extranodal tumour with regional node involvement or primary gastrointestinal tumour whith or without involvement of associated mesenteric nodes, that is completely resectable.
- **Stage III**: Multiple extranodal or nodal tumour on both sides of the diaphragm, any intrathoracic or intra-abdominal tumour, retroperitoneal involvement (except primary gastrointestinal tumour, that is completely resectable), bone lesion, paraspinal or epidural involvement.
- **Stage IV**: Central nervous system or bone marrow involvement.

Risk stratification takes into account disease stage, resectability of the tumor, and serum lactate dehydrogenase (LDH) levels (Tzotzola et al., 2021). Recent guidelines also highlight the prognostic significance of *TP53* mutations, which are associated with poorer outcomes (Cairo, 2024).

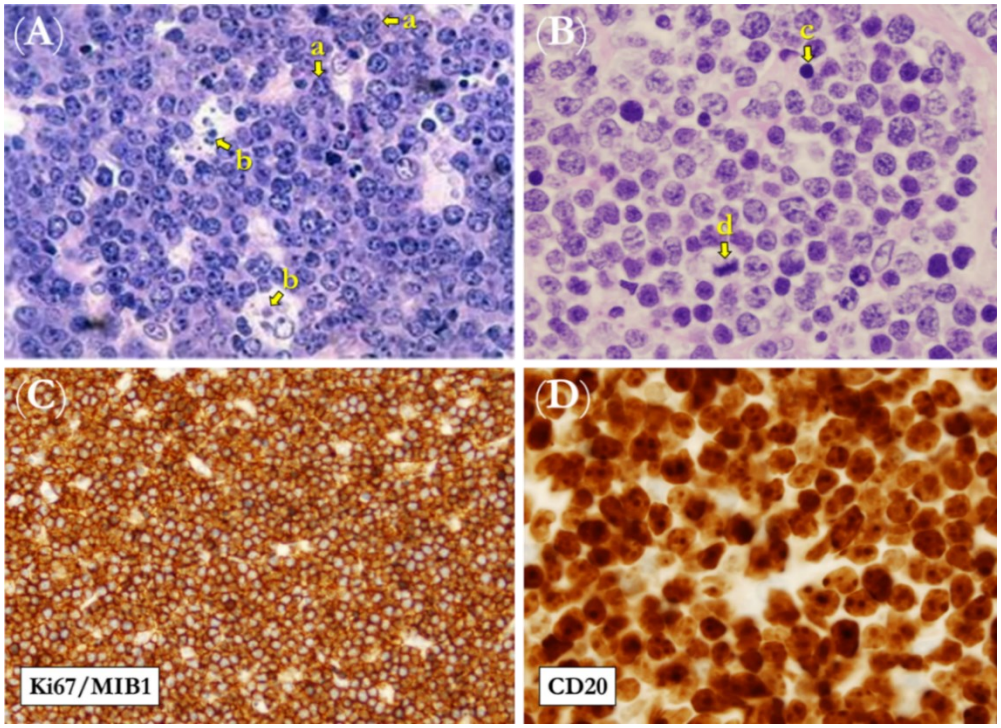

Figure 1. Sheets of monotonous intermediate-sized cells with a "starry sky" appearance. The cells are packed together (cobblestone pattern), with space between them due to retracted cytoplasm. The nuclei are round with finely granular chromatin, and multiple nucleoli. Numerous mitoses and apoptotic bodies are present, indicating rapid proliferation of tumor cells. Numerous macrophages are present, phagocytosing apoptotic material. The macrophages have a bright, abundant cytoplasm, giving a tumor a "starry sky" appearance. (**A**), (**B**) Typical morphological picture (**a**) round nuclei with dispersed chromatin and numerous nucleoli; (**b**) macrophages with phagocytized debris (tingible bodies macrophages); (**c**) apoptosis, and (**d**) mitosis. (**C**) CD20 is an immunohistochemical marker of the cluster of differentiation for B-lymphocytes, and (**D**) Ki67/MIB1 is a proliferation marker labeling all tumor cells.

The sample shown in **Figure 1** was treated according to the WHO classification of tumours (WHO, 2024). The sample is a soft tissue mass in the left inguinal region of a 30 y/o male patient. An excisional biopsy was performed. The tissue was fixed in 10% buffered formalin and embedded in paraffin. Paraffin tissue blocks were prepared. 3 μm-Thick slides were cut and stained with H&E (**B**) and Giemsa (**A**). Immunostaining with CD20cy, clone L26, DAKO (**D**), and Ki67, clone MIB-1, DAKO (**C**), was performed for diagnostic purposes.

4. Treatment and Prognosis

Most cases of Burkitt lymphoma and DLBCL can be successfully treated with standard chemotherapy but a risk-adapted approach is used. Low-risk patients typically receive intensive chemotherapy alone, reaching overall survival up to 97% (Gardenswartz & Cairo, 2024). Patients with higher-stage disease (III or IV) or elevated LDH levels receive a combination of chemotherapy and rituximab, an anti-CD20 monoclonal antibody, reaching 1-year event-free survival of more than 90% (Minard-Colin et al., 2016).

Given these excellent outcomes, recent efforts have focused on treatment de-escalation. This includes reducing cumulative doses of chemotherapy or replacing certain cytotoxic agents with rituximab, aiming to minimize long-term toxicity without compromising cure rates (Egan et al., 2019).

However, prognosis is considerably poorer in cases of relapse. Several factors influence outcomes in relapsed Burkitt lymphoma and DLBCL, including initial disease stage, LDH levels, and the extent and timing of relapse. Relapse occurring within six months of initial treatment is particularly associated with a worse prognosis (Jourdain et al., 2015).

Treatment of relapsed disease may include re-induction chemotherapy, rituximab, checkpoint inhibitors, chimeric antigen receptor T-cell (CAR T-cell) therapy, and, if remission is achieved, high-dose chemotherapy followed by hematopoietic stem cell transplantation (Gardenswartz & Cairo, 2024). Despite aggressive salvage strategies, overall survival in relapsed cases remains low, with cure rates ranging from 27% to 36% (Gardenswartz & Cairo, 2024; Rigaud et al., 2019).

5. Pediatric treatment of Burkitt lymphoma and diffuse large B-cell lymphoma in Slovenia – Conclusion

In Slovenia, patients with Burkitt lymphoma and DLBCL are treated according to the currently valid European protocol. Chemotherapy remains the mainstay of treatment, with the addition of Rituximab in high risk patients (**Figure 2**). Using this approach, we have achieved excellent event-free and overall survival rates.

Figure 2. Structure of Rituximab (Drugbank, 2005). Rituximab is a genetically engineered chimeric monoclonal antibody (*m*Ab) directed against the CD20 antigen on the surface of normal and malignant B lymphocyte cells. The antibody is an IgG1 *kappa* immunoglobulin containing sequences of the murine light and heavy chain variable region and human constant region sequences. It was originally approved by the US FDA in 1997 as a single agent for the treatment of patients with B-cell NHL, however has since been approved for a variety of diseases. On 28 November 2018, the US Food and Drug Administration (FDA) approved Truxima, the first biosimilar to Rituxan (rituximab). The chemical formula of the protein is $C_{6416}H_{9874}N_{1688}O_{1987}S_{44}$, and the average weight of the protein is 143859.7 Da (Drugbank, 2005).

Ongoing advances in molecular diagnostics and targeted therapies offer promising opportunities to further personalise and optimise treatment. Scientific innovations also open up the possibility of reducing treatment-related toxicity while maintaining high cure rates. By taking these developments into account, future protocols can become even more precise, effective and patient-centred. Building on this solid foundation, science will continue to lead the way to improved survival and quality of life for Burkitt lymphoma patients.

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Research

The Antimicrobial Activity of Plant-Derived Cannabinoid Suspensions from *Cannabis sativa* L. Against the Mixed Oral Microflora of Humans and Canine

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Abstract:
Cannabis sativa L. is a dioecious annual plant that is widely cultivated for its industrial, nutritional and medicinal uses. Historically used as a fibre plant and for therapeutic purposes, its modern importance lies in the production of biologically active compounds, particularly cannabinoids. Cannabis-derived compounds, particularly cannabidiol (CBD), have shown antimicrobial activity against both Gram-positive and Gram-negative bacteria. Preliminary studies suggest that cannabinoids may be more effective in reducing dental plaque than conventional oral care products. This study aims to evaluate the *in vitro* inhibitory effects of "pure" plant CBD and a full-spectrum cannabis isolate on polymicrobial cultures derived from the oral surfaces of healthy humans and canine to investigate their potential to prevent periodontal disease in both human and veterinary medicine. The cannabis isolate showed a slightly stronger activity, which is probably due to the synergistic effect of several cannabinoids, terpenes and other bioactive compounds. Factors such as potentially better solubility, the presence of minor cannabinoids (*e. g.* cannabigerol, CBG) and the membrane disruption caused by terpenes could contribute to this stronger effect. These results suggest that full-spectrum cannabis extracts may offer greater potential for oral antimicrobial applications than "pure" CBD alone.

Keywords: Plant, *Cannabis sativa* L., Cannabinoids, Human medicine, Veterinary medicine, Mixed oral microflora, Antimicrobial activity

1. Introduction

1.1. Cannabis sativa L.

Cannabis (*Cannabis sativa* L.) is a dioecious, annual flowering plant belonging to the family Cannabaceae, widely distributed due to its adaptability to environmental conditions. Historically valued for its industrial and medicinal properties, cannabis has been cultivated by humans since antiquity, serving as a source of fiber, oil, and therapeutic agents. In recent decades, scientific interest in this plant has significantly intensified due to its wide spectrum of biologically active compounds and therapeutic potential (Pečan et al., 2021).

The pharmacological properties of cannabis are primarily attributed to a unique group of secondary metabolites known as cannabinoids, of which over 140 have been identified to date (**Figure 1**). These compounds are synthesized and stored in trichomes, glandular structures especially abundant on the female inflorescences of the plant. Cannabinoids interact with the endocannabinoid system in the human body, modulating various physiological processes (Pečan et al., 2023; Appendino et al., 2008). Among them, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most extensively studied. THC is known for its psychoactive effects and therapeutic use in conditions such as chronic pain and nausea, acting as a partial agonist at CB1 and CB2 receptors (Pečan et al., 2021). In contrast, CBD exhibits no psychoactivity but offers anti-inflammatory, analgesic, antineoplastic, and neuroprotective effects, partly through modulation of oxidative stress and autophagic pathways (Pečan et al., 2023).

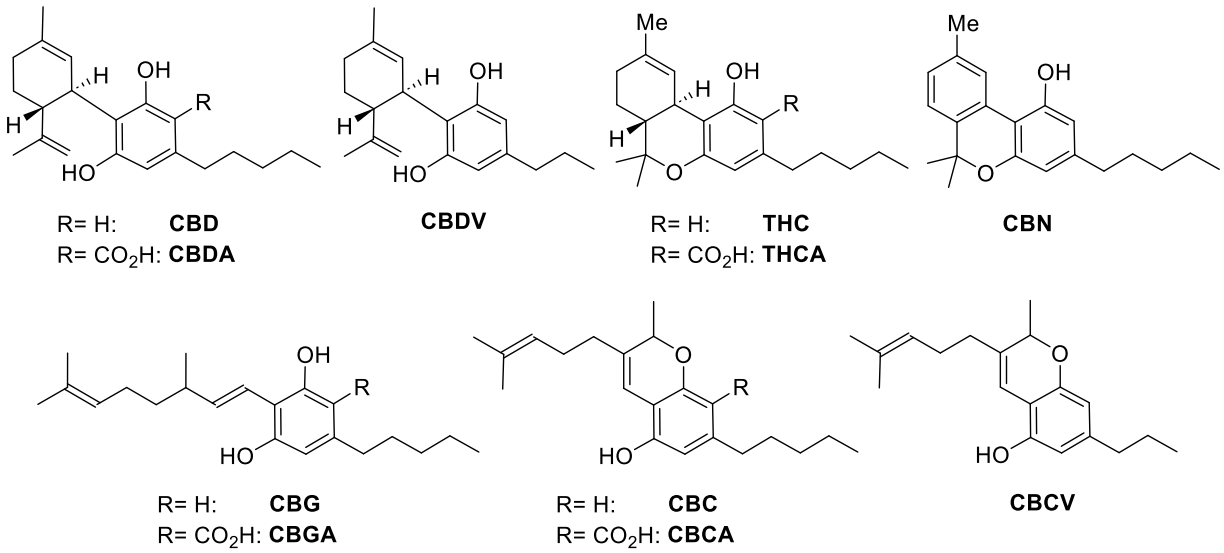

Figure 1. Structural formulas of some terpenes that may occur in cannabis isolate.

Beyond these two key molecules, cannabis also contains terpenes, flavonoids, and other phytochemicals with synergistic or individual bioactivity. These compounds contribute to the plant's potential in treating a range of conditions including antibiotic-resistant infections, dermatological disorders, and chronic inflammation. As a result, cannabis is being increasingly recognized not only in human medicine but also in veterinary science, particularly for dermatological applications. However, its therapeutic use remains subject to complex legal regulations that vary widely across jurisdictions (Mišič Jančar et al., 2024).

1.2. Oral microbioma, cannabinoids and antimicrobial activity

Oral microbiome is a complex microbial community composed by a variety of bacteria, archaea, viruses and fungi (Rajasekaran et al., 2024). Human and canine oral microbiome differs significantly, with 16.4% of taxa shared (Dewhirst et al., 2012). In healthy dogs, oral microbiota is composed by hundreds of bacterial species. *Porphyromonas* and *Corynebacterium* are the most prevalent genera (Šakarnytė et al., 2023). *Porphyromonas* species are increased in dogs suffering from periodontal diseases along with

Bacteroides and *Fusobacterium* (Santibáñez et al., 2021). Similar results were found in humans affected with periodontal disease, where *Fusobacterium, Porphyromonas, Leptotrichia* and *Prevotella* among others, were the most abundant genera (López-Martínez et al., 2020).

Inflammatory periodontal disease (gingivitis and periodontitis) is the most common disease involving the oral cavity of human adults and companion animals, and it is frequently induced by bacterial plaque and its derivate byproducts (Bellows et al., 2019; Martínez-García et al., 2021). As in humans, tooth brushing is the "gold standard" procedure to maintain a good oral heath in companion animals. However, due to the difficulty to be performed and the low owner compliance, alternative chemical anti-plaque retardants are needed (Gawor et al., 2025).

Plants are a wide source of natural bioactive compounds with antimicrobial activity (Chassagne et al., 2021). The resin obtained from the inflorescence of the plant *Cannabis sativa* L. contains more than 500 molecules (ElSohly et al., 2017). Cannabinoids and terpenes are the most abundant chemical groups in cannabis extracts, and both have proven to exert antimicrobial activity against pathogenic bacteria (Schofs et al., 2021). In particular, cannabidiol, a 314 Da phytocannabinoid, is the most studied cannabinoid in view of its safety profile and wide range of pharmacological effects (Izzo et al., 2009). CBD has shown an antibacterial activity against Gram-positive and Gram-negative bacteria (Blaskovich et al., 2021). Additionally, cannabinoids were found to be more effective than conventional commercial oral care products in reducing the bacterial burden of dental plaques from human sources *in vitro* (Stahl & Vasudevan, 2020). Hence, cannabis extracts and purified cannabinoids could be useful to reduce bacterial plaque formation and therefore to prevent periodontal disease in human and veterinary medicine.

The main goal of the present work was to preliminary asses the *in vitro* inhibitory effect of pure CBD and a complete cannabis isolate on a polymicrobial culture obtained from dental surfaces of a healthy human and dog, respectively.

2. Materials and Methods

2.1. Experimental suspensions of plant material

Two polymicrobial culture samples, collected from the dental surfaces of a healthy human and dog were studied. The CBD sample was a white powder with 99.3% purity. In contrast, the full-spectrum isolate—free of psychoactive components such as THC—was partially crystalline and amber-colored. Both samples were donated by Herbana, Ltd.

Fourier transform infrared (FTIR) spectroscopy is used to determine the molecular structure and composition of organic compounds by analyzing their absorption of infrared light. It helps to identify functional groups such as hydroxyl (–OH), cyano (–CN), carbonyl (–C=O) and amino ($–NH_2$) based on their characteristic absorption bands (Larkin, 2011). This technique can be used to confirm the identity of compounds, detect impurities, analyze the composition of polymers, etc. In our case, it was used to confirm the identity of compounds. In this study FTIR spectroscopy (Bruker, Tensor II) was used to determine the cannabinoid content in the full spectrum isolate sample (**Figure 2**).

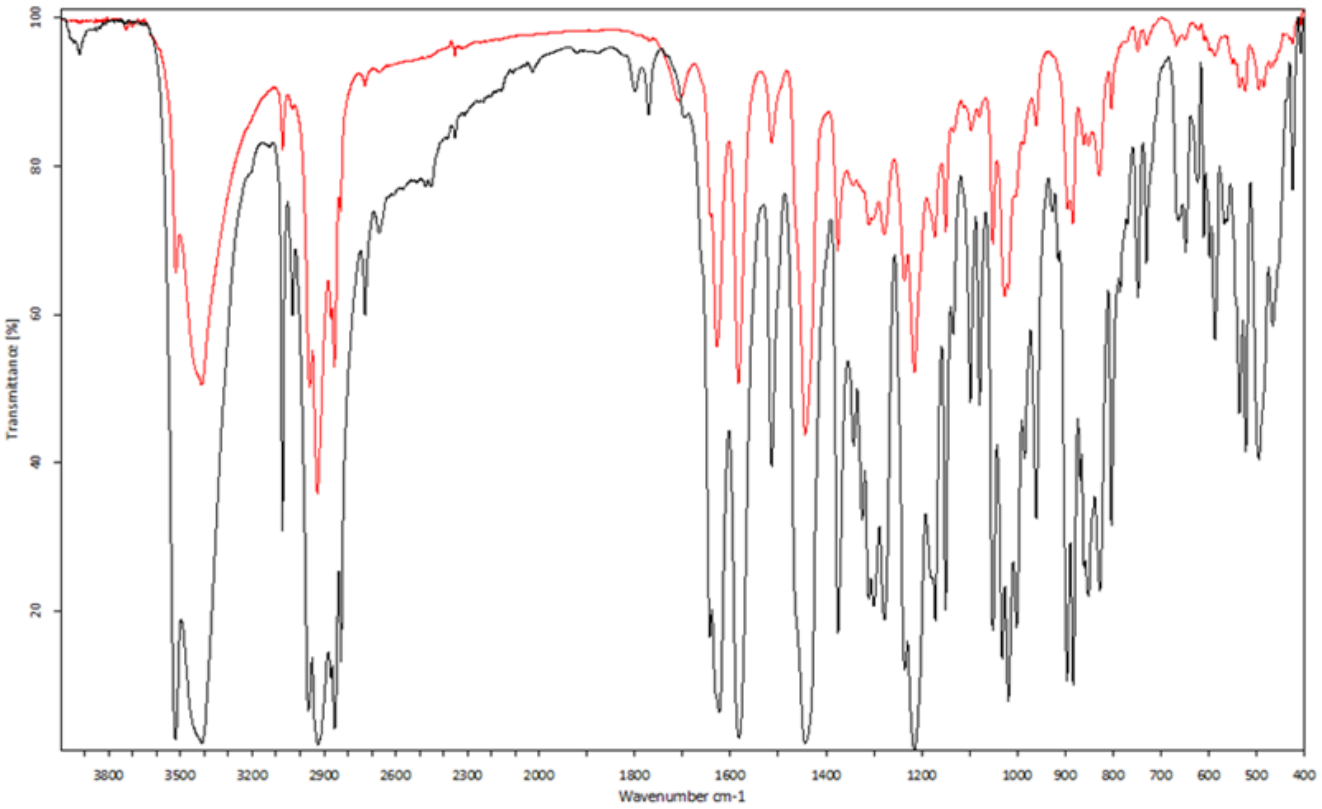

Figure 2. FTIR spectrum of the chemically "pure" CBD compound (black) and of isolate of cannabinoids (red).

FTIR spectra of a chemically pure cannabidiol (CBD) sample (black) and a full-spectrum cannabinoid isolate lacking psychoactive components such as THC (red) are presented in **Figure 2**. The spectral profiles of both samples exhibit strong similarities, particularly in key absorption regions corresponding to characteristic functional groups. A broad absorption band around 3400 cm^{-1} in both spectra indicates the presence of hydroxyl (–OH) groups, consistent with the phenolic structure of CBD and related cannabinoids. The C–H stretching vibrations observed between 2950 and 2850 cm^{-1} suggest aliphatic hydrocarbon chains, which are typical features of cannabinoids. Furthermore, the presence of peaks near 1600–1500 cm^{-1} is attributed to C=C stretching in aromatic rings, a core component of the cannabinoid structure. The fingerprint region (1500–500 cm^{-1}), which contains complex vibrational patterns unique to molecular structure, shows highly overlapping signals between the two samples. This spectral similarity implies that the full-spectrum isolate contains CBD and likely other non-psychoactive cannabinoids with comparable structural features. The lack of distinct additional peaks suggests the absence of unrelated impurities, supporting the hypothesis that the isolate is composed primarily of structurally similar phytocannabinoids. Overall, the FTIR data confirm the chemical relatedness of the two samples and support the use of the full-spectrum isolate in comparative bioactivity studies.

3.2. Antibacterial activity

The antimicrobial activity of the plant-derived cannabinoid samples was evaluated using the agar diffusion method. Suspensions of the active compounds were prepared in a 0.1% aqueous solution of the co-solvent dimethyl sulfoxide (DMSO) and applied onto a solid growth medium inoculated with the model microbial culture.

The antimicrobial activity of the cannabinoid samples on the oral microflora of human and canine was therefore tested on nutrient agar (solid medium) in Petri dishes. 22.5 g Plate Count Agar (PCA; Vegitone, Millipore) was suspended in 1 L of deionized water. The contents were heated with intensive stirring until a homogeneous suspension was formed, then sterilized at 121 °C for 30 minutes and poured onto Petri dishes in an aseptic environment.

Oral swabs were collected from the tooth surfaces of a healthy 34-year-old male (co-author of this study) and a healthy 1-year-old Weimaraner dog. The collected biocultures were transferred onto agar plates using sterile cotton swabs moistened with physiological saline solution.

Then, under an aseptic atmosphere, round filter discs (*diameter* = 0.5 mm) made of quantitative filter paper (MN 640W, Macherey-Nagel, Germany) were placed on each culture medium. A 10 µL of the respective samples were applied directly onto the surface of each disc as follows:

- different concentrations (5, 50 and 100 µg/mL) of suspensions of (*i*) cannabinoid CBD and (*ii*) full spectrum isolate prepared in 0.1 % aqueous DMSO solution;
- negative control: (*i*) saline solution (0.9% NaCl, B. Braun, Germany), (*ii*) deionised water, (*iii*) 0.1% aqueous DMSO solution (Merck, Germany); and
- positive control: 10% aqueous hydrogen peroxide solution (H_2O_2) (Merck, Germany), which has a proven antimicrobial effect.

The culture samples prepared as described were incubated in a Heratherm IGS60 GP incubator at 37 °C and evaluated after 24 and 48 hours. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones, defined as the clear areas surrounding the filter discs where microbial growth was absent. Each concentration of the cannabinoid samples was tested in triplicate against the model oral microflora to ensure reproducibility and statistical reliability of the results.

3. Results

In our study, the DMSO was used as a co-solvent at a final concentration of 0.1%, a level widely recognized in the literature as having negligible effects on biological systems. Higher concentrations have been shown to adversely affect cell viability and metabolic activity (Tunçer et al., 2018). Our results showed normal microbial growth in the negative control in the absence of active compounds (**Table 1**). This proves that that DMSO at this concentration did not interfere with microbial viability.

As expected, the microorganisms on the oral swab outgrew the diffusion discs with three different negative controls (saline, deionised water, 0.1% DMSO solution) after 24 hours of observation, as these samples provide the microorganisms with an environment in which they can grow and multiply undisturbed (**Table 1**). The highest zone of inhibition of microbial growth on diffusion discs was achieved with a positive control (10% H_2O_2). Interestingly, 10% H_2O_2 inhibits the growth of microorganisms in dogs better than in humans (inhibition zone after 24 hours 8.5 cm *vs*. 3.6 cm).

Table 1. The results of the inhibition zones for individual samples, taking into account the measurement error.

No.	Sample	Inhibition zone diameter [cm]			
		Human		Canine	
		24 h	48 h	24 h	48 h
1	10% H_2O_2	3.6 ± 0.1	3.0 ± 0.1	8.5 ± 0.0	8.5 ± 0.0
2	5 µg/mL CBD	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
3	50 µg/mL CBD	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
4	100 µg/mL CBD	0.8 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	1.0 ± 0.2
5	5 µg/mL isolate	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0
6	50 µg/mL isolate	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0
7	100 µg/mL isolate	1.0 ± 0.1	0.8 ± 0.1	1.5 ± 0.1	0.8 ± 0.1
8	Saline	0	0	0	0
9	Deionized water	0	0	0	0
10	0.1% DMSO	0	0	0	0

In both samples, the chemically "pure" CBD and the isolate, the inhibition zone of microbial growth were larger at higher concentrations. Both samples showed the greatest effect on the model microorganisms at the highest concentration tested (100 μg/mL) and the least at the lowest concentration (5 μg/mL).

The CBD concentration of 5 μg/mL had a moderate effect on human microorganisms after 24 hours (inhibition zone 0.6 cm), and after 48 hours the microorganisms grew slightly beyond the inhibition zone (0.4 cm). A similar effect was also observed on the growth of canine microorganisms after 24 hours (inhibition zone 0.6 cm) and after 48 hours (inhibition zone 0.5 cm).

Compared to pure CBD, the isolate with a concentration of 5 μg/mL had a slightly stronger effect on the growth of microorganisms in both humans (inhibition zone after 24 hours 0.7 cm, after 48 hours 0.6 cm) and canine (inhibition zone after 24 hours 0.9 cm), but in canine the effect was no longer present after 48 hours (inhibition zone 0 cm); the microorganisms had outgrown the inhibition zone.

At a CBD concentration of 50 μg/mL, the effect was better than at a concentration of 5 μg/mL and was the same after 24 hours as after 48 hours; in humans, the inhibition zone at both time points – 24 and 48 hours – was 0.8 cm and in canine 0.7 cm after 24 hours and 0.6 cm after 48 hours.

Taking into account the measurement error, the isolate with a concentration of 50 μg/mL had a very similar effect on human microorganisms as CBD (inhibition zone 0.8 cm), but after 48 hours the microorganisms had grown slightly more in humans (inhibition zone 0.6 cm) and beyond the inhibition zone in canine.

A CBD concentration of 100 μg/mL had the same effect on human microorganisms after 24 hours as a concentration of 50 μg/mL (inhibition zone 0.8 cm), but after 48 hours the microorganisms grew slightly beyond the inhibition zone (0.7 cm). In canine, the activity after 24 hours was twice as high as at a concentration of 50 μg/mL (inhibition zone 1.5 cm compared to 0.7 cm), but after 48 hours the difference decreased (1.0 cm compared to 0.6 cm).

The isolate with a concentration of 100 μg/mL was slightly more active in humans after 24 hours (inhibition zone 1.0 cm); after 48 hours, the zone of inhibition decreased by about 20% (inhibition zone 0.8 cm). In canine, the isolate was as active as CBD (inhibition zone 1.5 cm), and after 48 hours the inhibition zone was reduced by almost half (to 0.8 cm).

From these results we can conclude that the cannabis isolate is slightly more antimicrobially active against microorganisms from the oral cavity of humans and canine than pure CBD.

4. Discussion and Conclusion

The results of this study indicate that the full-spectrum cannabis isolate demonstrated slightly greater antimicrobial activity against oral microflora of both human and canine origin compared to pure cannabidiol (CBD). This enhanced effect is likely attributable to the synergistic interaction between cannabinoids, terpenes, and other bioactive constituents present in the isolate, supporting the hypothesis that complex phytochemical mixtures may exert broader or more potent antimicrobial effects than isolated compounds. These findings suggest that full-spectrum hemp extracts could offer increased utility in oral antimicrobial applications relative to purified CBD alone. Future studies should investigate the antimicrobial efficacy of concentrations exceeding 100 μg/mL to determine dose-dependent effects. Additionally, expanding the sample size to include oral swabs from a larger and more diverse cohort of individuals would be essential to validate and generalize these preliminary findings.

There are several reasons why hemp isolate, which contains more than just pure CBD, may be more effective in fighting bacteria. Cannabis extracts contain a mixture of cannabinoids such as CBD, CBG and CBC, along with terpenes and other natural plant compounds (Farha et al., 2020). These components can work together to enhance the antibacterial effect, a phenomenon known as a synergistic effect. Some terpenes, such as pinene, limonene and caryophyllene, have natural antibacterial properties and can interact with cannabinoids to enhance their overall effect. However, the presence of these terpenes in an isolate depends on how it is processed, as some volatile compounds may be lost during isolation (Sionov

et al., 2022; Palmieri et al., 2021; Khan et al., 2014). In addition, certain minor cannabinoids such as cannabigerol (CBG) have shown a strong antibacterial effect, even against antibiotic-resistant bacteria such as MRSA, which is not the case with "pure" CBD (Farha et al., 2020). Some studies also suggest that full-spectrum cannabis extracts are more effective at breaking down bacterial cell walls (membranes) or disrupting quorum sensing; the way bacteria communicate – making them less resistant and enhancing the antibacterial effect (Sionov et al., 2022; Coelho et al., 2025; Aqawi et al., 2020).
Since microorganisms play a central role in oral health and disease in both humans and animals, finding ways to control harmful bacteria while supporting beneficial ones is important for improving dental health in all species.

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Conflicts of Interest: The authors declare no conflict of interest.

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Review

Characterization of the Cytotoxicity of Materials in Dental Medicine

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Abstract

The increasing use of synthetic and metallic materials in modern dentistry has raised growing concerns about their potential biological impact on oral and systemic health. Many of these materials can release toxic components into the oral environment due to incomplete polymerization, mechanical wear, or enzymatic degradation. Resin monomers such as TEGDMA, BisGMA, and EGDMA have shown dose-dependent cytotoxic effects, often through mechanisms involving oxidative stress and apoptosis. Similarly, metals such as nickel, mercury, cobalt, and components of titanium and zirconium implants can provoke allergic or systemic reactions and lead to cellular damage. Furthermore, impression materials and substances like eugenol also demonstrate cytotoxic effects. Cytotoxicity can vary based on the dosage, duration of exposure, and combination of materials used. This review is based on a systematic analysis of peer-reviewed literature from major biomedical databases, focusing on studies published between 2000 and 2024 that examined the cytotoxicity of dental materials in vitro and in vivo. It emphasizes the importance of standardized testing (ISO 10993, ISO 14971, ISO 7405) to ensure material safety and guide clinical decision-making in dentistry.

Keywords: Cytotoxicity; Dental materials; Resin monomers; Oxidative stress; Metal alloys; Biocompatibility

1. Introduction

Biocompatibility of dental materials is essential for ensuring clinical safety and long-term success in dental treatments. To be considered clinically safe, dental materials must exhibit high biocompatibility, which includes the absence of cytotoxic, pro-oxidative, inflammatory, and mutagenic effects, as well as the ability to support normal tissue repair without interfering with physiological processes. Cytotoxicity is defined as a specific aspect of toxicity that refers to a substances ability to damage or kill cells, often measured in vitro (Shahi et al., 2019). The aim of this review is to characterize the cytotoxicity of commonly used dental materials, such as resin composites, metal alloys, impression materials, cements, and others. Understanding the cytotoxic potential of these materials is essential for optimizing clinical choices and ensuring patient safety.

The first national system for reporting adverse effects of dental materials was established in Norway in 1993. Between 1993 and 1999, a total of 899 reports were received, of which 253 were referred for clinical evaluation. According to the study, amalgam was responsible for the highest number of adverse reactions, followed by metals and resin-based materials (Lygre et al., 2003).

2. Design of the Review

A systematic literature search was conducted using the PubMed and Web of Science databases, focusing predominantly on articles published between 2000 and 2024. These databases were selected due to their comprehensive indexing of peer-reviewed biomedical literature and extensive coverage of dental, toxicological, and clinical research. The search strategy included combinations of the following keywords: cytotoxicity, dental material, biocompatibility, resin monomers, metal alloys, impression materials, toxicity, in vitro, and in vivo. Inclusion criteria were: (1) clinically documented case reports describing adverse biological reactions during or after the use of dental materials, (2) studies that assessed the cytotoxic effects of dental materials, (3) both in vitro and in vivo studies involving human or animal models, and (4) peer-reviewed articles published in English.

3. Types of dental materials and associated cytotoxic risks

Resin-based dental materials, such as composite materials, bonding agents, resin-based cements, acrylic resins, sealants, are not inert in the oral environment and can release various components, initially due to incomplete polymerization and later as a result of mechanical wear and enzymatic degradation (Emmler et al., 2008; Wiertelak-Makala et al., 2024). These materials remain in prolonged and close contact with the oral tissues in multiple ways. This includes direct contact with dentin, enamel, or adjacent gingiva, as well as indirect contact with the dental pulp via dentin tubules or with soft tissues through saliva (Wiertelak-Makala et al., 2024). This prolonged exposure increases the likelihood of adverse biological responses. Based on a national survey of adverse reactions to dental materials conducted in the USA, resins were identified as the leading cause of adverse reactions among dental technicians (Scott et al., 2004). Some studies have shown that the most common resin monomers, EGDMA (ethylene glycol dimethacrylate), BisGMA (bisphenol A-glycidyl methacrylate), TEGDMA (triethylene glycol dimethacrylate) cause dose-dependent cytotoxicity. Among these, TEGDMA is considered the most cytotoxic, primarily due to its ability to induce oxidative stress, deplete intracellular glutathione (GSH), impair mitochondrial function, and activate apoptotic signaling pathways, such as caspase activation and MAPK cascade (Schweikl et al., 2005; Eckhardt et al.; 2009; Stanislawski et al., 2003). Considering that BisGMA is a derivative of bisphenola-A, which is known to be associated with developmental issues of the genital organs, immune system dysfunction, thyroid disturbances, and neurological development problems in children, its cytotoxicity is understandable (Jung et al., 2023). Moreover, several studies have demonstrated that these resins can interfere with hormonal signalling. It has also been demonstrated that resins can interact with hormone receptors and negatively affect human health by disrupting the normal endocrine function (McLachlan,1993). Therefore, adverse reactions of dental materials can be classified as either local or systemic. Local adverse reactions are assessed from two distinct aspects: mucosal toxicity and pulpal toxicity. Resin-based dental materials can cause numerous adverse effects on the oral mucosa, including epithelial proliferation, asthma, oral lichenoid reaction, pain, burning sensations, epithelial desquamation, contact allergies, etc.

(Marquardt et al., 2009; Syed et al., 2015). In more severe cases, systemic symptoms may also develop. There have also been reports of generalized neuropathy after 14 years of exposure to methacrylates (Sadoh et al., 1999). Possible effects of monomers on cells include apoptosis, loss of cell viability, oxidative stress, glutathione depletion, and reduced mitochondrial activity. It has been established that the cytotoxicity of a dental material can be influenced by several factors, including the material's dosage, duration of exposure, and the combination of materials used during treatment. For example, an in vitro study demonstrated that combining Durafill (microfilled composite resin) with Dycal (calcium hydroxide-based dental material) did not significantly alter cytotoxicity. In fact, the rate of cell death decreased to approximately 15% when Flow Line (flowable composite) was combined with Dycal. However, the combination of MTA (mineral trioxide aggregate) with Durafill increased the cytotoxicity to approximately 85% (Agnes et al., 2017). Special Clinic of Children's Dentistry in Sweden reported an isolated case in which adverse effects such as asthma and urticaria occurred following fissure sealing (Hallström, 1993)

Alloys are frequently used in dentistry due to their strength and durability. Among all metals, nickel is the most common sensitizer, known to cause immediate allergic reactions (Syed et al., 2015). The Co-Cr (cobalt-chromium) alloy also contains metals such as manganese, molybdenum, and nickel, which have been shown to exert cytotoxic effects on human growth factors and osteoblasts, primarily through increased production of reactive oxygen species (ROS) (Shahi et al., 2019). The first reported case of a metal allergy in dentistry was associated with amalgam fillings in the oral cavity. The toxicity of mercury was the main reason for the eventual replacement of amalgam. Allergic reactions may present as urticaria, rhinorrhea, and swelling, as well as erythematous, erosive, and atrophic changes or ulcerative lesions. In severe cases can lead to life-threatening conditions such as laryngeal edema, anaphylaxis, and cardiac arrhythmias (Karabucak et al.,2007; Ramnarayan et al., 2014). Mercury exposure has also been linked to oral lichenoid lesions and burning mouth syndrome (Syed et al.. 2015). In addition, mercury can enter the bloodstream after being absorbed from saliva and can cause toxicity in various organs. It is well known for its neurotoxic and nephrotoxic effects. It induces different signaling pathways leading to cell death, DNA damage and liver dysfunction (Shahi et al.,2019). After the placement of titanium dental implants, orthodontic appliances, or endoprostheses, 56 patients developed severe health problems, including muscle and joint pain (Müller et al., 2006). Titanium and zirconium implants can form an oxide layer upon exposure to oral fluids. The accumulation of titanium and zirconium ions in tissue, particularly in lymph nodes and lung tissue may occur as a result of corrosion, degradation of the oxide layer, and the subsequent release of ions into the oral cavity. The accumulation of titanium particles within lysosomes and macrophages has been reported to trigger hypersensitivity reactions (Chaturvedi et al., 2013; Mitchell et al., 1990).

Due to their small size and chemical properties, silver nanoparticles (AgNPs), which are gaining momentum in dentistry because of their antimicrobial and anti-inflammatory characteristics, have the potential to cross the blood-brain barrier. Once in the bloodstream, they can reach the central nervous system (CNS) and exert neurotoxic effects (Wang et al., 2023). Within the cell, AgNPs can generate reactive oxygen species (ROS), leading to oxidative stress that may damage celulular components and potentially cause inflammation, apoptosis, or necrosis (Rohde et al., 2021). Studies have shown that silver nanoparticles exert cytotoxic effects by modulating and disrupting key intracellular signaling pathways.

Dental anesthetics are divided into two basic chemical groups, esters or amides. Most sensitization reactions are attributed to ester anesthetics, as one of their degradation products is the antigenic agent p-aminobenzoic acid (Canfield et al., 1987). Eugenol, polyethers, and polysulfides, which are used in the impression-taking process in dental medicine, have also been shown to be cytotoxic. A retrospective report has also documented a case of fatal anaphylactic shock caused by an alginate impression (Gangemi et al., 2009). In pediatric dentistry, eugenol, used in combination with zinc oxide for root canal filling after pulpectomy, has been shown to cause toxicity. Its use has been associated with the induction of oxidative stress, disruption of cell membranes, and disturbance of cellular homeostasis (Roberts et al., 2014; Khan et al., 2011). Eugenol has also been reported to exhibit antiplatelet activity through the inhibition of the cyclooxygenase-2 (COX-2) enzyme in humans. It is widely

accepted that eugenol can induce cytotoxicity in pulp fibroblasts of deciduous teeth in a concentration-dependent manner (Escobar-García et al., 2016).

4. Evaluation of cytotoxicity

Before being approved for routine clinical use, dental materials must undergo a structured evaluation of their toxicity and biocompatibility. This evaluation includes in vitro testing, in vivo studies, and ultimately, clinical trials. These procedures are performed in accordance with international standards: ISO 10993 for biological evaluation of medical devices, ISO 14971 for risk management (Carden et al., 2021), and ISO 7405, which is specifically designed for the evaluation of dental materials (Murray et al., 2007). In vitro cytotoxicity tests represent the first step in the evaluation process. They are used to identify toxic effects, quantify the dose of released substances, and monitor the biological response of cells to those substances (Shahi et al., 2019). One commonly used method is the agar diffusion test, where the material is placed on an agar medium over a monolayer of cultured cells. The diffusion of toxic agents is observed through zones of decolorization or cell lysis (Murray et al. 2007). If a material passes in vitro testing, it proceeds to in vivo testing, typically conducted on animal models to assess both local and systemic reactions. These studies provide essential insights into tissue compatibility and systemic toxicity, though they are limited by ethical and legal concerns. Finally, clinical trials in human subjects are conducted to confirm safety and biocompatibility in real-life conditions. However, due to ethical constraints, these studies are limited in scope and are generally conducted only after extensive preclinical validation (Shahi et al., 2019).

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Review

Encapsulation of Essential Oils via Spray Drying: Recent Developments in Wall Materials, Emulsion Technologies, and Food Applications

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Abstract:
The encapsulation of essential oils represents a promising strategy to overcome their inherent volatility, chemical instability and limited water solubility, which limit their wider application in food and nutraceutical products. This review provides a comprehensive examination of recent advances in wall materials and emulsion technologies used for the microencapsulation of essential oils by spray drying. Traditional wall materials such as maltodextrin, gum arabic and whey protein isolates have been extensively studied; however, novel biopolymers have proven to be effective alternatives that allow for improved encapsulation efficiency and controlled release. Improved emulsion stability can be achieved by high pressure homogenisation and ultrasonic treatment. They have facilitated the production of stable emulsions with a uniform droplet size distribution, which is crucial for efficient encapsulation. Nanoemulsions and Pickering emulsions have great potential to produce very stable microcapsules with essential oils compared to conventional emulsions. Advances in spray drying technology, such as electrostatic spray drying and nanospray drying, have further improved the bioavailability of essential oils and optimised the release kinetics. The applications of spray-dried essential oils have shown significant potential for extending shelf life, reducing synthetic additives and improving flavour in various foods, as well as for the development of functional food supplements. Future research should focus on process optimisation, scalability and evaluation of release behaviour under real storage and consumption conditions.

Keywords: Essential oil, Spray drying, Encapsulation, Wall material, Emulsion

1. Introduction

Essential oils (EOs) represent a diverse group of secondary metabolites derived from various parts of aromatic plants (S. Sharma et al., 2021). These oils are characterized by their complex composition, including low molecular weight lipophilic compounds such as aliphatic hydrocarbons, phenyl propanoids, terpenoids, and phenolic constituents. Additionally, EOs contain a variety of oxygenated compounds like aldehydes, ketones, esters, oxides, and alcohols. Despite their strong lipophilic and volatile nature, which renders them almost insoluble in water, EOs are widely utilized for their antioxidant, anti-inflammatory and antimicrobial properties (Amorati et al., 2013; Chouhan et al., 2017; Miguel, 2010). However, due to their instability and lipophilicity, only a limited number of EOs are commercially viable, with approximately 300 types being used out of the 3000 known varieties (Dima & Dima, 2015).

EOs are volatile and heat-sensitive compounds that can degrade when exposed to light, air, and moisture. This susceptibility to oxidation and environmental factors limits their practical applications. To address these challenges, encapsulation is employed as a strategy to enhance the stability of EOs. This technique involves encasing the oils in a protective coating, which helps to maintain their integrity during handling, processing, and storage (Veiga et al., 2019).

Spray drying is indeed the most used encapsulation technique in the food industry. This method involves converting liquid mixtures into dry powders by rapidly drying them with a hot gas. It is highly effective for encapsulating active ingredients, including essential oils, as it helps to preserve their stability and extend their shelf life (Piñón-Balderrama et al., 2020).

This review focuses on exploring different wall materials, including novel and emerging options, as well as novel emulsion techniques used for encapsulating essential oils. It also discusses new spray drying technologies, recent scientific advancements, future perspectives, and how spray-dried essential oils are applied in the food industry.

2. Wall Materials for Spray Drying of Essential Oils

2.1. Selection Criteria for Wall Materials

The selection of suitable wall materials for the encapsulation of essential oils in the food industry is based on several decisive criteria. Firstly, the chemical compatibility between the wall material and the core substance is crucial to avoid undesirable interactions that could affect product stability. The wall material must provide effective protection of the active ingredient against environmental influences such as light, heat, oxygen and moisture, which can lead to degradation. Controlled release behaviour is also an important prerequisite to enable a targeted and sustained release of the encapsulated active ingredient under certain physiological conditions such as pH changes or enzymatic activity (Weisany et al., 2022).

Good solubility and dispersibility of the wall material are crucial to achieve homogeneous distribution of the active ingredient and to maintain emulsion stability during processing. The rheological properties of the material must support efficient microencapsulation, especially in spray drying, where viscosity and flow behaviour influence droplet formation and drying performance (Tavares et al., 2022). Sensory neutrality in terms of odour, taste and colour is important to ensure that the wall material does not alter the organoleptic properties of the final food product. Finally, the selected material should be food safe, comply with regulatory standards (e.g. GRAS status), be economically viable for industrial production and be both biodegradable and bioavailable to ensure sustainability and effective delivery of the encapsulated compound (Shishir et al., 2018).

2.2 Common and Novel Wall Materials

Various carrier materials are used for the encapsulation of essential oils (**Figure 1**), which are selected according to compatibility with the encapsulation methods, target applications and stability requirements. Natural polysaccharides — such as maltodextrin, gum arabic, chitosan, alginate and pectin — are commonly used due to their biodegradability, low toxicity and good thermal stability (Fernandes et al., 2014). Proteins such as whey protein, casein, gelatine and zein also serve as efficient carriers as they are biocompatible and can be released in a controlled manner, although they are pH and temperature dependent

(Chen et al., 2015; Laina et al., 2024). Lipid-based carriers, both solid and liquid offer high encapsulation efficiency and prolonged release profiles (Yammine et al., 2024).

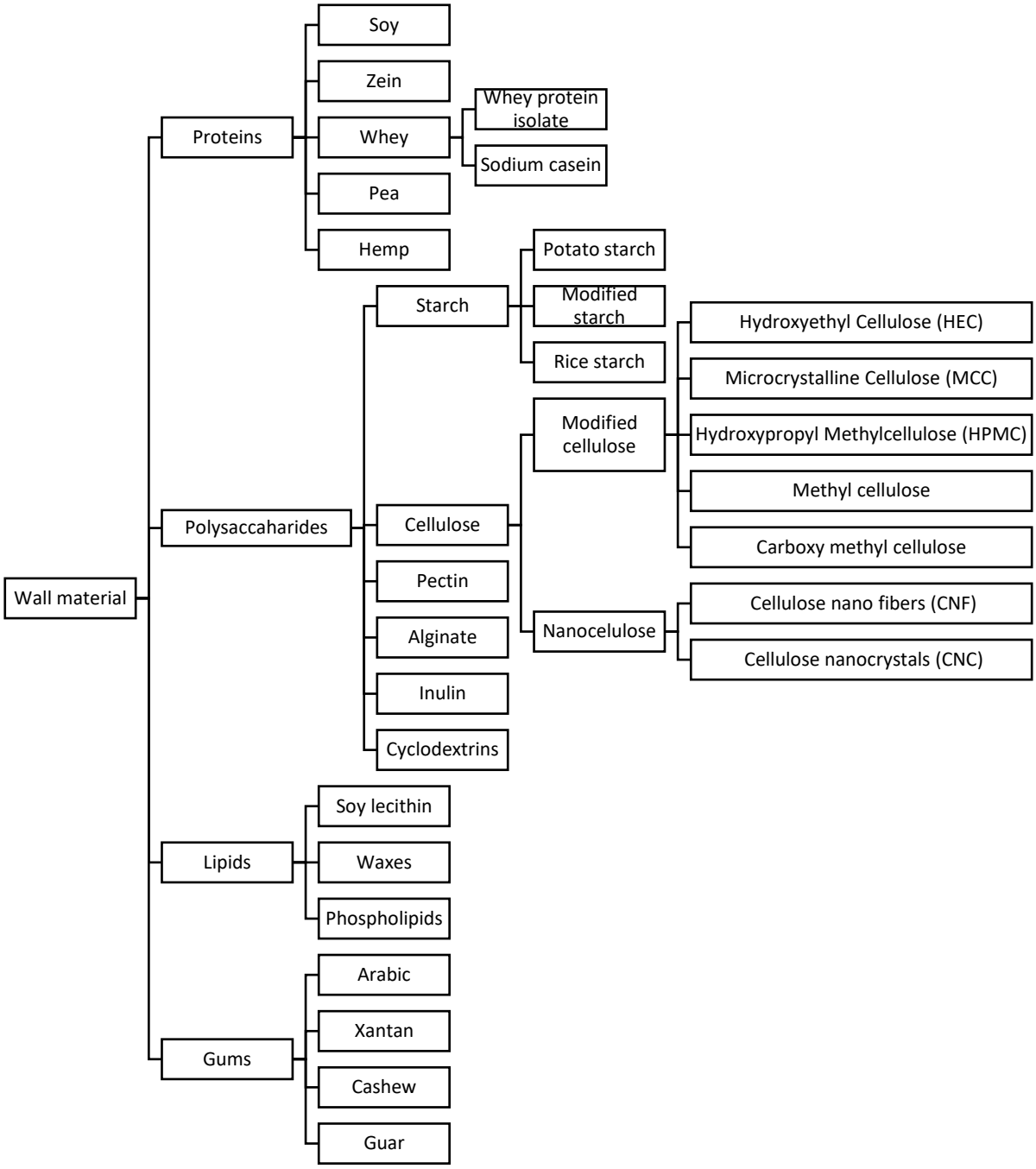

Figure 1. Wall materials used for encapsulation od essential oils

In recent years, novel wall materials have been investigated. The study by Kurek and Singh (2020) reports that among the various carrier materials investigated — combinations of maltodextrin with hemp, pea and rice proteins — the highest encapsulation efficiency was achieved with rice protein in combination with maltodextrin. This formulation outperformed the formulations with hemp and pea proteins, suggesting that rice protein maltodextrin is the most effective wall material for spray-dried encapsulation of hemp seed oil. Also, in another comparison study on spray-drying orange essential oil, it was concluded that formulations containing more than 50% chemically and thermo-mechanically modified rice starch as the primary wall material achieved the highest encapsulation efficiency

versus those using native rice starch, maltodextrin, or protein (Márquez-Gómez et al., 2018). Recently, novel material such as chachafruto flour have been explored for essential oil encapsulation due to their natural emulsifying properties (Daza et al., 2025). At concentrations of 3% and 4%, chachafruto flour significantly improved emulsion stability over 168 hours at room temperature, attributed to its starch and 20.5% protein content.
Overall, the choice of wall materials has a significant impact on the physicochemical properties, release mechanisms and functional performance of EO-loaded particles. Biocompatibility, biodegradability, regulatory status and economic feasibility are key criteria in optimising the selection of wall materials for food applications.

3. Emulsion Design for Improved Encapsulation

Emulsions are critical to the efficiency and stability of essential oil microparticles produced by encapsulation processes such as spray drying. Key physicochemical parameters—including droplet size, zeta potential, surface tension, rheological behaviour, and microstructure—are commonly evaluated to predict emulsion stability (Campelo et al., 2017). Maintaining emulsion stability from homogenization through to drying is essential to ensure the quality and performance of the resulting microcapsules, particularly in food and pharmaceutical applications.
High-speed homogenisation is generally used to produce coarse emulsions by mechanically reducing the droplet size. High pressure homogenisation (HPH) further refines the droplet size and improves the uniformity and stability of the emulsion. Another method for producing fine emulsions is ultrasonic treatment, which is particularly suitable for heat-sensitive essential oils due to its lower heat load (Lakshmayya et al., 2023). A study by Campelo et al. (2016) found that homogenisation followed by ultrasonic treatment was more efficient in emulsion formation than the homogenisation process alone.

3.1. Nanoemulsions & Pickering emulsions

Nanoemulsions and emulsions are colloidal dispersions consisting of two immiscible liquids and are often used in food systems. A key distinguishing feature is the droplet size, with emulsions having a droplet diameter of more than 200 nm, while nanoemulsions are characterised by droplets smaller than 200 nm (McClements, 2011). Nanoemulsions significantly enhance the properties of essential oils by increasing their stability, bioavailability and antimicrobial efficacy (K. Sharma et al., 2022). Due to their small droplet size, nanoemulsions provide a larger surface area that allows better interaction with microbial cell membranes, thereby improving their antimicrobial activity. They also protect essential oils from environmental factors such as temperature, light and oxygen that can degrade their active ingredients, ensuring their functionality over a longer period of time. In addition, nanoemulsions enable a more controlled and sustained release of essential oils, prolonging their antimicrobial effect in food. Compared to conventional emulsions, nanoemulsions are optically transparent and thermodynamically unstable, but their small droplet size provides greater stability against phase separation and creaming. In addition, nanoemulsions improve the dispersibility and solubility of essential oils in aqueous food systems, which is a challenge with conventional emulsions (Pathania et al., 2018). They also help to improve the sensory properties of foods by minimising the strong flavours and odours associated with essential oils due to better encapsulation and lower volatility.
Overall, nanoemulsions are superior to standard emulsions when it comes to effectively and efficiently utilising the bioactive properties of essential oils in food and pharmaceutical applications. There is still scepticism in the food industry about the use of nanoparticles, which requires a thorough investigation of their uptake, entry into the food chain and distribution under both in vitro and in vivo conditions (Maurya et al., 2021). Such comprehensive assessments are essential to ensure the safe and effective use of essential oil–based nanoemulsions in food systems.
Pickering emulsions are a class of emulsions stabilised by solid particles that irreversibly adsorb at the oil–water interface and represent an alternative to conventional surfactant-based systems (Ming et al., 2023). They enhance the stability of essential oils by preventing droplet coalescence through electrostatic and steric mechanisms, reducing droplet size and

are usually produced by high-energy processes such as homogenisation or ultrasonic treatment (Souza et al., 2021). The solid particles at the interface of Pickering emulsions improve the resistance of oil droplets to coalescence and degradation during high-temperature spray drying. This stabilization contributes to the formation of more uniform and structurally robust microparticles after drying. Moreover, Pickering-based systems offer better encapsulation efficiency and protection of sensitive bioactives, such as essential oils, compared to conventional emulsions (Meng et al., 2024). Therefore, integrating Pickering emulsions into spray-drying encapsulation strategies presents a promising approach for improving the stability and functionality of the final powdered products.

4. Emerging Spraying Techniques

Electrospraying, nano spray drying and electrostatic spray drying are emerging encapsulation and drying techniques that utilise electrical forces to process sensitive bioactive compounds such as essential oils (Jayaprakash et al., 2023). In electrospraying, a high voltage is applied to a liquid fed through a nozzle, creating an electric field that atomises the liquid into fine droplets. These droplets dry quickly on their way to a grounded collector, forming micro- or nano-sized particles with high encapsulation efficiency without the need for high temperatures. Oregano essential oil was encapsulated into thermally stable chitosan nanoparticles using the electrospraying technique, achieving high encapsulation efficiency and monodisperse particles (Yilmaz et al., 2019). Electrospraying significantly enhanced the antifungal and fungistatic activity of the essential oil against *Alternaria alternata*, suggesting electrospraying as a promising method for controlled-release antimicrobial applications in food industry.

Nano spray drying, on the other hand, uses a modified spray drying setup with a vibrating mesh and an electrostatic particle collector. Nano-sized particles (less than 100 nm) are produced by forcing the liquid through laser-drilled holes in a vibrating mesh, while an electrostatic collector captures the ultra-fine particles and ensures a narrow size distribution (Jafari et al., 2021). Plati et al. (2021) used a nano-spray drying technique to encapsulate oregano essential oil in whey protein isolate–maltodextrin matrices. The resulting nanoparticles showed significantly improved antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* compared to the pure essential oil, emphasising their promising potential for food preservation.

In electrostatic spray drying, a pre-charged nozzle is used to atomise the material into droplets and then an electrostatic field is applied in a drying chamber to aid drying and collection. The principle is to create an electrostatic force that repels the liquid surface, allowing the solvent to evaporate at lower temperatures and protecting heat-sensitive materials (Jayaprakash et al., 2023). Highly sensitive compounds such as essential oils can be effectively encapsulated using electrostatic spray drying, which allows particle formation at low temperatures under controlled voltage conditions. Although the application of this technique for the encapsulation of essential oils is still limited, it shows considerable potential for the preservation of thermolabile bioactives.

5. Applications in Food Industry

Encapsulated essential oils can be used in various areas of the food industry; first and foremost, they can be used as food preservatives due to their antioxidant and antimicrobial properties.

Thyme essential oil has been encapsulated in chia mucilage and used in meat sausage as a partial or total substitute for sodium nitrate and nitrite (A. S. Souza et al., 2025). TEO from the capsules inhibited the growth of coagulase-positive staphylococci, E. coli, mesophilic aerobic bacteria and Salmonella. In another study by Radünz et al. (2020), in which TEO was encapsulated, it was concluded that TEO can be used as a natural preservative in hamburger-like meat products. Rosemary essential oil was encapsulated using whey protein isolate and inulin as the encapsulation matrix, effectively extending the shelf life of Minas Frescal cheese (Fernandes et al., 2017).

Spray-dried essential oils also have great potential in the flavour industry, where they can be used as flavour enhancers. In a study by Mehran et al. (2020), mentha essential oil was

encapsulated in gum arabic and inulin. The results showed that SEO microcapsules can be used in various foods as flavourings and in chewing gum.

The essential oil of hops (*Humulus lupulus* L.) (HEO) has shown various applications in hot beverages (Su et al., 2023). HEO was encapsulated in modified starch and the controlled release from the capsules was determined. HEO microcapsules showed significant potential, highlighting the suitability of essential oil microcapsule formulations as promising flavouring agents for use in a range of hot beverage systems.

The dietary supplement sector is expanding rapidly and encapsulated essential oils have also proven to be functional ingredients in human nutrition. Spray-dried powder containing encapsulated oregano essential oil has been used to produce tablets (Partheniadis et al., 2019). Tablets with encapsulated essential oil successfully inhibited Gramme-negative and Gramme-positive bacteria and provided a controlled-release form of oral administration.

6. Conclusions

This review highlights the significant advances that have been made in the encapsulation of essential oils through the development of innovative wall materials and advanced spray drying techniques. Various biopolymers, including proteins, polysaccharides and lipids, have been investigated as encapsulation matrices to improve encapsulation efficiency, oxidative stability and controlled release properties. New wall materials such as chia mucilage, hemp, pea and rice proteins and chachafruto flour have shown promising results in improving the protective function of microcapsules. Novel emulsion production methods, including ultrasonic treatment and high-pressure homogenisation, have helped to produce more stable emulsions with uniform droplet size prior to spray drying.

Recent technological advances in spray drying, such as electrostatic spray drying and nanospray drying, have expanded the possibilities for producing microcapsules with improved structural and functional properties. These innovations have made it possible to better preserve the bioactivity of essential oils and to better control the release kinetics in food and dietary supplements. The integration of encapsulated essential oils into food systems has demonstrated the potential for natural preservation, flavour enhancement and the development of functional products. Overall, spray drying technologies hold promise for expanding the use of essential oils in the food industry while maintaining product safety, stability and sensory quality.

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Research

Maxwell-Heaviside Description of Curvature Waves: A Second Generation Model

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Abstract:

A model is presented to describe curvature waves in analogy with derivation of light waves in free space. Starting from Maxwell-Heaviside equations, the differential wave equations for electric and magnetic fields are derived, and their solution, i.e. sinusoidal depedence on space and time at precisely defined speed - determined by the permittivity and permeability of free space - is given. This formalism is then applied to the gravitational and kinetic fields subjected to the curvature of space. Massive bodies are described as curvature lumps - regions with given average space curvature distinguished from the background space curvature. Companion kinetic field is represented by the deviation of the angular frequency of the curvature lump from the baseline. The identity of the companion field was indicated from dimensional analysis.

Keywords: Gravitation; Curvature; Gravitational waves; Curvature waves; Speed of gravitational waves; Maxwell equations; Heaviside

1 Introduction

Following empirical investigations, gravitational, electromagnetic, the weak and strong nuclear interactions were outlined as fundamental physical interactions. In line with the assumption that physical laws are universal, a common origin of these interactions is being sought theoretically and experimentally, but an unified interaction theory has not yet been acknowledged (Weinberg, 1980). While experiments involving electromagnetic fields are accessible within the macroscopic scale, gravitational and nuclear interactions present challenges as regards experiments. The theory of general relativity indicates that gravitational interaction is connected to the curvature of space (Einstein, 1916). Following this idea, the analogy with derivation of the light waves from Maxwell-Heaviside equations (Heaviside, 1894) and the first generation model of gravitation described in (Kralj-Iglič, 2025), here we derive the equations describing the waves of the gravitational and kinetic fields. The velocity of the waves turns out to depend on two constants, the gravitational permittivity of space ε_G and the kinetic permeability of space μ_K. First we present the formalism with which the light waves are derived from the Maxwell-Heaviside equations and then we use this formalism for derivation of the "gravitokinetic" waves.

2 Maxwell - Heaviside model of electromagnetism

James Clark Maxwell constructed his theory based on the vector potential of the magnetic field $\mathbf{A}$ and scalar potential of the electric field ψ (Hunt, 2012). Oliver Heaviside re-expressed the Maxwell's theory in a more concise form based directly on the electric field $\mathbf{E}$ and magnetic field $\mathbf{H}$ (Heaviside, 1894; Hunt, 2012). Heaviside then used his energy-flow theorem and derived what he called the "second circuital law," which related the curl of $\mathbf{E}$ directly to the time derivative of a "fitting partner" in the Maxwell's first circuital law ($\mathbf{H}$), and the curl of $\mathbf{H}$ to $\mathbf{E}$ and its time derivative (Hunt, 2012). By combining these expressions with Maxwell's expressions for the divergence of the electric displacement $\mathbf{D}$ and the magnetic induction $\mathbf{B}$, Heaviside arrived at the compact set of four differential vector relations that are now known as Maxwell's equations. Heaviside published the energy-flow theorem and built his model of electromagnetism in a series of papers (Hunt, 2012). As the form of the equations was of importance to us, by nominating them as the Maxwell-Heaviside equations we indicate the Heaviside's important contribution to the simplicity and clarity of the formulation.

3 Theory

3.1 Description of light by electric and magnetic field

We take that the source of the field $\mathbf{D}$ is charge q,

$$\nabla \cdot \mathbf{D} = \varrho_{\mathrm{el}}, \tag{1}$$

where

$$\rho_{\mathrm{el}} = \frac{\mathrm{d}q}{\mathrm{d}V}, \tag{2}$$

and V is the volume. For convenience of scaling, we introduce a constant ε_0 and a field $\mathbf{E}$ so that

$$\mathbf{D} = \varepsilon_0 \mathbf{E}. \tag{3}$$

Proportionality constant $\varepsilon_0 = 8.9 \times 10^{-12}$ As/Vm is called the permittivity of free space. The magnetic field $\mathbf{B}$ has no monopole sources

$$\nabla \cdot \mathbf{B} = \mathbf{0} \tag{4}$$

with proportional field $\mathbf{H}$

$$\mathbf{B} = \mu_0 \mathbf{H} \tag{5}$$

where $\mu_0 = 4\pi \times 10^{-7}$ Vm/As is called permeability of free space.

The vortex equations are

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial \mathbf{t}}. \tag{6}$$

and

$$\nabla \times \mathbf{H} = \mathbf{j_{el}} + \frac{\partial \mathbf{D}}{\partial \mathbf{t}}, \tag{7}$$

where

$$\mathbf{j}_{\text{el}} = \frac{\mathrm{d}}{\mathrm{d}S} \left(\frac{\mathrm{d}q}{\mathrm{d}t}\right) \mathbf{I}_{\text{el}} \tag{8}$$

is the density of mass current, S is the cross section area and $\mathbf{I}_{\text{el}}$ is the unit vector in the direction of the current. The movement in straight lines can be included in considering rotation with respect to limiting small curvature.

Consider there are no charges and therefore no current so that

$$\nabla \cdot \mathbf{D} = 0. \tag{9}$$

and

$$\mathbf{j}_{\text{el}} = 0. \tag{10}$$

Applying a double vector product with vector nabla on the vortex equations, we obtain

$$\nabla \times \nabla \times \mathbf{H} = \nabla \times (-\frac{\partial \mathbf{D}}{\partial t}). \tag{11}$$

and

$$\nabla \times \nabla \times \mathbf{E} = \nabla \times (\frac{\partial \mathbf{B}}{\partial t}). \tag{12}$$

The double vector product of some vector $\mathbf{X}$ reads

$$\nabla \times \nabla \times \mathbf{X} = \nabla(\nabla \cdot \mathbf{X}) - \nabla^{\mathbf{2}}\mathbf{X} \tag{13}$$

so that with Eqs.(**??**) and (10)

$$\nabla \times \nabla \times \mathbf{H} = -\nabla^2 \mathbf{H}, \tag{14}$$

and

$$\nabla \times \nabla \times \mathbf{E} = -\nabla^2 \mathbf{E}. \tag{15}$$

Combining Eqs.(36) and (40), and (12) and (15) yields

$$-\nabla^2 \mathbf{H} = \nabla \times (-\frac{\partial \mathbf{D}}{\partial t}), \tag{16}$$

$$-\nabla^2 \mathbf{E} = \nabla \times (-\frac{\partial \mathbf{B}}{\partial t}). \tag{17}$$

Changing the consecutive order of operations in the right hand side of Eq.(18) and (29), and Eq.(42) and (29) yields

$$\nabla^2 \mathbf{H} = (\frac{\partial(\nabla \times \mathbf{D})}{\partial t}), \tag{18}$$

$$\nabla^2\mathbf{E} = (\frac{\partial(\nabla \times \mathbf{B})}{\partial t}). \tag{19}$$

Considering Eqs.(6) and (7), we get

$$\nabla^2\mathbf{H} = \varepsilon_0\mu_0\frac{\partial^2\mathbf{H}}{\partial t^2} \tag{20}$$

and

$$\nabla^2\mathbf{E} = \varepsilon_0\mu_0\frac{\partial^2\mathbf{E}}{\partial t^2} \tag{21}$$

with periodic solutions with respect to time and space

$$\mathbf{E} = \mathbf{E_0}\sin(\mathbf{k}\cdot\mathbf{r} - \omega\mathbf{t}) \tag{22}$$

and

$$\mathbf{H} = \mathbf{H_0}\sin(\mathbf{k}\cdot\mathbf{r} - \omega\mathbf{t} + \delta) \tag{23}$$

where $\mathbf{k}$ is the wave vector, $\mathbf{r}$ is the displacement, δ is the phase lag and ω is the angular frequency of the waves.

The velocity of the light waves c is given by

$$c = \sqrt{\frac{1}{\varepsilon_0\mu_0}} = 3\times10^8\text{m/s}. \tag{24}$$

3.2 Description of gravito-kinetic waves

Assuming that the space is isotropic, we take that the source of the field $\mathbf{G_C}$ is a lump of the space curvature,

$$\nabla\cdot(\mathbf{G}_C - \mathbf{G}_{C,0}) = \langle H\rangle, \tag{25}$$

where H is the mean curvature of the space

$$H = \frac{1}{2}(C_1 + C_2), \tag{26}$$

C_1 and C_2 are the two principal curvatures and $\mathbf{G}_{C,0}$ is the baseline curvature of the space. For convenience of scaling, we introduce a constant ε_G and, analogously with Maxwellian notation, call it gravitational permittivity of the space (Nyambuya, 2015)

$$\mathbf{G}_C = \varepsilon_G\mathbf{g}. \tag{27}$$

We introduce companion kinetic fields $\mathbf{K}$,

$$\nabla\cdot(\mathbf{K} - \mathbf{K}_0) = 0 \tag{28}$$

and κ

$$\mathbf{K} = \mu_\mathbf{K}\kappa \tag{29}$$

where μ_K is a proportionality parameter coupling the kinetic fields that we call kinetic permeability of the space and $\mathbf{K}_0$ is the baseline kinetic field.

The vortex equations are

$$\nabla\times(\mathbf{g} - \mathbf{g}_0) = -\frac{\partial(\mathbf{K} - \mathbf{K}_0)}{\partial t}. \tag{30}$$

and

$$\nabla\times(\kappa - \kappa_\mathbf{0}) = \mathbf{j}_C + \frac{\partial(\mathbf{G}_C - \mathbf{G}_{C,0})}{\partial t}, \tag{31}$$

where $\mathbf{j}$ is the density of the current of curvature lumps

$$\mathbf{j}_C = \frac{\mathrm{d}}{\mathrm{d}S}\left(\frac{\mathrm{d}\langle C\rangle}{\mathrm{d}t}\right)\mathbf{I}_C, \tag{32}$$

$\mathrm{d}S$ is cross section element, t is time and $\mathbf{I}_C$ is the unit vector in the direction of the current. Subtraction of the baseline parameters $\mathbf{G}_{C,0}$ and $\mathbf{K_0}$ denotes that the expected undulations will be about these values.

Consider that there are no curvature lumps and therefore

$$\nabla\cdot(\mathbf{G}_C - \mathbf{G}_{C,0}) = 0 \tag{33}$$

and

$$\mathbf{j} = 0. \tag{34}$$

Applying a double vector product with vector nabla, we obtain from Eq.(30),

$$\nabla\times\nabla\times(\mathbf{g}-\mathbf{g}_0) = \nabla\times\left(-\frac{\partial(\mathbf{K}-\mathbf{K}_0)}{\partial t}\right). \tag{35}$$

and

$$\nabla\times\nabla\times(\kappa-\kappa_0) = \nabla\times\left(-\frac{\partial(\mathbf{G}_C-\mathbf{G}_{C,0}}{\partial t}\right). \tag{36}$$

The double vector product reads,

$$\nabla\times\nabla\times(\mathbf{g}-\mathbf{g_0}) = \nabla(\nabla\cdot(\mathbf{g}-\mathbf{g}_0)) - \nabla^2(\mathbf{g}-\mathbf{g_0}) \tag{37}$$

and

$$\nabla\times\nabla\times(\kappa-\kappa_\mathbf{0}) = \nabla(\nabla\cdot(\kappa-\kappa_0)) - \nabla^2(\kappa-\kappa_0) \tag{38}$$

and so that with Eqs.(33) and (28)

$$\nabla\times\nabla\times(\mathbf{g}-\mathbf{g}_0) = -\nabla^2(\mathbf{g}-\mathbf{g}_0) \tag{39}$$

and

$$\nabla\times\nabla\times(\kappa-\kappa_0) = -\nabla^2(\kappa-\kappa_0) \tag{40}$$

Combining Eqs.(35) and (39), and Eqs.(36) and (40), respectively, yields

$$-\nabla^2(\mathbf{g}-\mathbf{g}_0) = \nabla\times(-\frac{\partial(\mathbf{K}-\mathbf{K}_0)}{\partial t}). \tag{41}$$

and

$$-\nabla^2(\kappa-\kappa_0) = \nabla\times(-\frac{\partial(\mathbf{G_C}-\mathbf{G_{C,0}})}{\partial t}). \tag{42}$$

Changing the consecutive order of operations in the right hand sides of Eqs.(41) and (42), and considering Eqs.(27) and (29), and Eqs.(30) and (31) yields

$$\nabla^2(\mathbf{g}-\mathbf{g}_0) = \varepsilon_G\mu_K\frac{\partial^2(\mathbf{g}-\mathbf{g}_0)}{\partial t^2} \tag{43}$$

and

$$\nabla^2(\kappa-\kappa_0) = \varepsilon_G\mu_K\frac{\partial^2(\kappa-\kappa_0)}{\partial t^2} \tag{44}$$

with periodic solutions in dependence of time and space

$$\mathbf{g} = \mathbf{g}_0 + \Delta\mathbf{g}\sin(\mathbf{k_G}\cdot\mathbf{r}-\omega t) \tag{45}$$

and

$$\kappa = \kappa_{\mathbf{0}} + \mathbf{\Delta}\kappa \sin(\mathbf{k_G} \cdot \mathbf{r} - \omega \mathbf{t} + \delta) \tag{46}$$

where $\Delta\mathbf{g}$ and $\Delta\kappa$ are the amplitudes, $\mathbf{k_G}$ is the wave vector, $\mathbf{r}$ is the displacement, δ is the phase lag and ω is the angular frequency of the waves. The velocity of the waves c_G is given by

$$c_G = \sqrt{\frac{1}{\varepsilon_G \mu_K}}. \tag{47}$$

4 Estimation of fields and constants

We made dimensional analysis of the model. The dimension of the curvature is 1/m. It follows from Eq.(25) that the field $\mathbf{G}$ is dimensionless. Further, it follows from Eq.(31) that the dimension of κ is m/s. We take that the kinetic field is angular frequency

$$\mathbf{K} = \Omega \tag{48}$$

with dimension 1/s. It follows from Eq.(30) that the dimension of $\mathbf{g}$ is m/s^2 representing gravitational acceleration. Using Eqs.(27) and (29) yields also the dimensions of the proportionality constants ε_G (s^2/m) and μ_K (1/m), respectively. The dimensions of the fields and constants are concisely given in Table 1. It can be verified by using Eq.(47) that the velocity of gravitational waves has the correct dimension m/s. It can be noted that the dimension of the mass (kg) is not involved in the model.

Table 1. Dimensions of the fields and constants.

Quantity	Dimension
$\mathbf{G}$	
$\mathbf{g}$	m/s^2
$\mathbf{K} = \Omega$	1/s
κ	m/s
ε_G	s^2/m
μ_K	1/m

To estimate the constant ε_G we consider the definition of gravitational field by the mass (Nyanbuya, 2015; Kralj-Iglič, 2025),

$$\nabla \cdot \mathbf{G} = \rho, \tag{49}$$

where

$$\rho = \frac{\mathrm{d}m}{\mathrm{d}V}, \tag{50}$$

m is mass and V is volume. The companion field was introduced as (Kralj-Iglič, 2025)

$$\mathbf{G} = \gamma \mathbf{\Gamma} \tag{51}$$

with

$$\gamma = \frac{1}{4\pi G}, \tag{52}$$

where G is the gravity constant $6.67{\cdot}10^{-11}$ Nm^2/kg^2 so that (Nyanbuya, 2015; Kralj-Iglič, 2025)

$$\gamma = 1.193 \times 10^9 \text{ kg s}^2/\text{m}^3 \tag{53}$$

To link the constants γ and ε_G, we connect the mass and the curvature lump,

$$m = \lambda_{Cm} \langle C \rangle V \tag{54}$$

where λ_{Cm} is an unit constant

$$\lambda_{Cm} = \mathrm{kg/m^2} \tag{55}$$

so that

$$\mathbf{g} = \lambda_{Cm}\mathbf{\Gamma} \tag{56}$$

and (Kralj-Iglič, 2025)

$$\varepsilon_G = \gamma/\lambda_{Cm} = 1.193 \cdot 10^9 \mathrm{s^2/m}. \tag{57}$$

While the gravitational permittivity ε_G could be estimated from the gravitational acceleration on the Earth, estimation of the constant μ_K would require the data on the effect of the movement of a massive flux on another massive flux or the measurement of the velocity of the gravitational waves. Instead we estimated μ_K from Eq.(47). Some results of the determination of the velocity of the gravitational waves (experimental and theoretical) and the estimated μ_K are shown in Table 2.

Table 2. Velocity of gravitational waves and the corresponding kinetic permeability of space. $c = 3 \times 10^8$ m/s.

Reference	Velocity	μ_K (1/m)
Van Flanders (1998)	2×10^{10} m/s	2.10×10^{-30}
Whitfield (2003)	c	9.31×10^{-27}
Fomalont and Kopeikin (2003)	$1.06\ c$	8.19×10^{-27}
Kopeikin and Fomalont (2006)	c	9.31×10^{-27}
Luo et al. (2013)	$\leq 1.3 \times 10^{14}$ m/s	49.3×10^{-27}
Moffat (2014)	$> c$	$>9.31 \times 10^{-27}$
Nyanbuya (2015)	c	9.31×10^{-27}
Cornish et al. (2017)	(0.55 - 1.42) c	(30.4 - 4.45) $\times 10^{-27}$
Liu et al. (2020)	(0.97 - 1.01) c	(9.78 - 9.02) $\times 10^{-27}$
de Rham and Tolley (2020)	$> c$	$<9.31 \times 10^{-27}$
Ito (2023)	$> c$	$<9.31 \times 10^{-27}$
Dai and Stojkovic (2024)	$>> c$	$<<9.2 \times 10^{-27}$
Delgado et al. (2025)	$> c$	$<9.31 \times 10^{-27}$

5 Discussion and Conclusions

We considered gravitation due to the curvature lumps of the space affected by the movement. Following the elegant Maxwell-Heaviside equations originally derived for the electric and magnetic fields, we have stated the gravitational field as a source of a space lump with notably higher average mean curvature than the background. We have introduced a companion kinetic field. Dimensional analysis showed that the unit of this field is 1/s and indicated that the field could be the angular frequency. This seems reasonable as rotational motion produces acceleration that is at the essence of the gravitational effect. We have derived the wave equation and expressed the velocity of the gravitational waves by two parameters: gravitational permittivity and kinetic permeability. We have estimated gravitational permittivity from the Earth's gravitational acceleration. We have estimated the kinetic permeability from gravitational permittivity and velocity of gravitational waves reported in the literature. While some authors claim that the velocity of the gravitational waves equals that of the light, some experimental and theoretical works report considerably different values (Table 2).

We have made a step forward from the 1.st generation model (Kralj-Iglič, 2025) to formulate the equations only with the quantities having units of length and time thereby indicating that the companion field is kinetic. Mass is not explicitly included in the model which is in the spirit of Einstein's notion that the gravitational waves are the movements of the coordinate system (Einstein, 1916). Analogy with Maxwell-Heaviside equations for the gravitational field has been previously proposed by Nyanbuya (2015). The companion field was nominated the gravitomagnetic field and the waves were nominated gravitomagnetic waves. However, in contrast with our model, Nyanbuya (2015) included in his description the mass m and introduced with further development of the theory the Lorenz-like equation including the scalar and vector gravitomagnetic potentials. Our presentation of the Maxwell-Heaviside equations is essentially equivalent to the initial presentation of Nyanbuya (2015), specifically regarding the gravitational field, but deviates in identification of the companion field. Nyanbuya's elaboration of the companion field is based on the formalism of the general theory of relativity, however it retains mass as one of the parameters. Our elaboration of the companion field based on dimensional analysis is simple but includes a bold step in eliminating mass from the formulation of the gravitational field. Instead of a black box parameter - mass - we indicate the origin of the increased density of the substance, i.e. the curvature effects. In this regard, packing of highly curved space formations is associated with greater mass. For simplicity, we have introduced the packing of space with an average value of the curvature, however, the dimension of the parameter $\langle H \rangle$ (i.e. 1/m) was sufficient to eliminate mass and identify as a possible companion field the angular frequency $\mathbf{K} = \Omega$.

The attempts to better understand the origin of the gravitational effects include some modern theories proposing that they derive from entropy (Verlinde, 2011) or torsion of the space-time (Aldrovandi and Pereira, 2013), or thermodynamic effects (Padmanbhan, 2010). In theories involving energy flux or fields (non-mass sources), gravitational effects were described without explicitly emphasizing mass as the sole source, instead focusing on energy -momentum distributions, which can include non-mass energy forms; frame - dragging and the Lense - Thirring effect (Lense and Thirring, 1918) was introduced as the relation of the angular momentum/rotation (which relates to angular frequency) and space-time topology and curvature. Lense and Thirring (1918) formulated the weak-field and slow - motion description of the effect of frame dragging of inertial frames around rotating masses on the orbit of a particle around the spinning body. However, to our best knowledge there are no conclusive results yet on relevant experimental evidences.

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7 Conflicts of Interest

The authors disclose no conflict of interest.

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Research

Transformative Educational Strategies with Artificial Intelligence: Redefining Access to Information and the Evolving Role of Educators

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Abstract:

Artificial Intelligence (AI) is fundamentally transforming educational paradigms through unprecedented personalization of learning experiences, adaptive assessment methodologies, and administrative automation. This comprehensive analysis examines AI as a disruptive social process that revolutionizes how humans interact with information while simultaneously redefining educators' roles within educational contexts. The evidence demonstrates that sophisticated AI frameworks (comprising input processing layers, inference engines, and dynamic content delivery mechanisms) create individualized learning pathways that enhance student engagement and achievement while addressing longstanding educational challenges. As AI systems increasingly assume routine instructional and administrative tasks, educators are evolving from knowledge transmitters to learning facilitators, guides, and designers of AI-enhanced experiences. This transformation requires careful navigation of ethical considerations including equity, privacy, algorithmic bias, and cultural relevance, alongside significant policy adaptations and institutional restructuring. This approach concludes that effective AI integration depends not merely on technological capabilities but on human choices that prioritize educational values of human flourishing, social equity, and critical thinking development

Keywords: Artificial intelligence in education; Transformative learning; Personalized learning; Educator roles; Educational equity

1. AI as a disruptive social process in education

The integration of Artificial Intelligence into educational systems constitutes a disruptive social process that transcends mere technological innovation to fundamentally reconfigure educational paradigms, methodologies, and institutional structures. To conceptualize this disruption, it is essential to examine AI not simply as a tool but as a socio-technical system that transforms existing educational frameworks and practices.

The theoretical understanding of AI as a disruptive force in education draws from complementary perspectives including disruptive innovation theory, sociocultural learning theories, and critical theoretical frameworks. Through these lenses, AI emerges as a transformative agent that initially addresses underserved educational needs before gradually reshaping mainstream educational practices. This process reconfigures not only technological infrastructures but also social relationships, knowledge construction processes, and power dynamics within educational contexts.

Recent research in modern education indicates that AI is fundamentally transforming educational environments through personalized learning experiences, adaptive assessment methodologies, and administrative automation (Ahamed, 2025). The integration of sophisticated AI technologies across diverse educational contexts has demonstrated remarkable potential to address longstanding challenges in teaching and learning through continuous behavioral analysis, cognitive mapping, and dynamic curriculum generation (Ahamed, 2025). These capabilities enable the creation of individualized learning pathways that significantly enhance student engagement and achievement outcomes (Ahamed, 2025).

The multi-layered technical frameworks of these systems (comprising input processing layers, inference engines, and content delivery mechanisms) enable precise identification of knowledge gaps and misconceptions, facilitating targeted interventions when they matter most in the learning process (Ahamed, 2025). Meanwhile, predictive analytics provide early warning of academic difficulties, allowing for proactive support strategies that meaningfully improve retention and success rates for diverse student populations (Ahamed, 2025).

This theoretical framework conceptualizes AI in education as a disruptive social process characterized by transformation of information accessibility, reconfiguration of educational roles and relationships, restructuring of institutional practices, and emergence of new educational possibilities and ethical considerations. This conceptualization guides subsequent analysis of how AI is reshaping information management, transforming educator roles, and enabling novel educational strategies.

2. Historical context and evolution

The emergence of AI as a transformative force in education follows a trajectory of technological developments that have progressively expanded educational possibilities. From early computer-assisted instruction to contemporary machine learning applications, educational technologies have evolved from simple programmed instruction to sophisticated adaptive systems that respond to individual learner characteristics. The current wave of AI educational applications represents a qualitative shift from previous technologies through capabilities for natural language processing, pattern recognition, and adaptive personalization that more closely approximate human cognitive processes. This evolution has accelerated in recent years as advances in machine learning, particularly deep learning approaches, have enhanced AI's ability to recognize patterns in complex data and generate increasingly sophisticated responses.

Another line of research in education emphasizes that recent advances in artificial intelligence technologies, particularly machine learning and deep learning, have shown great potential in predicting academic achievement, improving teaching strategies, and supporting decision-making within educational institutions (Esakkiammal & Kasturi, 2024). These technological developments have coincided with increased recognition of the limitations of standardized, one-size-fits-all educational approaches, creating fertile ground for AI integration that promises more personalized and adaptive learning experiences.

3. AI's impact on information access and management, democratization and personalization

The integration of AI technologies into educational contexts has fundamentally transformed how information is accessed, processed, organized, and utilized by both learners and educators. This transformation extends beyond merely increasing available information; it represents a qualitative shift in human-information relationships with profound implications for teaching and learning processes.

AI has significantly democratized access to educational information through sophisticated search algorithms, recommendation systems, and translation tools that transcend linguistic, geographic, and cultural boundaries. Simultaneously, AI enables unprecedented personalization of information delivery through systems that adapt content presentation based on individual learning profiles, preferences, and needs.

Research demonstrates that through continuous behavioral analysis, cognitive mapping, and dynamic curriculum generation, AI systems create individualized learning pathways that significantly enhance student engagement and achievement (Ahamed, 2025). These technologies identify knowledge gaps and misconceptions, facilitating targeted interventions that provide relevant information at optimal moments in the learning process (Ahamed, 2025). This capability transforms traditional information access from a standardized, one-size-fits-all approach to a dynamic, responsive system attuned to individual learning trajectories.

4. Transformation of information processing and knowledge construction

Beyond access, AI is transforming how information is processed and converted into knowledge. Natural language processing and machine learning algorithms analyze, synthesize, and generate content at scales and speeds previously unimaginable. In educational contexts, these capabilities enable automated summarization of complex materials, generation of customized learning resources, real-time analysis of student work with formative feedback, and sophisticated cross-referencing of concepts across disciplines.

The multi-layered technical frameworks of AI systems (comprising input processing layers, inference engines, and content delivery mechanisms) enable sophisticated information processing that supports knowledge construction in novel ways (Ahamed, 2025). These systems identify patterns, relationships, and contradictions within vast information repositories, potentially enhancing critical thinking and metacognitive awareness among learners who engage with AI-mediated information.

5. Challenges in information evaluation and critical literacy

While AI expands information access and processing capabilities, it simultaneously creates new challenges related to information evaluation, verification, and critical literacy. The proliferation of AI-generated content necessitates enhanced skills in distinguishing reliable from unreliable information sources, recognizing bias in algorithmic recommendations, and critically evaluating AI-produced analyses.

Some studies emphasize the necessity for AI literacy, prompt engineering proficiency, and enhanced critical thinking skills in educational settings (Walter, 2024). As AI becomes increasingly integrated into information ecosystems, educators must facilitate development of these meta-cognitive capabilities alongside traditional subject-matter expertise. This necessity highlights the evolving nature of information literacy in AI-enhanced educational environments.

6. The evolving role of educators in AI-enhanced learning environments. from knowledge transmitters to learning facilitators

The integration of AI technologies into educational contexts is catalyzing a profound transformation in the role of educators. As AI systems increasingly assume routine tasks related to information delivery, assessment, and administrative functions, educators are navigating a significant reconfiguration of their professional identity and practice.

Traditionally, educators have been positioned as primary sources and transmitters of knowledge, delivering standardized content to groups of learners. The emergence of AI as an information provider and content generator necessitates a shift toward roles focused on

facilitating learning processes rather than primarily delivering content. This transition reflects a broader epistemological shift from knowledge as a product to be transferred to knowledge as a process to be facilitated.

Research indicates that AI enables personalized educational materials, virtual consultations, and virtual reality simulations, improving student understanding and experience (Thorat et al., 2024). In this context, educators become designers of learning experiences who strategically integrate AI tools to support differentiated learning pathways while providing the human guidance, motivation, and context that remain essential to effective education.

7. Augmentation rather than replacement, new competencies and professional development

Despite concerns about AI replacing educators, evidence suggests that the most effective educational models involve human-AI collaboration rather than substitution. The integration of AI technologies requires a nuanced understanding of how human and artificial intelligences can complement each other, with each contributing distinct capabilities to the educational process.

Studies indicate that AI can track student progress, analyze feedback, streamline administrative processes, and provide ongoing support, enhancing educational outcomes (Thorat et al., 2024). However, the same research emphasizes that ethical, regulatory, and equity considerations require attention for responsible AI deployment (Thorat et al., 2024). This suggests that educators play crucial roles in ensuring ethical implementation, contextualizing AI-provided information, cultivating critical thinking, and providing socio-emotional support that remains beyond AI capabilities.

The evolving educational landscape requires educators to develop new competencies beyond traditional pedagogical expertise. These include AI literacy and technological fluency, data interpretation and learning analytics, design thinking for AI-enhanced learning environments, ethical reasoning regarding AI implementation, and facilitation of human-AI collaboration.

Some research show the necessity for AI literacy, prompt engineering proficiency, and enhanced critical thinking skills among educators (Walter, 2024). This necessitates comprehensive educator training and curriculum adaptation to align with evolving societal structures (Walter, 2024). The development of these competencies requires systematic professional development opportunities and revision of teacher education programs to incorporate AI-related knowledge and skills.

8. Transformative educational strategies with AI and personalized learning at scale

The integration of AI into educational contexts enables transformative strategies that reconfigure traditional approaches to teaching and learning. These strategies leverage AI capabilities to create more personalized, adaptive, and effective educational experiences while addressing longstanding challenges in educational practice.

AI technologies enable unprecedented personalization of educational experiences by adapting content, pace, and pedagogical approaches to individual learner characteristics, preferences, and needs. Unlike previous efforts at individualization that were limited by human capacity constraints, AI systems can implement personalization at scale across large and diverse student populations.

Research indicates that AI has the potential to offer personalized educational materials that improve student understanding and experience (Thorat et al., 2024). Through continuous behavioral analysis, cognitive mapping, and dynamic curriculum generation, AI systems create individualized learning pathways that significantly enhance student engagement and achievement (Ahamed, 2025). These systems adapt not only content presentation but also learning activities, feedback mechanisms, and assessment approaches based on real-time analysis of student performance and engagement.

The personalization capabilities of AI address the fundamental educational challenge of meeting diverse learner needs within standardized systems. By tailoring educational experiences to individual learning profiles, AI-enhanced personalization has the potential to improve learning outcomes while increasing educational equity and inclusion.

9. Adaptive assessment and intelligent tutoring, feedback and support systems

Traditional assessment approaches often provide limited information at infrequent intervals, constraining their utility for informing instructional decisions and supporting learning. AI-enhanced assessment systems enable more continuous, formative, and adaptive approaches that provide actionable insights for both educators and learners.

Studies show that AI can improve data analysis accuracy and risk assessment, providing deeper operational insights into educational processes (Sari et al., 2024). AI-driven approaches, leveraging machine learning, natural language processing, and advanced analytics, offer pathways to overcome limitations in adaptability, scalability, and the ability to provide personalized feedback (Mahamuni et al., 2024). These technologies enable automated grading, adaptive testing, and real-time feedback mechanisms that significantly enhance assessment practices (Mahamuni et al., 2024).

AI-enhanced tutoring systems provide personalized guidance and support that complement human teaching. These systems deliver targeted instruction, practice opportunities, and feedback in response to individual learning needs, creating supplementary learning pathways that extend beyond traditional classroom interactions. Research documents the effectiveness of autonomous tutoring systems, personalized education channels, and data-based analysis in supporting personalized learning (Kara and Sevim., 2013). AI technologies can track student progress, analyze feedback, streamline administrative processes, and provide ongoing support, enhancing educational outcomes (Thorat et al., 2024). These intelligent tutoring systems are particularly valuable for providing additional practice opportunities, reinforcing foundational skills, and supporting learners who may benefit from alternative explanations or approaches.

While not replacing human educators, intelligent tutoring systems extend educational support beyond the constraints of classroom time and teacher availability. This extension is particularly valuable for learners who require additional assistance or who benefit from learning opportunities outside traditional educational schedules and settings.

10. Challenges, Ethical Considerations, Equity and Access Challenges

While AI offers transformative potential for education, its integration raises significant challenges and ethical considerations that must be addressed to ensure responsible and equitable implementation. These concerns extend beyond technical issues to encompass social, cultural, ethical, and institutional dimensions of AI in educational contexts.

The integration of AI into education presents risks of exacerbating existing educational inequalities if access to AI-enhanced learning experiences is unevenly distributed. Digital divides related to technological infrastructure, internet connectivity, device availability, and digital literacy can create disparities in who benefits from AI in education.

Research identifies significant limitations in AI educational implementation, including poor contextual adaptability of AI models and insufficient integration of emerging technologies (Esakkiammal & Kasturi, 2024). These limitations are often more pronounced in resource-constrained educational environments, potentially widening gaps between well-resourced and under-resourced educational settings.

Addressing equity concerns requires intentional efforts to ensure that AI implementation in education prioritizes underserved populations and contexts. This includes developing AI applications that function effectively with limited technological infrastructure, creating multilingual AI systems that serve linguistically diverse populations, and designing AI tools that accommodate diverse learning needs and cultural contexts.

AI-enhanced education typically involves the collection, analysis, and storage of substantial learner data, raising important questions about privacy, security, and data ethics. The use of sensitive information about learning patterns, behaviors, and challenges necessitates robust frameworks for protecting learner privacy while enabling beneficial educational applications.

Studies emphasize concerns about privacy and regulatory compliance as persistent challenges in AI educational implementation (Thorat et al., 2024). Issues related to data collection consent, data ownership, appropriate data use, and protection against security breaches require careful consideration in AI educational applications. Additionally, questions about the long-term implications of creating comprehensive digital learning

profiles that may follow learners throughout their educational and professional trajectories warrant serious ethical reflection.

11. Algorithmic bias and fairness

AI systems reflect the data used in their development and the assumptions embedded in their design, potentially perpetuating or amplifying biases related to race, gender, socioeconomic status, language, and other characteristics. These biases can affect various aspects of AI-enhanced education, including content recommendations, performance assessments, and predictive analytics.

Research highlights algorithmic bias as a significant ethical concern in AI educational applications, alongside issues of overreliance and accuracy (Thorat et al., 2024). Biased algorithms can adversely impact educational experiences and outcomes for marginalized groups, potentially reinforcing rather than challenging existing inequities in educational systems.

Addressing algorithmic bias requires diverse development teams, representative training data, regular bias audits, and transparent explanation of algorithmic decision processes. Additionally, educational institutions must maintain human oversight of AI systems and establish mechanisms for challenging or correcting algorithmic decisions that may disadvantage particular learner groups.

12. Implications for educational policy and practice, curriculum and pedagogical reform

The transformative impact of AI on education asks for significant reconsideration of educational policies and practices at multiple levels. From classroom-level instructional approaches to national educational policies, the integration of AI requires thoughtful adaptation to harness benefits while addressing challenges.

The emergence of AI necessitates fundamental reconsideration of what is taught and how teaching occurs. Curricula must evolve to incorporate AI literacy, emphasize uniquely human capabilities, and prepare learners for collaboration with intelligent technologies.

Some research indicates that AI literacy, prompt engineering proficiency, and enhanced critical thinking skills are essential components of contemporary education (Walter, 2024). Educational curricula should systematically develop these capabilities while also emphasizing creativity, ethical reasoning, interpersonal skills, and other distinctively human attributes that complement rather than compete with AI capabilities.

Pedagogical approaches must likewise evolve to effectively integrate AI tools while preserving essential human elements of education. This involves designing learning experiences that strategically combine AI-enhanced personalization with social learning, teacher guidance, and opportunities for creative expression. Effective pedagogy in AI-enhanced environments requires thoughtful decisions about when to employ AI tools and when to prioritize human interaction and guidance.

13. Professional Development and Teacher Education

The changing educational landscape requires comprehensive approaches to professional development for practicing educators and reformation of teacher education programs to prepare future teachers for AI-enhanced learning environments.

Studies emphasize the need for comprehensive educator training to align with evolving societal structures influenced by AI (Walter, 2024). Professional development should focus not only on technical skills related to AI tool utilization but also on pedagogical strategies for AI integration, ethical frameworks for AI implementation decisions, and approaches to developing student AI literacy and critical thinking.

Teacher education programs must be reformed to incorporate knowledge about AI capabilities and limitations, skills for designing AI-enhanced learning experiences, and ethical frameworks for navigating AI implementation decisions. This preparation should position future educators as informed, critical users of AI technologies who can effectively guide learners in AI-enhanced environments while advocating for responsible AI development and implementation.

14. Institutional structures and processes

Educational institutions must adapt organizational structures and processes to effectively integrate AI technologies. This includes developing new roles and departments, establishing governance frameworks, and creating collaborative spaces for AI implementation planning.

Research indicates that AI can streamline administrative processes and provide deeper operational insights, enhancing organizational effectiveness (Sari et al., 2024). However, realizing these benefits requires intentional redesign of institutional structures and processes rather than simply layering AI onto existing organizational arrangements.

Effective institutional adaptation involves creating clear governance structures for AI initiatives, establishing data management frameworks that balance innovation with privacy protection, and developing collaborative teams that bring together educational, technical, and ethical expertise. Additionally, institutions must establish ongoing evaluation processes to assess AI impact and inform continuous improvement of implementation approaches.

15. Conclusion

The integration of AI into educational contexts represents a transformative social process that is fundamentally reshaping how humans access and manage information while simultaneously redefining the role of educators. This transformation extends beyond mere technological adoption to encompass profound shifts in educational philosophy, practice, and institutional structures.

The evidence examined demonstrates that AI is enabling unprecedented personalization of learning experiences through continuous behavioral analysis, cognitive mapping, and dynamic curriculum generation (Ahamed, 2025). These capabilities create individualized learning pathways that enhance student engagement and achievement while addressing longstanding challenges in teaching and learning (Ahamed, 2025). Simultaneously, AI is transforming information accessibility through sophisticated input processing, inference engines, and content delivery mechanisms that facilitate targeted interventions and support (Ahamed, 2025).

As AI assumes routine tasks related to information delivery, assessment, and administrative functions, the educator's role necessarily evolves from primary knowledge transmitter to learning facilitator, guide, and designer of AI-enhanced experiences. This evolution requires educators to develop new competencies including AI literacy, prompt engineering proficiency, and enhanced approaches to developing critical thinking (Walter, 2024). Far from diminishing the importance of educators, AI integration highlights the essential human elements of education: relationship building, ethical guidance, creative inspiration, and culturally responsive teaching.

The transformative educational strategies enabled by AI-including personalized learning at scale, adaptive assessment systems, intelligent tutoring, enhanced collaboration, and immersive learning environments-offer promising approaches to longstanding educational challenges. However, their effective implementation requires careful navigation of significant ethical considerations related to equity, privacy, algorithmic bias, learner autonomy, and cultural relevance.

Looking forward, educational systems must adapt policies and practices to effectively integrate AI while preserving and enhancing educational values. This adaptation involves curriculum reform, professional development, institutional restructuring, equity-focused policies, appropriate regulatory frameworks, and strategic research priorities. Through thoughtful navigation of these complex changes, educational systems can harness AI's transformative potential while ensuring that technology serves human flourishing rather than narrowly defined efficiency or performance metrics.

The path forward requires ongoing dialogue among diverse stakeholders, commitment to inclusive and equitable implementation approaches, and willingness to continuously assess, adapt and refine practices in response to emerging insights and challenges in education.

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Research

Experimental Scientific Values in Inclusive Education as a Challenge for Teachers and Academic Researchers in the Sustainable Education Process

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Abstract:
This article explores children's natural curiosity about science and the factors that encourage their interest, drawing on the educational philosophy of Maria Montessori. It emphasizes the importance of hands-on, inquiry-based learning to foster children's interest in science. Special attention is given to children with special needs, explaining legal definitions and educational adaptations required for inclusive learning. The discussion emphasizes the need for equitable, stimulating science education that is accessible to all learners. The main objective is to present an initiative that introduces science to children with special needs through hands-on experiments in a model elementary school. Around 150 pupils from various adapted and special education programs participated in engaging, sensory-rich activities led by researchers from the Jožef Stefan Institute. The activity highlights the importance of integrating scientific values into inclusive education to improve learning equity and sustainability. It emphasizes the need for collaboration between educators and researchers in creating accessible and meaningful science experiences for all pupils.

Keywords: Inclusive education, Science, Experimental work, Natural sciences, Sustainable education process, Collaboration

1. Introduction

1.1. Children and science – Are children interested in science and what motivates them to be more interested in scientific topics?

According to Maria Montessori's theories, children are believed to have a natural interest in science (Montessori, 1995). Child's curiosity should be nurtured through hands-on, exploratory learning. Her observation was that children are instinctively drawn to understanding the world around them and science provides a way to satisfy this drive by engaging their senses. In Maria Montessori' s theories, children begin to take an interest in science during their school years. At this time, they begin to research tirelessly and ask questions because they want to know and understand something. This is the time when the abstract part of the child's mind is organised. During this time, children begin to ask questions that are often philosophical in nature (*e. g.* Why are leaves green? Why does the sun set and the moon rise in the evening? etc.) because their curious minds want to know and understand something (Montessori, 1995).

1.2. Who are children with special needs?

Children with special needs are defined in the *Placement of Children with Special Needs Act* (ZUOPP-1, 2011; ***Law 1***) as children with intellectual disabilities, blind and visually impaired children or children with visual impairments, deaf and hard of hearing children, children with speech and language disorders, physically disabled children, children with long-term illnesses, children with learning disabilities in certain areas, children with autistic disorders and children with emotional and behavioural disorders (***Law 1***).

Children who have been identified as having special needs require adapted implementation of education and training programmes with additional professional support or adapted education and training programmes or special education and training programmes. These children require adapted spaces and resources that meet the guidelines for adapted programme implementation (***Law 1***). The educational programme can also be adapted to enable children with special needs to acquire an equivalent standard of education by adapting the curriculum, organisation, methods of knowledge assessment and evaluation, methods of external knowledge assessment, progression and timetable of lessons (***Law 1***).

2. Science experiments in Roje Primary School

We wanted to bring science closer to children with special needs. As part of a technical day, we demonstrated some experiments to the pupils of Roje Primary School. The experiments were attended by children who attend the development department of the school's kindergarten, pupils who attend an adapted education and training programme and pupils who attend a special education and training programme (Roje Primary School, n. d.). Approximately 150 pupils attended the presentation: 6 from the school's kindergarten development department, 90 pupils attending an adapted education and training programme and 54 pupils attending a special education and training programme. Researchers from the Jožef Stefan Institute led an educational discussion while conducting scientific experiments. All experiments were designed so that pupils could participate, gain hands-on experience and relate the experiments to real-life situations (Jeran et al., 2024).

The session began with a stimulating discussion about air and its components (National Geographic, n. d.; National Oceanic and Atmospheric Administration, n. d.). More developed pupils eagerly participated in the discussion about the components of air and the methods of isolating them. Many of them already had knowledge of the gaseous composition of air. After this theoretical foundation, the most important components of air (nitrogen and oxygen) were illustrated by practical demonstrations.

All participants, including those with special needs, were particularly fascinated by liquid nitrogen, which made a remarkable first impression due to the smoke produced by the rapid condensation of water vapour around the cold liquid. All pupils also had the opportunity to see and observe liquid nitrogen up close. They were also encouraged to blow into the cold liquid to create a cold and refreshing mist (**Figure 2** (**c**)). Simple experiments were used to investigate the practical effects of the extremely low temperature

(-196 °C) (Liquid nitrogen, n. d.). Remarkable demonstrations was the observation of how a balloon deflates when immersed in liquid nitrogen and then re-inflates to its original size at room temperature – an illustration of how gases behave at different temperatures (**Figure 2** (**a**)). At room temperature, the air molecules inside the balloon move quickly and take up a larger volume. When the balloon is cooled with liquid nitrogen, the air molecules slow down as they lose heat. This leads to condensation and deflation of the balloon. If you shine a torch, you can also observe the liquefied air at the bottom of the shrunken balloon. When the balloon is warmed back up to room temperature, the air inside heats up, expands and the balloon returns to its original size (Jeran et al., 2024).

The Leidenfrost effect on the skin was used to illustrate the significant temperature difference between liquid nitrogen and the human body (Adda-Bedia et al., 2016). When liquid nitrogen briefly comes into contact with the skin, part of it evaporates immediately and forms an insulating layer of gas. The remaining liquid then rolls over this gas layer without direct contact. With prolonged contact, however, there is a risk that this gas layer will dissolve, which can lead to frostbite or more serious injuries. Suitable protective equipment is therefore essential when working with liquid nitrogen (University of Reading, 2023). You will need special gloves, as normal laboratory gloves can become brittle on contact with the cold gas, which can lead to skin ulcers. We have also demonstrated the effect of liquid nitrogen on these standard gloves, which has further emphasised their fragility under such conditions, as they become brittle and break under physical stress. To mimic the effect on biological material and to show young people the damaging effects of liquid nitrogen, we also demonstrated the freezing of green leaves in liquid nitrogen, which solidified and became like glass – the water in the cells turned to ice. These frozen leaves broke easily and showed the profound effect that low temperatures have on biological material. All pupils eagerly participated in this experiment and learnt first-hand about the effects and dangers of liquid nitrogen on biological matter (**Figure 2** (**b**)).

Building on these experiments, we discussed the topic of storing liquid nitrogen. We showed the large vessels (dewar flasks) that we used to transport the liquid nitrogen (University of Reading, 2023). These vessels are well insulated to prevent heat exchange and loss of nitrogen. However, They are not sealed, as closing the container would lead to a rapid increase in gas pressure inside the container (University of Reading, 2023). To demonstrate this problem, we have shown the consequences of trapping a small amount of liquid nitrogen in a plastic bottle with a rubber stopper (Jeran et al., 2024). The trapped liquid nitrogen boils continuously, causing the gases in the sealed container to expand and the pressure to rise. When the liquid nitrogen turns to gas, the volume increases more than a hundredfold, generating enough force to eject the stopper. In an alternative demonstration, a bottle with a rubber stopper was used, pierced by a thin tube through which the nitrogen gas quickly escapes and forms a large cloud. This experiment is often referred to as a "nitrogen fountain" (**Figure 2** (**e**)).

We have then moved on from physical changes in matter to chemical reactions and transformations. Fire is a chemical reaction that everyone recognises and which is seen as an important step in human development (New Scientist, n. d.; Stauffer et al., 2008). In a discussion with more verbal pupils, we summarised that fire is a chemical reaction in which fuel and oxygen are converted into carbon dioxide, water and heat, creating new, more stable products. However, this simplified explanation does not do justice to the complexity of this reaction, in which there can be many different products, depending primarily on the fuel and the availability of oxygen (Stauffer et al., 2008). The latter is particularly important as it can lead to incomplete combustion and material residues. We have demonstrated the different cases of combustion by burning different materials – dried bread, cotton, nitrocellulose. In the first case, dried bread does not really burn well when we try to set it on fire. The participating pupils realised that this material does not burn well. At school we are taught about the "fire triangle", three things that are needed for a fire: fuel, oxygen and heat (Palcon, n. d.). In this experiment we provided all three, but sometimes they are not all available in sufficient quantities. We modified this experiment by adding more oxygen to the mixture. To do this, we condensed air from the atmosphere onto a metal cone filled with liquid nitrogen. The condensed air contained a higher proportion of oxygen due to its higher boiling point. As soon as we had collected

enough oxygen, we soaked the dried bread in it and then set it alight. In the first experiment, the dried bread did not burn, even though it was constantly lit with a lighter, while in the second experiment the fire burned very brightly and independently. We explained to the participants that more oxygen leads to a more complete reaction. We also backed this up with two other fire reactions, one with normal cotton (celulose), the other with nitrocellulose cotton. Whilst cotton burns quite well, it leaves a solid residue, indicating incomplete combustion. We repeated the same experiment with nitrocellulose cotton, which burned almost immediately and astonished the audience (**Figure 2** (**h**)). This particular cotton contains nitro groups (-NO_2) which act as an oxidising agent – a 'source' of oxygen (Tramšek et al., 2023). Coming back to the fire triangle (**Figure 1**), this means that we have both the fuel and the oxygen in a single compound, the only requirement to start a fire is heat (Palcon, n. d.). This is a typical feature of compounds used as explosives, of which nitrocellulose is one. We have also observed that no solid residues are produced in this reaction, as the solid is completely converted into gaseous products. With these experiments, we have shown the influence of oxygen on both the speed of the reaction and its completeness.

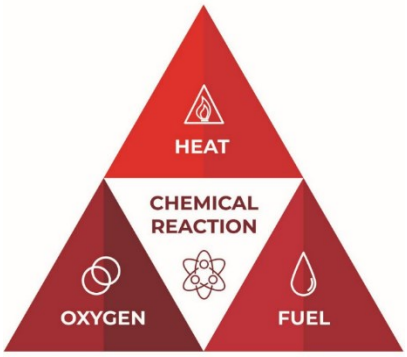

Figure 1. Oxygen, heat, and fuel are frequently referred to as the "fire triangle" (Palcon, n. d.).

We have continued the visually attractive experiments by showing two different flame reactions. As the name suggests, this is a combustion reaction, but it involves different cations that give the flame a unique colour. In the first example, we poured a solution of spirits into a glass bottle, closed it, shook it well and quickly poured out the contents. The empty bottle contained only spirit vapours, which quickly ignited and resulted in a slow-moving orange wall of flame. The orange colour comes from the sodium Na^+ from the salt, which is excited by the flame and emits the orange light when it releases the energy (Tramšek et al., 2023). As sodium is very common in nature, most fires have an orange colour. A similar experiment was carried out in a flask by placing aluminium foil in a solution of hydrochloric acid and copper(II) sulphate (Fleming, 2014). The aluminium reacted quickly with the hydrochloric acid and produced flammable hydrogen gas. This gas was then ignited, producing a bright, swirling blue-green flame that burned until all the aluminium was consumed. As in the previous experiment, the metal cation, in this case copper, produces the distinct colour when excited by the fire. We then explained that this effect is common with fireworks, which contain many different materials that produce different colours.

Having recognised that light can be produced by means other than fire, we have turned our attention to other luminescence reactions. The classification of luminescence is usually based on the nature of the emitted energy. Bioluminescence, which is often observed in natural systems, is often the enzymatic catalysis of the oxidation of a substrate (e.g. the oxidation of luciferin catalysed by luciferase in fireflies) (Marinko et al., 2024). Photoluminescence comprises two phenomena: fluorescence, which occurs under continuous energy input (usually radiation), and phosphorescence, which can persist even after the energy source is removed. We have introduced the fluorescence of various dyes, including fluorescein, a component of fluorescent markers (**Figure 2** (**f**)). Chemiluminescence, the generation of electromagnetic radiation as light through a

chemical reaction, was illustrated using the reaction between luminol and hydrogen peroxide (Jeran et al., 2020).

After the light reactions, we next investigated more colourful transformations. We carried out a reaction in which a lollipop was dissolved in a sodium hydroxide solution with potassium permanganate (Prolongo & Pinto, 2018). As the sugar of the lollipop dissolved, we observed a series of colour changes, each corresponding to a different oxidation state of the manganese (Prolongo & Pinto, 2018). This series of redox reactions involves the continuous transfer of electrons from the glucose to different manganese compounds, with each step in this chain manifesting itself in a distinct colour change (**Figure 2** (**g**)). Manganese lends itself well to this demonstration due to its numerous stable oxidation states (from +2 to +7), each of which has a unique colour (Prolongo & Pinto, 2018; Jeran et al., 2024).

In the following experiment, we investigated several simultaneous chemical changes (Royal Society of Chemistry, n. d.; Liu et al., 2021). First, we prepared a solution of calcium chloride and fluorescein in a large beaker. Then we used a syringe to introduce a steady stream of sodium alginate solution. The viscous alginate quickly reacted with the calcium ions and formed a solid, string-like compound. As the original solution contained fluorescein, this alginate string also exhibited fluorescent properties (Royal Society of Chemistry, n. d.). Interestingly, the fluorescence of the filament disappeared when it was cooled with liquid nitrogen, demonstrating that there is a significant temperature effect even with non-biological materials.

Finally, we performed a straightforward reaction with ferric chloride ($FeCl_3$) and potassium thiocyanate (KSCN) (MEL Science, n. d.). We asked volunteers to draw on paper with a cotton swab soaked in pale orange $FeCl_3$ solution. After they had completed their drawings, we sprayed them with KSCN solution, which resulted in a dark red image. This colour change resulted from the rapid reaction between $FeCl_3$ and KSCN, producing the more stable, red-coloured $Fe(SCN)_3$ (Jeran et al., 2024). Given the simplicity of this experiment, all pupils were encouraged to try it out for themselves (**Figure 2** (**d**)).

Figure 2. Observing the properties of liquid nitrogen: (**a**) interaction of liquid nitrogen with air from an inflated balloon, (**b**) freezing plant tissue in liquid nitrogen and testing for brittleness, (**c**) participants blowing into a container of liquid nitrogen and (**e**) nitrogen fountain – gas escaping through a hole. (**d**) Artistic creation with chemical reactions – reaction between iron trichloride and potassium thiocyanate, the product is a red colored complex. (**f**) Demonstrating a series of chemiluminescence reactions to simulate the phenomenon of light in fireflies and jellyfish. (**g**) Observation of the oxidation states of manganese with a lollipop in dilute aqueous sodium hydroxide solution. (**h**) Ignition of nitrocellulose cotton.

3. Conclusion

The integration of scientific values into inclusive education represents both a profound challenge and a critical opportunity for educators and academic researchers. Incorporating these values, such as objectivity, critical inquiry, and evidence-based practice, increases the effectiveness and equity of educational approaches. Teachers must constantly adapt to the diverse needs of pupils while maintaining rigorous academic standards, and researchers must support this process through interdisciplinary collaboration and innovation. Ultimately, incorporating scientific values into inclusive education is essential to promoting a more sustainable, equitable and resilient education system that leaves no learner behind. Such approaches are important for both educational progress and the academic community, and we will continue our work.

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List of regulation documents

Law 1: Zakon o usmerjanju otrok s posebnimi potrebami (ZUOPP-1) (*Engl*. Placement of Children with Special Needs Act). Available from https://pisrs.si/pregledPredpisa?id=ZAKO5896

Research

An Analysis of Music Intervention in the Management of Anxiety by Spontaneous Mandala Drawing

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Abstract:
Spontaneous mandala drawing has demonstrated therapeutic potential in reducing anxiety. This exploratory study addressed the research question: Does instrumental ambient music enhance the physiological effects of spontaneous mandala drawing? To answer that, three female graduate-level volunteers (ages 29–48) participated in eight sessions (four conducted without music and four with instrumental background music) in which pre- and post-measurements of systolic and diastolic blood pressure and heart rate were obtained. Non-parametric statistical analysis (t-tests and non-parametric Mann–Whitney U test) revealed no significant differences between the Music and No Music conditions across the three physiological variables. Effect sizes were negligible, suggesting that ambient music neither enhanced nor interfered with the observed physiological changes. Heart rate exhibited minor but more consistent shifts, potentially indicating its greater sensitivity as a short-term anxiety marker. The raw data and individual plots revealed substantial inter-individual variability, particularly in systolic blood pressure. These findings highlight the subjective and context-dependent nature of music appreciation and artistic engagement. While limited by a very small sample size (n = 3), this study provides preliminary insights into the role of ambient music in mandala-making interventions. No significant differences were found between conditions, underscoring the therapeutic value of spontaneous mandala drawing itself and highlighting the importance of individual variability in art-based interventions. Given the exploratory nature of this study and the individualized responses observed, these findings highlight the value of contributing to interdisciplinary dialogues on art-based methodologies, embodiment, and emotional regulation.

Keywords: Mandala drawing; Mandala therapy; Anxiety reduction; Music intervention.

1. Introduction

1.1. Therapeutic Mandalas

Mandalas, well-known as symbolic diagrams long used in Eastern spiritual traditions as tools for meditation and healing, were introduced to psychotherapy in the early 20th century by the Swiss psychiatrist Carl Gustav Jung (Jung, 1973). Jung encouraged patients to draw within circles to access unconscious material and transform internal chaos into coherence by recognizing them as expressions of the *Self* that bring order, balance, and psychological integration (Jung, 1973).

In Brazil, psychiatrist Nise da Silveira observed that patients with schizophrenia spontaneously drew mandalas during therapeutic art sessions. Influenced by Jung, she understood imagery as a language of the unconscious. She was key in advancing clinical art as a respected, non-invasive therapeutic approach to mental illness (Mello, 2009).

In the 1970s, the art therapist Joan Kellogg analysed over 11,000 mandalas from psychiatric patients and developed *The Archetypal Stages of the Great Round*, a model of 12 stages of human development, later expanded with a *Stage 0*, representing interconnectedness (Kellogg, 1977). Based on her theory of the Great Round, Kellogg created the Mandala Assessment Research Instrument (MARI®), a psychological tool for evaluating one's mental state, relationships, and overall well-being. A valuable contribution not only for therapists but to individuals interested in self-knowledge through the exploration of the 12 archetypal stages to reduce anxiety and increase self-awareness was proposed by Susanne Fincher (Fincher, 2010).

1.2. Mandalas and Anxiety Management

The growing urgency to address mental health challenges has highlighted the importance of accessible, non-invasive interventions. Depression and anxiety remain the most prevalent mental disorders globally, including in Brazil, according to the Global Burden of Diseases Study (GBD 2019 Mental Disorders Collaborators, 2022). Their incidence increased further during the COVID-19 pandemic (COVID-19 Mental Disorders Collaborators, 2021). Mandala colouring has shown promise as a means of reducing anxiety in diverse clinical settings, including among COVID-19 patients (Khademi et al., 2021; Kirca & Daglı, 2024; Kirka et al., 2024; Sadeqi et al., 2024). Mandala art therapy has also been linked to increased hope among psychiatric inpatients (Kim et al., 2018).

However, following Jungian principles, drawing spontaneous mandalas, rather than colouring pre-designed patterns, offers a deeper psychological benefit. This process encourages active imagination, self-reflection, and integration of unconscious material, going beyond mindfulness-based stress relief.

A recent meta-analysis confirmed that mandala colouring and free drawing reduce anxiety, though evidence suggests spontaneous mandala drawing may foster greater insight and emotional expression (Jakobsson-Støre & Jakobsson, 2022; Campenni & Hartman, 2019). Mandala drawing helped patients with dementia to externalize thoughts and emotions otherwise difficult to articulate (Couch, 1997), and in individuals with intellectual disabilities to reduce stress (Schrade et al., 2011)

Henderson et al. (2007) validated mandala drawing as a non-verbal method for accessing unconscious content with participants with PTSD who experienced greater symptom reductions than controls, suggesting mandala drawing could supplement or even substitute verbal trauma therapies.

The healing power of spontaneous mandalas was also observed among older female cancer survivors (Yakar et al., 2021) and hospitalized adolescents with cancer (Gürcan & Turan, 2021). The MARI® test has also been applied in clinical settings, including among heart disease and cancer patients. Bruscia and Shultis (2017) found that patients often selected stages reflecting existential concerns about death while simultaneously expressing a creative will to survive. Their spontaneous mandalas revealed efforts to cope with illness and maintain meaning in life.

Despite the growing evidence supporting mandala drawing as a therapeutic tool, little is known about how background music may influence its effects. This study investigates

whether instrumental ambient music modulates physiological indicators of anxiety—specifically systolic and diastolic blood pressure and heart rate—before and after spontaneous mandala drawing sessions.

2. Material and Methods

2.1. Participants

Three female volunteers aged between 29 and 48 (37.7 ± 9.6) participated; two were master's candidates and one was a postdoctoral researcher at the Centre of Health Sciences, Federal University of Rio de Janeiro, between March and May 2023.

2.2. Procedure

The 40-minute workshops were held in a quiet study room, where participants had access to all necessary materials, including paper, oil pastels, a notebook, and a pencil. The workshop followed four steps:
(i) a welcoming moment, allowing participants to share their feelings and state of mind from that day or week, and to achieve a baseline for the physiological variables;
(ii) relaxation and grounding through breathing exercises, followed by the initial measurement of blood pressure and heart rate;
(iii) a 20-minute mandala drawing session, followed by the final measurement of blood pressure and heart rate; and
(iv) a sharing and reflection period.
Before and after the drawing activity, the systolic (S) and diastolic (D) blood pressure and heart rate (HR) were measured with a digital wrist blood pressure monitor. The results were analysed as the difference between the initial (I = right before drawing) and final (F = immediately after) measurements of the systolic ($\Delta S_{BP} = S_{BP}^{F} - S_{BP}^{I}$) and diastolic ($\Delta D_{BP} = D_{BP}^{F} - D_{BP}^{I}$) blood pressure and heart rate ($\Delta HR = HR^{F} - HR^{I}$). Two experimental conditions were established: mandala drawing without (Group I) and with (Group II) background ambient music.
Following the recommendation of Joan Kellogg (1973), oil pastel was chosen for its balanced fluidity – more expressive than pencil (a highly controlled medium) and more manageable than watercolour or gouache (which are more difficult to control). Sheets of A3 paper were cut into 29.7 cm squares, providing a large surface for free emotional expression. A circle was lightly drawn with a black pencil in the centre of the square. Participants were instructed to use the circle as a guide rather than a constraint, with the freedom to draw inside, outside, and/or across the circle.
After completing their drawings, participants were encouraged to observe their work closely from different angles, slowly rotating the paper to discover personal meanings through the lines and colours, and start journaling.
For the sessions with music, a playlist of instrumental compositions was curated, including works by Ryuichi Sakamoto, Kitarō, René Aubry, Alexis Ffrench, Ólafur Arnalds, Will Ackerman, and Brian Eno, among others.

2.3. Statistical Analysis

Data analysis was performed in Excel with 24 observations for each physiological measure (systolic and diastolic blood pressure, and heart rate), divided into two groups: Group I (no music, n = 14) and Group II (with music, n = 10). Descriptive statistics (means, standard deviations, medians, and ranges) were calculated for each group. Independent samples t-test and non-parametric Mann–Whitney U test were conducted to compare the groups. Cohen's d was calculated to estimate effect sizes and assess practical significance. Statistical significance was set at $\alpha = 0.05$.

2.4. Ethical Approval

Ethical approval was obtained from the Research Ethics Committee of the Institute of Psychiatry, Federal University of Rio de Janeiro (approval no. 5,925,361). Participation required accepting an electronic informed consent (e-IC) form, which was accessed before completing the registration form. The e-IC outlined the study purpose, confidentiality guarantees, voluntary participation, and the option to withdraw without data retention. Participants were informed that their contributions would include registering the physiological data (blood pressure and heart rate). The e-IC also included details about the workshop's structure, duration, and scheduling. Upon acceptance, participants were directed to complete the registration form.

3. Results

Anxiety levels were evaluated by physiological variables such as changes in systolic and diastolic blood pressure and heart rate (Condominas et al., 2025). The data analysis revealed considerable inter-individual variability in physiological responses to the mandala drawing sessions, both with and without background music. This variability is evident in the raw data, particularly in the change scores for systolic blood pressure (ΔS_{BP}), which ranged from -9 to +13 mmHg in Condition I (No Music) and from -7 to +22 mmHg in Condition II (Music). Similar dispersion was observed for diastolic pressure (ΔD_{BP}), ranging from -5 to +13 mmHg in Condition I and from -6 to +15 mmHg in Condition II. Both variables suggest that participants responded to the sessions in markedly individual ways. The variation in the changes in heart rate (ΔHR) was less pronounced, ranging from -8 to +4 bpm in the No Music condition and -7 to +5 bpm in the Music condition.

The variability of the data was related to one participant who consistently showed strong emotional responses to the mandala drawing exercises, which were often accompanied by physiological fluctuations. Her drawings were rich in symbolic content, and she occasionally became visibly moved, sometimes even crying, when interpreting her own work. This participant was undergoing personal and academic stress at the time of the study, which may have amplified the emotional resonance of the sessions. Notably, she reported improved emotional well-being during the workshop period and completed her Master's degree shortly thereafter.

Despite these personal and emotional differences, inferential statistics showed no significant differences between the Music and No Music conditions across all three physiological variables: systolic blood pressure ($\boldsymbol{\Delta S_{BP}}$), diastolic blood pressure ($\boldsymbol{\Delta D_{BP}}$), and heart rate ($\Delta HR$). Results from both parametric (independent t-test) and non-parametric (Mann–Whitney U) tests confirmed this lack of statistical difference. Additionally, effect sizes (Cohen's d) were small, suggesting negligible practical differences between groups.

Table 1 presents the descriptive and inferential statistics for each variable. **Figures 1** and **2** summarize the results, emphasizing the wide variability in individual responses. Notably, ΔS_{BP} values exhibited the most pronounced spread, particularly under the Music condition, as seen in the scatter (**Figure 1**) and violin (**Figure 2**) plots.

Table 1. Statistical analysis of the effect of background music during mandala drawing on decreasing anxiety

Variable	Background Music		Independent t-test		Mann-Whitney Test		Cohen's *d*
	no	with	t	p	U	p	
ΔS_{BP}	-0.43 ± 5.15	0.90 ± 9.16	-0.41	0.685	69.5	1.000	-0.19
ΔD_{BP}	2.21 ± 4.90	2.30 ± 6.02	-0.04	0.971	74.5	0.814	-0.02
ΔHR	-1.43 ± 3.61	-0.50 ± 3.50	-0.63	0.535	59.5	0.555	-0.26

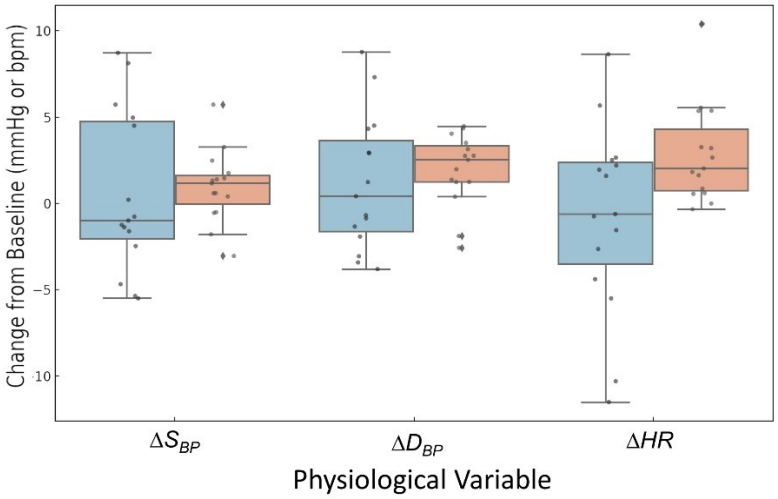

Figure 1. Changes in systolic blood pressure (ΔS_{BP}), diastolic blood pressure (ΔD_{BP}), and heart rate (ΔHR) following spontaneous mandala drawing, without (blue) and with (orange) instrumental music. Each dot represents one individual measurement. Outliers and individual variability are especially evident in ΔS_{BP} under the Music condition. No significant differences were observed between conditions.

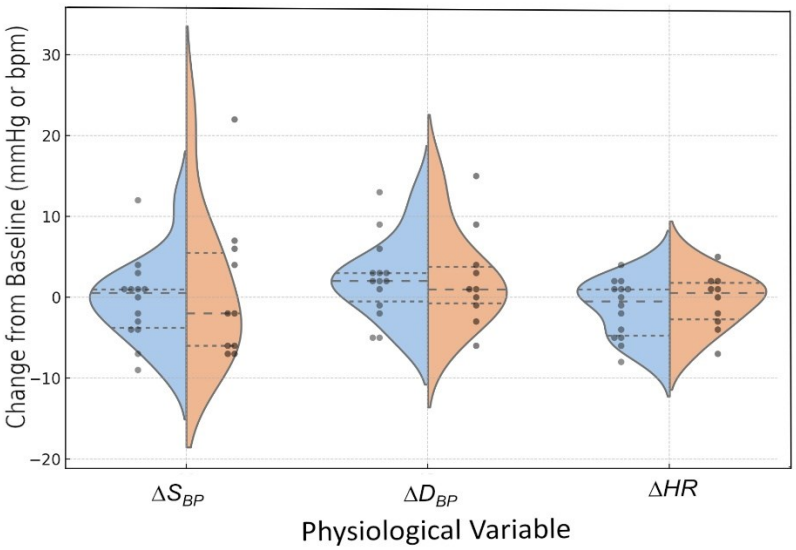

Figure 2. Violin plots showing the distribution of pre–post differences in systolic blood pressure (ΔS_{BP}), diastolic blood pressure (ΔD_{BP}), and heart rate (ΔHR) due to spontaneous mandala drawing sessions. The left half of each violin represents the condition without music, and the right half represents the condition with music. Despite individual variability, especially in ΔS_{BP}, no statistically significant differences were observed between conditions.

4. Discussion

This exploratory study investigated whether instrumental ambient music modulates the physiological effects of spontaneous mandala drawing on anxiety. While all participants showed changes in systolic and diastolic blood pressure and heart rate after the sessions, statistical analyses revealed no significant differences between the Music and No Music conditions. Effect sizes were negligible, suggesting that background music neither enhanced nor diminished the observed physiological responses.

These results are important in understanding the role of environmental factors, such as ambient sound, in therapeutic art practices. Ambient music is often assumed to be inherently calming, but in this context, it did not produce a measurable difference in physiological markers of anxiety. Interestingly, two participants reacted oppositely to the same playlist during one session: one expressed deep enjoyment, while the other was visibly disturbed. The latter described the music as irritating and intrusive; her mandala, typically light and harmonious, appeared dark and chaotic, reflecting her discomfort. She later reported that the playlist had negatively impacted her focus and emotional state during the session. Notably, this participant enjoyed the playlists in the other three music sessions, highlighting how subjective and context-dependent music appreciation can be.

This suggests that the core therapeutic value may lie in the spontaneous creation rather than in external stimuli accompanying the process.

One participant demonstrated intense emotional engagement during the workshops, accompanied by marked physiological variation across sessions. Her mandalas were often deeply symbolic and prompted strong personal insights. Although this case does not represent the group statistically, it highlights the profoundly individual nature of expressive art therapy. For her, the workshops appeared to catalyze a psychological turning point, coinciding with improved coping and the eventual completion of her Master's dissertation. This anecdotal evidence underscores the need to balance group-level analysis with individual-level observations when evaluating creative therapies.

Despite the small sample size, the violin plots allow the visualization of the variation in measurements, suggesting that heart rate has a smaller variation than blood pressure (systolic and diastolic). Data obtained from another group of participants showed the same pattern, strengthening the suggestion that heart rate measurement has a better outcome than systolic and diastolic blood pressure. The wide dispersion in physiological responses, particularly in systolic blood pressure, also reflects the complex and personal nature of anxiety regulation. It is plausible that individual factors, such as emotional state, personality traits, or life circumstances, played a stronger role than the presence or absence of background music.

Several limitations must be acknowledged. The small sample size (n = 3) reduces statistical power and limits generalizability. Furthermore, the sample consisted of graduate-level women in a high-stress academic environment, which may not reflect broader populations. Scheduling constraints, common among graduate students and researchers, also introduced challenges to participant continuity and data consistency.

Despite these constraints, the study contributes to the growing interdisciplinary conversation around STEAM-based mental health approaches. By combining artistic expression, therapeutic frameworks, and statistical analysis, this work illustrates the value of mixed-methods inquiry in evaluating non-verbal interventions. This exploratory study aimed to determine whether background music influences the physiological impact of spontaneous mandala drawing on anxiety. The absence of significant differences suggests that instrumental music neither enhances nor disrupts the benefits of the activity in this small sample. This finding is important from a therapeutic perspective. While music is commonly used to aid relaxation, it may not be necessary when the goal is to reduce anxiety through spontaneous artistic expression. Furthermore, the variability seen in physiological responses, especially from one participant, highlights the deeply personal and idiosyncratic nature of art-based interventions.

Nonetheless, the study adds value to the interdisciplinary dialogue within STEAM fields. By incorporating statistical analysis, visual data exploration, and psychological interpretation, this work models a holistic approach to investigating non-verbal therapies.

5. Limitations

Although only three participants completed both experimental conditions, the repeated-measures design allowed intra-individual comparison across sessions. Rather than aiming for statistical generalization, this exploratory framework prioritized the observation of personal responses to artistic engagement under different auditory conditions. Considering the significant heterogeneity in responses, the study aligns with idiographic and n-of-1 approaches, emphasizing variability, subjectivity, and context in therapeutic research. Expanding the participant pool in future studies could help assess how broadly these patterns apply while preserving attention to individual dynamics.

6. Conclusions

Spontaneous mandala drawing appears to be an effective tool for reducing anxiety, regardless of the presence of background music. In this exploratory study, instrumental ambient music did not significantly enhance or interfere with physiological indicators of anxiety—systolic and diastolic blood pressure and heart rate—suggesting that the core therapeutic benefit lies in the act of artistic expression itself.

It is worth noting that individual responses varied widely. One participant showed strong emotional engagement and notable physiological fluctuations. At the same time, another exhibited a rare adverse reaction to the music in one session, reflected in her comments and the visual qualities of her mandala. These findings underscore the highly personal nature of art-based interventions and the subjective experience of music.

Heart rate changes were less intense than blood pressure shifts, suggesting they may serve as a more direct and sensitive indicator of short-term emotional regulation. Future studies might consider including heart rate variability or other dynamic physiological measures to capture subtle shifts in anxiety.

While no significant differences were found between music and no music conditions, the small sample size (3 participants) prevents broad generalization. These findings should be interpreted as preliminary insights, emphasizing individual variability and personal factors in art-based methods. Nevertheless, this study contributes to ongoing interdisciplinary efforts to integrate artistic processes into mental health strategies. Further research with more diverse populations and larger samples will help clarify how individual differences mediate the therapeutic effects of spontaneous mandala creation, with or without music.

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Research

Foresight, Forecast and Futurology in Reverse: Towards Cultural Sustainability via STEAM

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Abstract:
The overall focus of this discussion is the integration of STEAM (Science, Technology, Engineering, Arts, Mathematics) approaches into future thinking, structured around three key dimensions. First, it examines the conceptual distinctions between foresight, forecasting, and futurology, highlighting how each concept frames the anticipation of the futures in different ways. Second, it introduces a reverse approach through the concept of "Retrosight" - a perspective in which alternative futures are not projected linearly but imagined creatively via art-based STEAM practices. It explores the SMS (Stories based on Music about Scientists) method (STEAM approach), which involves composing original music based on poems written by scientists (17th-20th centuries), poetry or musical compositions dedicated to them with digital images. SMS is a narrative method that integrates music and storytelling to humanize scientific futures and reconnect with enduring questions. Third, the discussion illustrates how creative methods can be applied in practice - through illustrated art experiments that map uncertainty and reframe how we approach complex future challenges. The role of science, education, and planetary health is central to this exploration, drawing on original SMS compositions using poetry by James Maxwell, Francis Bacon, and Alfred Nobel. These SMS songs offer a creative lens through which to explore questions still relevant today with philosophical and scientific concern. As recognized by the WHO (World Health Organisation) and the European Commission, Art is essential not only for health and homeostasis, but it serves as a key component of cultural sustainability. STEAM-based SMS experiments can be ideally applied to futures studies from a management perspective grounded in framework of Retrosight.

Keywords: Cultural sustainability; Management perspective; Futures studies; Stories based on Music about Scientists; Retrosight; STEAM

1. Introduction

1.1. SMS method as a STEAM approach: definition

This paper builds on research into the Foresight through STEAM approach with a focus on the role of Art practices. It aims to develop foresight´s alternative models using the SMS method (Stories based on Music about Scientists) as STEAM approach introduced in previous research publications (Istileulova, 2023). In short, the SMS method, which emerged in 2012, combines three elements of storytelling: (1) music, (2) poetry (written by scientists or about scientists of the past), and (3) image.

Thus, this paper includes the art-innovative practices of STEAM approach applied in Foresight with:

1. Original music (composed specifically for the poem)
2. Poems written by scientists (from the 17th to 20th centuries), or poetry dedicated to notable scientists, based on historical or biographical research
3. Digital visual images, such as videos, drawings, pictures, engravings, photos, etc

Thus, SMS is a narrative method that integrates music, poetry, and visual imagery about scientists to inspire innovation.

We can say that SMS is a story-singing to humanize scientific futures and reconnect with enduring questions. While grounded in scientific research, the method also allows space for poetic imagination, weaving fact with interpretation to create emotionally resonant narratives. The method uses the Art to connect the past with the present and to explore ideas about the future - and, in reverse, to reimagine the past through the lens of future possibilities.

What distinguishes this method (for instance, from songs) is that the poetic narrative is grounded in original research: each story is based on research constructed from historical, biographical, and conceptual study of selected scientists and their work.

The story-singing is primarily written in English and Russian, with some texts available in other languages (examples: https://www.youtube.com/@aleona_von_sultanova).

Next, we introduce the concept of foresight as a multidisciplinary function to further illustrate how it can be leveraged within the SMS method.

1.2. Foresight as a multidisciplinary function

Foresight as a multidisciplinary function

Foresight is rooted in an American technological forecasting or tradition; which was mainly developed in relation to strategic military studies at the RAND (Research and Development Corporation) in the USA during the 1940s and 1950s (Jantsch, 1967). Jantsch's 1967 work is often seen as the conceptual starting point for institutional foresight. The RAND, an American nonprofit global policy think tank, research institute, and public sector consulting firm expanded its research into civilian fields: education and international affairs. The 1972 report "The Limits to Growth" by the Club of Rome (Meadows et al., 1972), a global think tank headquartered in Switzerland, used systems analysis and modelling to forecast environmental and economic challenges, and highlighted the importance of long-term planning and foresight.

David Fidler (2011) approaches foresight not just as a futures-thinking tool, but as a multidisciplinary, strategic function integrated across several fields - especially from a strategic management perspective.

Fidler (2011) considered that the growth of Foresight or Futures Studies as a discipline has been less robust than the internal logic of the field, indicating an opportunity for its theoretical renewal. Fidler´s foresight domains include strategic management, leadership, sustainability and corporate responsibility, innovation management, policy, governance, risk and resilience management, marketing and consumer insight, change and organizational learning, technology and R&D (Research and Development) strategy. "Foresight" can also be approached from various perspectives of branding, when we consider it as part of strategic marketing, cultural positioning and identity management or the Arts-related innovative practices within STEAM.

This theoretical renewal is suggested here with the Art-embedded methods of STEAM approach with the FFF (Foresight-Forecast-Futurology) triangle.

2. Literature: Foresight, Forecast and Futurology

An interesting fact is that while working on technology futures - under various labels such as "anticipation," "forecasting," and "futures studies" - I&M (Irvine and Martin) introduced the term "foresight", partly as a humorous counterpoint to "hindsight" (Miles, 2010). "Foresight" was just one among many words used to describe long-range forward-looking activities included in a list, known as "futures studies, futures research, futurology, futuristics, futurics, foresight, forecasting, prognostics" (Marien, 1984). FFF (Foresight, Forecast and Futurology) are three overlapping but distinct approaches in futures studies. They differ in their purpose, methods, and philosophical orientation. Forecasting answers: "What is most likely to happen?", Futurology answers: "What could happen?" or "What should happen?", and Foresight – "What is plausible, and how should we respond?" However, here we suggest defining all these concepts as FFF or 3F orientation, because SMS method can approach all three of them. Moreover, the need for all varieties of foresight are greater than at any point in history (Fidler, 2011; Miles, 2010).

In the book of a futurist Peter Schwartz (1997), "*Art of the long view: planning for the future in an uncertain world*", he also takes a holistic view, emphasizing the power of storytelling: he explores aspects of scenario planning and draws insights from disciplines such as anthropology, history, geopolitics, fiction writing, music, and other fields accessible to an intellectually curious mind. He discusses how artists and musicians perceive and express the world differently, and how this can inform scenario thinking. He emphasizes that musicians don't always "plan" in a linear way, but rather feel patterns, improvise, and shift tones dynamically - much like planners must do when facing an uncertain future. Schwartz (1997) considers that "Music is a window onto freedom in the future."

Mason (2025) emphasizes that each scenario requires specific research, although there are subjects that need to be studied at all times: science, technology, and music.

In the conceptual paper on "futures thinking for transforming" Sohail Inayatullah (2008) integrates the concepts (disowned future, alternative futures, alignment, models of social change, and uses of the future), pillars (mapping, anticipating, timing, deepening, creating alternatives, and transforming) and questions. One of his concepts is about the alternative futures: why do we believe that there is only one future, and often cannot see the alternatives, but if we start looking for alternatives, we may see new ones. Inayatullah´s pillar, - past, present and future - are mapped: "By mapping time, we become clearer on where we have come from and where we are going" (Inayatullah, 2008). This principle of Past, Present and Future´s pillar is also applied here. This temporal distinction is not just chronological - it leads us to apply different analytical dimensions to our understanding of future-oriented thinking.

Futurology was first introduced by Ossip Flechteim in his 1966 book "History and Futurology", and it was introduced more like "historical sociology''' (Sardar, 2010). Other futurologists like Pentti Malaska summarized it as the question "What Does "Knowledge of the Future" Mean"? (Sardar, 2010). Contemporary young futurists, such as Kian Bakhtiari, a widely recognized figure featured in Forbes, contribute actively to current discourses in futurology, strategic foresight, and emerging cultural trends. In his article "7 Cultural Trends for 2025 and Beyond" (Bakhtiari, 2025), he identifies major transformations such as post-institutional decay, AI (Artificial Intelligence) empires, rising individualism, and postponed futures, particularly within the realms of culture and marketing. (Bakhtiari, 2025). However, while these contributions offer valuable insights into commercial and socio-cultural dynamics, they tend to overlook essential dimensions of long-term futures thinking - namely, the role of the arts (including music, storytelling, and cultural heritage) in shaping imaginative visions of the future. Bakhtiari's work also does not engage with the ethical legacies of science. These gaps highlight the need for more integrative foresight approaches - such as those grounded in STEAM and narrative-based methodologies - with historical reflection, and artistic engagement as well as cultural sustainability.

Bibri (2018) suggests inclusion of time-horizon, users, approaches in forecasting, and backcasting. Backcasting as a scholarly and planning approach is increasingly used in futures studies in the fields related to urban sustainability as an alternative to traditional planning approaches and a formal element of future strategic initiatives.

We summarise various relevant dimensions to see the differences in FFF in **Table 1**.

Table 1. Differences in Forecasting, Futurology and Foresight: Comparison

Dimension	Forecasting	Futurology	Foresight
Goal	Predict likely future outcome	Explore possibilities	Guide decisions
Time horizon	Short-to mid-term: 1-5 years	Long-term: 20+ years	Mid- to long-term: 5+/30
Approach	historical data, trend analysis.	Theoretical, exploratory	Strategic, applied
Certainty	Higher (for short term)	Low	Medium
Users	Analysts, planners, economists, Businesses, meteorologists	Academics, futurists, visionaries	Policymakers,organisations, consultants, NGOs

3. Definition of "Retrosight" for STEAM approach

In SMS, the concepts of FFF (indicated in **Table 1**) is approached in reverse - as an attempt to reframe FFF by looking from the perspective of the Present toward the Past in order to define the Future. This approach has been in use in practice since 2012 within the SMS framework, when SMS songs as a storytelling about different innovations of Scientists of the Past were first introduced (Kralj-Iglič, 2025). We can explore future scenarios via back-casting, or retrosight and historiography (based on research about scientists) - taking a reverse path due to the nature of the SMS method, which aligns with the STEAM approach. For instance, Bibri (2018) applies backcasting as a scholarly and planning approach is increasingly used in futures studies as an alternative to traditional planning and a formal element of future strategic initiatives. Thus, in the backward-oriented approaches: Backcasting, Historiography, Retrosight/or Hindsight, the term "Retrosight" best reflects our art-related method and can therefore be effectively integrated into a STEAM-based method. While Retrosight is occasionally used in foresight and innovation studies to refer to learning through retrospective analysis, we propose a definition grounded in the arts and education. In the context of STEAM and Futures Studies, we define the concept "Retrosight" as a method of STEAM learning that draws on retrospective analysis - using the arts and historical reflection to inform forward thinking. This concept aligns with the SMS method, which uses artistic tools such as storytelling, music composition, and visual interpretation to revisit scientific legacies. Through Retrosight, the SMS method connects past narratives with present-day learning and future-oriented thinking, highlighting how the arts can serve as a reflective and imaginative force in futures education. The **Table 2** includes the suggested Framework FFF in Reverse) which reflects the illustration of the SMS model (**Figure 1**) which can be used in future studies.

Table 2. A FFF Trilogy in Reverse: suggested framework

Concept	Future-oriented	Backward-oriented equivalent
Forecasting	Predicting likely futures based on current data	Backcasting / Historical Reconstruction - inferring past causes from present evidence
Futurology	Exploring possible futures in speculative/ philosophical ways	Counterfactual Historiography - exploring "what could have happened" in the past
Foresight	Strategically anticipating possible futures	Hindsight/Retrosight: using past patterns to inform present and forward thinking

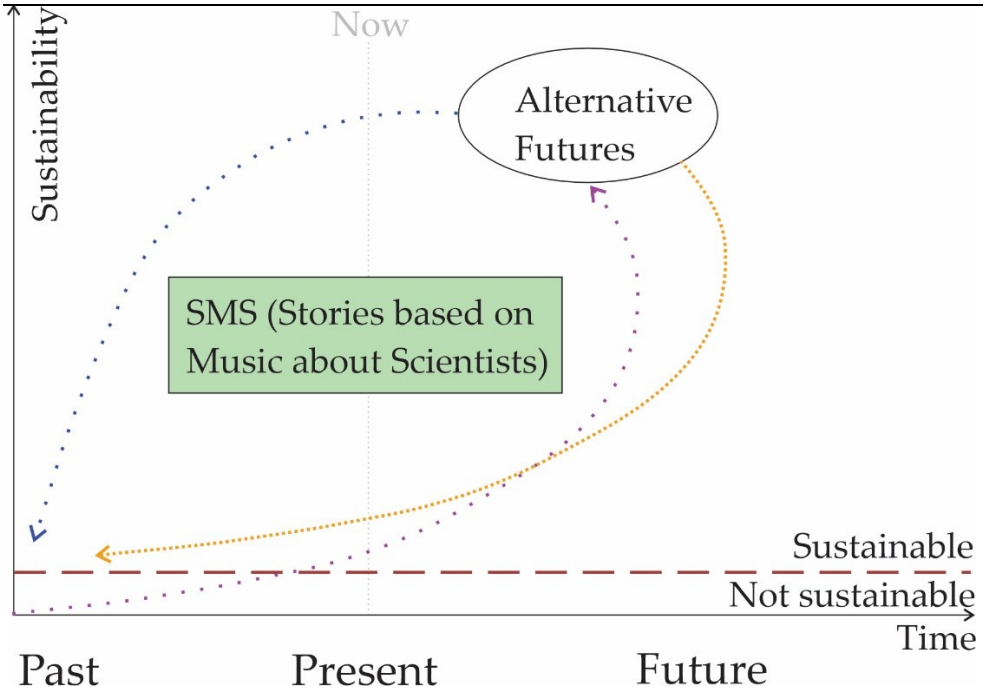

Figure 1. SMS model and Foresight with Alternative Futures. SMS is developed in Present (Now) to reflect the research about the idea of innovation of one of the scientists from Past time. This approach blends artistic expression with historical insight, offering a narrative bridge between past scientific thought and contemporary futures thinking. In life, no resource is 100% sustainable forever without careful management. Every resource- whether natural, human, or social—has limits. While physical resources degrade or exhaust over time, music, poetry, and art often endure across generations. They aren't consumed in the traditional sense, but rather transmitted, reinterpreted, and revived. SMS allows to approach from different angles of this FFF Triangle (Foresight-Futurology-Forecast). Therefore, we consider the SMS method a form of cultural sustainability.

Also, the SMS model can address the current and future uncertainties within the STEEP (Society, Technology, Economy, Environment, Politics) frame, as depicted in Terra Incognita (Goldin & Muggah, 2020) (**Figure 2**).

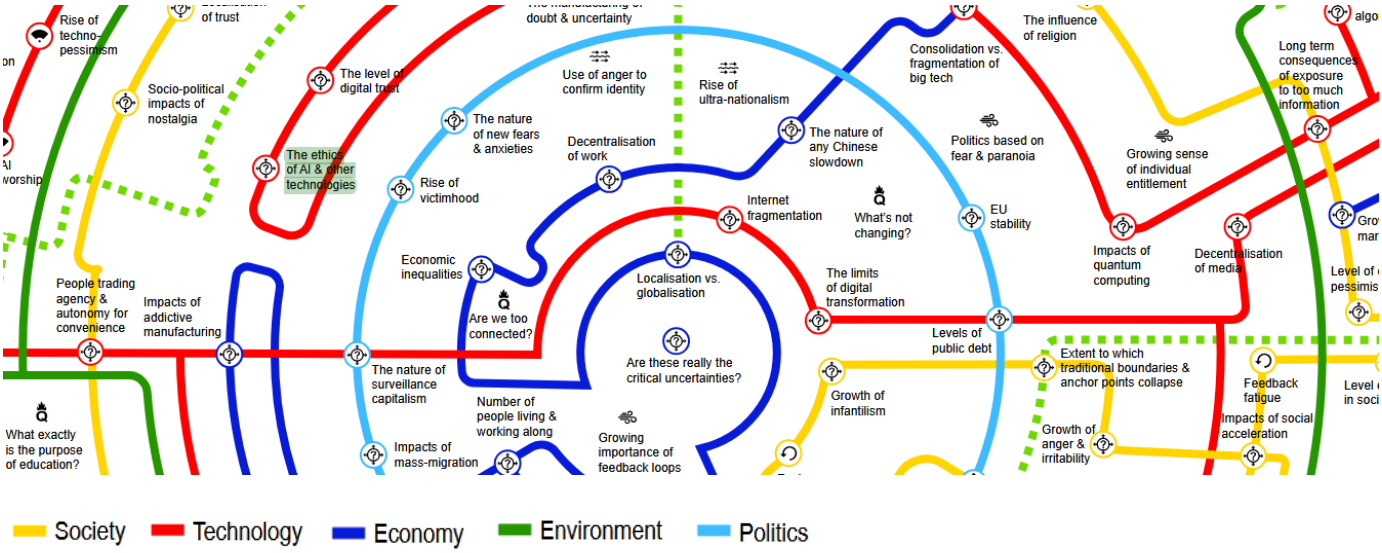

Figure 2. Mapping Uncertainties. A fragment. From (Goldin & Muggah, 2020).

4. Results

The results are a list of selected SMS songs, shown below. Each song includes three components: Music, Poem, and Image. All songs are freely shared. One SOS (Songs about Science) is included as an example of a preliminary version. It is close in form to SMS, but was not recorded in a studio and therefore does not include a video image.

SMS songs can be meaningfully connected to the "Map of Uncertainties" (**Figure 2**) by addressing key themes such as ethics, social responsibility, resource allocation, and the evolving role of science in society. Through creative storytelling, SMS offers a way to explore these uncertainties not just in contemporary contexts, but also through historical examples.

21th century:

1. "Where is my home" (in English): Reflection on migration by the modern scientists. https://www.youtube.com/watch?v=B4ZM3lzl1iU (This SMS introduces a composite image of a contemporary scientist in migration, inspired by the experiences of Jacob Bronowski, (1908–1974) – British-Polish mathematician, biologist, philosopher, and historian of science.

20th century:

2. "Crossing" (in English & Russian). Poem: Robert Oppenheimer (1904-1967), American physicist. https://www.youtube.com/watch?v=MOfArpU9OkI This version of SOS (Songs about Science) does not include an image (3rd component) for two reasons: It is performed live, without visual projection or professional studio editing. There is no budget for the professional studio production (therefore, digital artwork is absent).
3. "Spring is coming with water" (in Russian). Theory of Time: Nikolai Kozyrev (1908-1983), Soviet astrophysicist. https://www.youtube.com/watch?v=q5NC7Kc-UO8
4. "Star Selena" about Yuri Gagarin (1934-1968), Sergei Korolev (1906 – 1966), Konstantin Tsiolkovsky (1857 – 1935), Soviet astronauts and rocket scientists. https://www.youtube.com/watch?v=kyMi393_Y1I

19th century:

5. "You say I am a Riddle" (originally in English, translated into Russian by Aleona von Sultanova); Poem: Alfred Nobel (1833 - 1896), Swedish chemist, engineer, inventor, businessman, and philanthropist https://www.youtube.com/watch?v=vL0KI5Ow1Rg&list=RDvL0KI5Ow1Rg&start_radio=1
6. "Загадка«. (Riddle. Poem: Alfred Nobel (translated and performed in Russian with the different images): https://www.youtube.com/watch?v=6ZbaSAVtN1k
7. "Evening hymn": James Clerk Maxwell (1831-1879), Scottish physicist, mathematician and poet, https://www.youtube.com/watch?v=qyZIRCKG240

18th century:

8. "Morning Hymn" (Уже небесное светило) (in Russian). Morning Meditation or Ode to the Sun/Утреннее размышление или Ода Солнцу): Mikhail Lomonosov. https://www.youtube.com/watch?v=wysfY8a-YRM Poem: Mikhail Lomonosov (1711–1765), a Russian polymath (literature, education, chemistry, physics, mineralogy, history, art, philology)
9. "Road of a lifetime"or "Water" Johann Georg Gmelin (1709-1755), German naturalist and botanist. https://www.youtube.com/watch?v=daiSGLn-0GI
10. "Apples of Sievers" (in Russian) about Johann August Carl Sievers (1762–1795), a German-born botanist who explored Central Asia, Siberia, and other regions of the Russian Empire. Among the species described by Sievers is Malus sieversii, the ancestor of the domesticated apple. https://www.youtube.com/watch?v=i8Nw2nyPTmc

17th century:

11. "Baron Valvasor" (in Russian and Slovenian). Janez Vajkard Valvasor (1641 –1693), a natural historian and polymath from Carniola. https://www.youtube.com/watch?v=x7dsaxGGNLk

12. "Soul of Robot girl" or Romance about Alchemist (in Russian) about Jacob Bruce (1669 – 1735), scientist, inventor, diplomat statesman: https://www.facebook.com/1346167803/videos/pcb.10220238261770494/10220238242370009
13. "The world is a bubble". Poem: Francis Bacon (1561-1626), English philosopher, statesman, and scientist https://www.youtube.com/watch?v=c8v5-1S9S8k .

There are other SMS songs that were not recorded in a studio but performed live - some still include visual images during performance. These include:

"Love to Pigeon" (*Любовь к голубке*) — about Nikola Tesla (1856–1943), a Serbian-Croatian-American scientist; "Will You Come Along with Me" — based on the work of James Clerk Maxwell; "Glaube und Tat" — performed in German and Russian, with a poem by Immanuel Kant (1724–1804), a German philosopher and key figure of the Enlightenment in epistemology, metaphysics, and ethics; "The Chain of Connection" based on a poem by Alexander von Humboldt (1769–1859), a German polymath, translated into English by Bill Nuttle; "Galileo Galilej – Эти планеты", These Planets - about Galileo Galilei (in Russian); "Danish Erasmus" (*Датский Эразмус*) — about Erasmus Bartholin (1625 – 1698), a Danish physician and grammarian; "Poem on Medicine" — attributed to Avicenna (Ibn Sīnā) the renowned Persian philosopher and father of medicine (died 1037); "Omnis Mundi Creatura"- a medieval text by Alain de Lille (1125 – 1202), a French theologian and poet.

All SMSs sit at the intersection of various interdisciplinary and philosophical themes, and much broader than just one topic. According to STEEP, Alfred Nobel could be categorized into "Economy", yet the song reflects a much broader scope - particularly his philosophical outlook and inner conflicts. As with many SMS pieces, the subject transcends any single category, blending economic, ethical, environmental and existential as well as many other themes. Thus, SMS is more than just song writing - it is a narrative technique that integrates music and storytelling (story-singing) to humanize scientific futures and engage with the same questions we face today.

The role of science and education is central to this exploration based on the songs written on the lyrics of Francis Bacon (the 17th century´s English philosopher and statesman) that contain consideration of societal problems, and James Clerk Maxwell (the 19th century´s Scottish physicist and mathematician, famous for his theory of electromagnetism) that consider issues regarding environment.

17th-century scientists like Jacob Bruce, featured in "Soul of Robot Girl" (number 12), becomes a lens through which to examine the ethical dimensions of scientific ambition, now echoed in modern concerns about AI, ethics and emerging technologies (the Ethics of AI is highlighted in **Figure 2**, red line; Technology). Francis Bacon's philosophical vision in "The World is a Bubble" (number 13; pertains to the yellow line; Society in **Figure 2**) touches on uncertainties about knowledge, society, and the human condition, all central to today's debates on education, scientific authority, and values. By revisiting the lives and ideas of historical figures through SMS, we create a bridge between past and present uncertainties, showing that many of the questions we face today are deeply rooted in the scientific and philosophical legacies of earlier centuries.

The modern problems of migration are considered in "Where is my home?" (listed under number 1; light blue and dark blue lines in **Figure 2**) can be reflected based on Jacob Bronowski, British-Polish mathematician, biologist, philosopher, and historian of science (Edgerton, 2019). Bronowski is known for the influential BBC series "The Ascent of Man" (1973). May-Britt Moser - a Norwegian neuroscientist and Nobel Prize laureate in Physiology or Medicine (2014, with Edvard Moser and John O'Keefe) expressed that international collaboration and cultural openness are essential to science. She has emphasized the critical role of diverse collaboration and open scientific culture in advancing research: "We need all kinds of people doing science" - different people from different backgrounds bring different insights, highlighting the importance of inclusion and international teamwork (Danylova and Komisarenko, 2025).

SMS songs can be addressed to various topics outlined in the Map of Uncertainties - including ethical concerns, scientific responsibility, social impact, and the challenges of limited resources. They offer creative reflections on these themes through historical examples, making abstract uncertainties more tangible and relatable. Moreover, SMS songs go beyond the rational and pragmatic scope of the Map. These songs also capture the human, emotional, and existential dimensions of the scientific experience - feelings of wonder, doubt, loneliness, love, and inspiration. In this way, SMS provides a richer and more nuanced perspective, complementing analytical approaches with a poetic and deeply personal view of science and those who pursue it.

5. Discussion

The STEEP framework was introduced as a tool to explore current and future challenges. However, what is most significant is that many of the topics it includes have historical roots—issues that scientists in the past have already addressed or even solved. The goal of the SMS method is to spark new ideas, inspire innovation, and support more informed decision-making. This discussion demonstrates how creative methods like SMS can be applied in practice—through experiments that use artistic tools to map uncertainty and reframe our understanding of complex future challenges. The broader focus lies in the integration of STEAM approaches into futures thinking.

Mapping uncertainty becomes a pedagogical tool, helping learners connect emerging trends with historical patterns and cultural narratives. The SMS method provides a compelling example of how science and art can intersect to navigate the unknown. These songs offer a creative lens for exploring how scientists of the past were already engaging with questions that remain urgent today—such as education, citizenship, and planetary health. By giving musical form to their original poetic texts, the project invites us to revisit the past not as something fixed, but as a living dialogue - one that can inspire how we imagine and shape the future.

SMS aligns with the STEEP and supports the mapping of uncertainty in both present and future contexts. By applying Retrosight—a creative, backward-looking approach that begins with imagined futures and traces pathways back to the present—the method positions the Arts within STEAM as a strategic tool for futures-oriented learning and planning. Rather than viewing the past solely as history, Retrosight uses artistic expression to reinterpret past knowledge and experiences through the lens of future aspirations. As a management approach within the STEEP framework, it encourages broader reflection on how science, art, and education can work together to envision and co-create more inclusive and resilient futures. Inayatullah (2020) uses the term “walled past” as a metaphor for rigid institutional traditions, entrenched mindsets, and legacy structures that limit educational innovation. This "wall" prevents the emergence of transformative thinking by: 1. Reinforcing fixed schooling models (fixed ages, curriculum, standardized exams). 2. Maintaining conservative leadership responses—favoring continuity over creative alternatives. 3. Suppressing alternative futures in favor of the status quo, even when workshops initially generate bold visions.

In SMS, STEEP was used as a framework to illustrate how various scientists of the past time can reflect on contemporary challenges from different disciplinary perspectives. The SMS method offers a poetic and artistic response to STEP, inviting deeper reflection beyond conventional categories, where one field – Economy - is initially underrepresented, given the difficulty of addressing complex economic issues through song (although can be made in other styles, or to be re-directed towards Educational purpose). However, it still can be meaningfully reflected upon (in a broader sense). For example, an SMS composition on migration (primarily categorized under Politics, shown in light blue in **Figure 2**) can also be interpreted through an economic lens (dark blue in **Figure 2**), as migration is often driven by economic conditions. What matters is how these challenges were understood and addressed in the past - many of today’s issues, such as mass migration, have also historical precedents. SMS compositions reconnect with those past experiences, offering both emotional resonance and also insight. Additionally, these songs can be referenced in a way similar to destination branding, where music serves as a narrative tool to evoke place, memory, and identity.

6. Conclusions

The results of this work will most probably have profound consequences on the art practices of STEAM approach towards FFF, highlighting how each frames the anticipation of the future in different ways. Scientists might also try to introduce a reverse approach to future scenario-building, where alternative futures are not projected linearly but imagined creatively through art-based and STEAM-oriented practices. As recognized by the WHO (World Health Organisation) and the European Commission, Art is essential not only for health and homeostasis, but it serves as a key component of cultural sustainability.

The next step of this research is targeted toward the health or planetary health and sustainability issues with the cultural foresight dimensions. It may involve narrative foresight with arts-based methods, and cultural framing. The further research might be related to the very broad specter of topics: how various organisations and institutions respond to the art-related STEAM practices like SMS methods in different fields – in STEM disciplines, in Health and Medicine fields, in the area of Higher Education Management, Economics, and Finance, as well as Marketing and Branding (like Branding Cities or Branding Destinations). In addition, we can explore mapping uncertancies within a broader STEEP (Society, Technology, Economy, Environment, Politics) framework with Arts-related SMS method of STEAM. The contemporary and future challenges in social issues such as citizenship and migration (the migration issues were well present in the research of the 17-18-19 centuries due to migration of European population to USA, Africa and other continents) can be further explored. By reinterpreting uncertainty not as a limitation but as a creative space, the work suggests that science and education can prepare society for complex futures.

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Reflection

Z-STEAM activities at 13th Socratic Lectures Symposium, Con-cert at City Hall and Opera Coronation of Poppaea

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Abstract:
We report on the recent development within the Z-STEAM activities: the concert accompanying the 13.th Socratic Lectures symposium at the Trio Lorenz hall, palace Kazina on June 8, 2025, a concert at the City hall on May 9, 2025 and Opera Corronation of Poppaea at the Ljubljana Opera House on July 4 and 5, 2025. Two compositions were presented that were composed particularly for the 13th Socratic Lectures symposium, "Dedication to Science and the Arts", composed by the scientist Yelena Istileulova and "$E = mc^2$" by the musician Lara Oprešnik. Integration of science and music was demonstrated at its best practice. Z-STEAM activities are focusing on defining the added value of integration of science and art and on the respective improvements of curricula.

Keywords: STEAM; Science and music; Education innovations

1. Z-STEAM activities

STEM Education is an approach to learning that uses Science, Technology, Engineering, and Mathematics as access points for guiding student inquiry, dialogue, and critical thinking (Kralj-Iglič and Istileulova, 2025). STEAM integrates the Arts (music, poetry, visual arts, science (research and innovation) and culture (language, ideas, and beliefs) into the educational process with the aim to prepare workforce and literate citizens for a highly technology-based society (ESEP, 2025). It is stated in the European School Education Platform (ESEP, 2025) that understanding science, mathematics and technological and engineering practices, has become a global key priority for national education programmes (Kelley and Knowles, 2016). The United States´ Next Generation Science Standards (NGSS) enlist engineering design and practices into science education (NGSS Lead States, 2013). Germany created a national STEM forum to promote STEM education at all education levels, formal and informal (Nationales MINT (STEM) Forum, 2014). These activities are introduced with the aim to fulfill the contemporary needs that require strengthening the workforce in STEM areas to address global issues (Kelley and Knowles, 2016).

Socratic Lectures take part within the so-called Z-STEAM activities that include all possible support for effective scientific and educational performance. This includes adjustment of the curricula and examination methods, organization of Socratic Lectures symposia where students meet top scientists in the field, organization of cultural events, publishing activities and integration of art and science.

2. Z-STEAM activities at the Socratic Lectures symposia

Music was not integrated in the Socratic Lectures by planning or following some global educational trend. It happened by chance as in one of the early symposia around 2010, a chorus was practicing in a room next to the lecturing hall. Then, the symposia were centered on the undergraduate students of Medicine and the symposiums were in person, at the Faculty of Medicine, University of Ljubljana. Novelties in the examination process were already taking place. Scientists and experts generously donated their time and dedication to the students and the students were very supportive and active in discussion. They were completing the examination procedure, but the time of the symposium was already exceeded. By improvisation, the chorus Vox medicorum agreed to sing a song for the participants. After the last lecture, the chorus entered the lecturing hall and performed the song Ko boš prišla na Bled. It was a joyeous song perfectly fitted for the occasion, to lift up the spirit of the exhausted participants. Moreover, to the pleasure of the participants, it was announced that after listening to the music, all the students were merited the highest grade at the subject Biomechanics of the hip, for their dedication and support to the lecturers. This great experience indicated the necessity to include music in the symposia also in the future and indeed, music has been a part of all events since. It survived also during the COVID isolation period, moving online - together with the scientific part.

Figure 1. Program of the concert accompanying 13th Socratic Lectures Symposium 8.6.2025.

3. Internationalization and globalization of the Z-STEAM activities

Furthermore, the musical part was organized also within separate events that took place at the Castle Fužine, Museum of Architecture and Design, and continued in Naples, Italy, at the Palace Venezia, Ars Domus, Academy Enrico Caruso, and Vilino Mannina, and ultimately at the Palace Kazina, Academy of Music, University of Ljubljana.

Anita Prelovšek contributed substantially to the Socratic Lectures from the very beginning, by means of organizing and chairing sections, presenting lectures, writing papers, inviting guests, and performing music. Holding a PhD in Musicology, as well as being an excellent flutist by profession, she embodies all the qualities of the integration of science and art. Moreover, she established a link with the Russian Center of Science and Culture in Ljubljana where many excellent musicians originally from Russia were gathering at the concerts and other cultural events. Unfortunately, devastating war caused the centre to close; however, creation and friendship within the Socratic Lectures participants continues, involving those originally from Russia and Ukraine, as well as from other countries formerly included in the Soviet Union.

Yelena Istileulova is originally from Kazakhstan, she holds a PhD in Economical Sciences, but also a degree in classical music. Furthermore, as STEAM activities are in her scientific interest, she introduced them into the Socratic Lectures. Her idea is to write music on the stories on famous scientists and on the texts of these scientists. She performed and published songs featuring Baron Valvazor, Francis Bacon, Alfred Nobel, J. Robert Oppenheimer, Mikhail Vasiljevič Lomonosov and James Clerk Maxwell. For the first time she interpreted her own compositions on the concert accompanying 2nd Socratic Lectures in 2019. She supported Socratic Lectures also by organizing and chairing sessions, writing papers and editing the proceedings. Importantly, due to her personal invitations to the colleagues all over the world, she contributed significantly to the internationalization of the symposium, bringing to the symposium universities (including groups of students) from Ukraine, Kazakhstan and Georgia, as well as the lecturers from South America and Indonesia, thus up-grading the event to the global level. She established a link with the institutions such as EASE (EuropeAn network of steam Educators). Also, she participated at two concerts in Naples in 2024.

4. Creation of new music within the Z-STEAM activities

For the 13th Socratic Lectures symposium Yelena Istileulova composed a new song originally entitled: Hymn to Science and the Arts, and later – renamed to the Dedication to Science and the Arts (for voice and piano). She performed it herself as an opening composition of the concert. The text is writen in English and in Latin, and considers science, arts, music and Socratic questions. The presentation included also the video created by Yelena Istileulova.

The composition E=mc² by Lara Oprešnik was written for solo piano, inspired by the notorious equation, and interpreted into a musical, symbolic, and philosophical language. Using letters for the theme was previously done by Johann Sebastjan Bach, directly or transposed, as his name is composed of the letters that have a meaning in music (Stegall, 1965). Such approach was used also by others, and the idea of intruducing scientific contents was previously implemented by taking the letters of the genetic code (FEBS Communications, 2021). In the composition E = mc², symbols E (for energy), twice the C (for the speed of light), and E flat – mi (for mass), were used. From the initial sound of atoms, "nothingness," where the sound material only hints at the beginnings of energy, the composition gradually takes shape, gaining form, mass, and power. The various motives depict diverse forms of life. It develops into tonality, rhythm, harmonic, melodic, and dynamic clarity, while the strength of inner expression continuously shapes and builds until it reaches the peak of potential—the audio embodiment of the transformation of energy into matter. The ending signifies the disintegration of this matter, a return to the ineffable where the original theme appears as a reminiscence—changed through the experience of one's own process (in philosophy—through the experience of one's own life). The work thus is not only a study of a physical principle but also a contemplation on the cycle of life: on creation, existence, and transformation that transcend the physical (FE, 2025). The composition was performed by the author on the piano.

The third piece composed by integration of science and music was Cantique de Baruch Spinoza, by the author of this contribution. Cantique is a part of the Mass in E minor, which was written and performed publicly for the first time in 2017 at the Church of Assumption, Tromostovje, Ljubljana, and again at the 12th Socratic Lectures concert in January 2025. Originally, the mass was written for organ, orchestra, chorus and soloists, however, the partiture had to be adjusted for the musicians that were available at the event. As in January 2025 the concert was held at the Julij Betetto hall, the partiture was adjusted for organ, piano, flute, timpani and voice, while in June, the concert was held at the smaller Trio Lorenz hall and the partiture was adjusted for 2 pianos and voice. The text of the Cantique expresses the contents of the axiomatic presentation of Spinoza's philosophical model. It is written in Latin language: »Quid quod est in Deus est et nihil sine Deus esse. Deus est in omnium rerum causa imanens. Deus sive omnia. Dei atributa sunt eterna.« Spinoza's Ethics (Spinoza, 2004) expresses deep connection between science and religion. It is hard to understand why he was considered an apostate to the faith. Also it is hard to understand from where came the idea that science and religion are in contradiction. Considerable effort is needed to put on stage a new work written for an ensemble. The first performance in 2017 included organ (Dušan Ješelnik), two flutes (Anita Prelovšek and Veronika Kralj-Iglič), timpani (Špela Cvikl), soprano (Metka Penko Natlačen), tenor (Marjan Jarnjak) and a 4 voice chorus (Zimzelen). At the first glance the rhythm seems demanding and at the rehearsal, the musicians protested and required a conductor to help decrease the chaos. The success was partial and can be heard on the recording (Kralj-Iglič, 2025). The second performance at the Betetto hall included organ, timpani, flute and voice, and piano was

added, to take advantage of the availability of both, the organ and piano. However, the ensemble had only 15 minutes for rehearsal before the performance, which was not enough even to decide on registration of the organ. Therefore, the piece was performed again in June by an even smaller group (this time two pianos and voice). Timpani are crucial for this piece, however, the Trio Lorenz hall is rather small and the organ counterpart was missing, so it was decided to perform without timpani. It can be concluded from these experiences that practice and performance are necessary elements in obtaining feedback and improving the partiture in order to optimize the quality of the music performed. Furthermore, one can understand why there are multiple historical versions of scores that show significant differences between them, as the scores have to be adjusted to the cast and limitations (Kralj-Iglič and Prelovšek, 2025).

5. Slovenian vocal music

We outline the contribution of Elena Startseva Somun who performed in different ensembles as a pianist and also as a singer in the chorus Studenec Pivka (Irena Rep, Mateja Zadel, Elena Startseva Somun, Rafael Zorko, Renato Adam). The chorus presented Slovenian vocal music at its best.

7. Excellence in music

Notable were performances of professional musicians at the 13th Socratic Lectures symposium: Marina Igritskaya (soprano) accompanied by Yelena Istileulova, Neven Stipanov (bariton), and Branko Brezavšček (both accompanied by Elena Startseva Somun) and Lara Oprešnik (piano) (**Figure 2**). The performances were excellent, to the pleasure of the public.

Figure 2. Performances of excellent musicians were a true gift to the public. From the left: Lara Oprešnik, Elena Startseva Somun, Neven Stipanov, Marina Igritskaya in Yelena Istileulova at the rehearsal for the concert accompanying 13th Socratic lectures symposium 8.6.2025. Photo: Drago Videmšek.

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6. Young musicians

Young musicians from Musical school in Idrija, (Marina Žigon, Doroteja Poljanec, Matic Bogataj) performed on the piano. Arina Gusevskaya sang accompanied by Yelena Istileulova, Lan Stražišar Lamovšek played piano together with Alenka Stražišar Lamovšek, and Nuša Levec (who is supporting Socratic Lectures for many years) sang. Creations of young participants (**Figure 3**) are highly warranted.

Figure 3. Children played beautifully at the concert. From the left: Marina Žigon, Matic Bogataj, Arina Gusevskaya and Doroteja Poljanec at the rehearsal for the concert accompanying 13th Socratic lectures symposium 8.6.2025. Photo: Drago Videmšek.

At May 9, 2025 a concert within the Z-STEAM activities took place at the Red hall of the City hall, Ljubljana featuring Anita Prelovšek (flute), Branko Brezavšček (violin), Elena Startseva Somun (piano) and the author of this contribution as a guest. The program of the concert included Trio-sonata in B minor for flute, violin and piano by Georg Friedrich Handel, Old style suite for violin and piano by Alfred Schnittke, Sonatine for flute and piano by James Rae, Meditation from Souvenir d'un lieu cher op. 42 for violin, piano, Five small duettes op. 46 for flute, violin and piano, and Prelude in A-minor for piano by Davorin Zupanič Turković (first public presentation).

Figure 4. Concert at the City hall 9.5.2025; from the left: Elena Startseva Somun, Branko Brezavšček, Anita Prelovšek and Veronika Kralj-Iglič. Photo: Nevenka Gajšek.

8. Coronation of Poppaea at Slovene National Theatre Opera and Ballet

Within the interdisciplinary preparative project Nanostructurome led by the Faculty of Health Sciences, collaboration between science and music was officially established involving 9 partner faculties and Academy of Music of the University of Ljubljana. Within this collaboration, Academy of Music has put on stage at the Ljubljana Opera House the work composed by Claudio Monteverdi on the text of Francesco Busenello: Coronation of Poppaea. The premiere was on July 4, 2025 and the next day there was another performance. Representatives of the faculties involved at the project attended the performances. Music director and conductor was Egon Mihajlović, stage director Eva Hribernik, set designer Jaro Ješe, costume designer Andrea Quadri, lighting designer Andrej Hajdinjak, stage manager Višnja Fičor, make up artist Marijanka Sešek, hair stylist Tjaša Pugelj, women's hair stylist Meta Podkrajšek, language consultant Lucia Rizzello, assistant conductor and repetiteur Branimir Rezić, production assistant Vedran Kirin, assistant costume designer Neža Plestenjak, producers were Simon Dvoršak, Theresa Plut, Polona Kante, Katja Bogovič, Rebeka Dobravec, Maryna Dolibets, Lara Malenić and Črt Vodopivec. Technical team of the Opera and Ballet Ljubljana was led by Mislav Kuzmanić. Mentors of vocal soloists: Egon Mihajlović, Branimir Rezić, Pia Brodnik, Barbara Jernejčič Furst, Theresa Plut, Nuška Drašček and Matjaž Robavs.

Figure 5. Cast and a part of the orchestra of the Coronation of Poppea at Slovene National Theatre Opera and Ballet, July 5, 2025. Photo: Veronika Kralj-Iglič.

Baroque Orchestra of the Academy of Music consisted of Monika Toth, Vasilij Meljnikov, Hanna Kryvtsova, Marija Terezija Kolman, Zala Eva Kocjančič and Neža Feguš (violin), Tina Trivunović (viola), Kaja Kapus, Urša Roš (baroque cello), Stevan Jovanović (double bass), Domen Gvozdebovič, Eva Pišek (theorbo, baroque guitar), Branimir Rezić and Egon Mihajlović (harpsichord, portative organ).
Cast: Poppaea: Katarina Zorec/Talita Sofija Komelj, Neron: Ireneja Nejka Čuk, Octavia: Alessandra Tessaro, Ottone: Velten Schroeter, Drusilla: Isabel Gale, Seneca: Blaž Stajnko, Arnalta: Janja Teržan, Octavia's nurse: Eva Lavriha, Amor: Brina Vučković, Venus: Ronja Praprotnik, Virtue: Eva Kokot, Mercury: Aljaž Kolenko, young girl: Nives Hadžić, Lucan/first soldier/consult: Oleksandr Nastasiichuk, Freed man/second soldier/first student of Seneca: Fabijan Trbara, third student of Seneca/lictor/tribune: Bor Brenkuš Porenta.

The score features 28 singing characters and the production can use role-multiplying; the original Venetian production reported 11 singers: two female sopranos, three male sopranos (castratos), two contraltos (castratos), two tenors and two basses (Kralj-Iglič and Prelovšek, 2025). The production in 2025 reports 17 singers, 11 female (with Poppaea in alternation) and 6 male, however, 7 male singers can be seen in **Figure 4**.
On the 4th of July, it was a hot day; the reported temperature in Ljubljana was about 33 degrees. Elena Startseva Somun and myself were seated at the 2nd balcony (former standing area). Hot air is traveling up and it was gathering at the top. The sight on the stage from there is only partial and one cannot see the details for being high up, so the music is more exposed, in particular as the acoustics at this place is very good.
In preparation for the premiere, I was listening to different interpretations of the Corronation of Poppaea, and with time have accepted the peculiarities of the male soprano. The pitch of the Nero's part (being writen for the castrato) surprised me when I heard the opera for the first time. Today, technique allows men to sing the castrato parts, however, in this case, the color of the voice indicated that Nero was interpreted by a woman. On 5th of July, the temperatures went down to enable sharper listening, and my seat was in the first row of the parterre, in front of violins. I wished to see the performers closely. The orchestra shell was lifted so one could follow playing the instruments too. I noticed baroque instruments on the other side, and was sorry that I could not be closer to them.
Already the opening accords at the premiere announced energetic interpretation of the timeless music composed by Monteverdi. I sensed the essential frame interpreted by Egon Mihajlović at the harpsicord embeddeding the idea of the spiritus movens of the production. As described below, all the elements of the opera matched the excellence of the music written. Both days the music (the composition, singing and playing) was supreme. It was a pleasure to be immersed in the musical substance during the performances and also between them, listening in the memory the motifs so clearly and plastically presented. In particular, the bars of the arias such as Pur ti miro, pur ti godo; Non morir, Seneca, non morir, Seneca; Oni mal, oni mal diventa gioia, etc. It was a perfect combination: first to acknowledge the work as a whole feeling mostly the energy and the musical substance as a whole, then listen in mind to the echoes for one day, and finally consume the music again,

but closely and with more details (individual singing, acting, moving, playing, and the elements of directing and scenography). The stage directing complemented music in a sense that it outlined beauty and elegance. Rude and vulgar gestures were absent as well as annoying noises or screams. There was only one sound sticking out – i.e. Ottone throwing away a (fake) weapon, the object hitting the ground and producing a hollow noise. Light was focused on the singers. It enabled effortless following of the happening on stage. Some modern productions shock the audience with featuring blood and violence of murders taking place behind the libretto. Here everything was done classically: the person to be removed (even Seneca) was taken away in full standing, by moving the stage. By adjusting the story to the point to which the data were followed, the audience was left to believe that all but Seneca were alive. The acting upgraded the music considerably. While Poppaea's aspirations were fulfilled due to Nero's love for her, Octavia turned out desperate and vicious – conspiring the murder of Poppaea out of jealousy - and failing it. Ottone's miseries (being pressured to murder Poppaea in frustration between love, and offense for betrayal) were evident and the voice of Velten Schroeter was optimally suited for the role. Prologue outlines the ethical issues of Poppaea's rise to the throne. In contradiction, here they were not challenged but justified with the arguments of all three: Fortune, Virtu and Love. The costumes were made to original images (**Figure 4**), revival of shoes was lovely, sandals appeared more like jewels to complete the looks. The fashion of old Roman aristocrates allowed men to expose feet and legs which could, in particular in youth, add to the beauty and elegance of the ambient. Attention has been paid to the hairdo and accessoirs. From close, I could resolve widened shoulders and facial mask of Nero exhibiting a beard, but I could not overcome the fact that Nero was a woman disguised in a man, which was even more apparent from close by. Scenography was minimalistic and seemed more Shakespearean than Venetian. It is a question whether such scene was actually exposing the cast and the music even more than a hypothetical opulent one. The floor was bare, as used in rehearsals, exhibiting lining on the black-painted wood which made a particular impression, as there is a lot of content in those lines. All the cast and orchestra were excellent, in technique and in interpretation. Singing and playing was mutually supportive which contributed to the unification of music. The two nights devoted to Monteverdi/Busenello Coronation of Poppaea will be remembered as one of the best productions I have witnessed at the Ljubljana Opera House since I first set my foot in it more than 50 years ago.

9. STEAM and STREAM

Since the spontaneous onset of the STEAM approach, generous support of all who have donated to the Socratic Lectures, have led to focusing the activities to the benefit of students. An important element is involvement of the students in scientific production – i.e. publication of scientific results. Students are being actively involved in writing (STREAM) and publishing in the Proceedings of Socratic Lectures. While publication in scientific journals and participation in scientific meetings by default requires funding to cover the costs of editing, reviewing, formatting and publishing in open access mode, the Socratic Lectures since 2019 provided these services free of charge. The largest share

in these activities is merited to Anna Romolo who has designed, edited and formatted all 13 volumes of the proceedings. She is a custos of the Gallery Marguerite de Saint Champs featuring visual art between the scientific papers, yet another dimension of the collaboration between science and arts, which will be considered in more detail elsewhere.

10. Conclusions

While STEAM principles have been already widely implemented in the education up to high school level, their introduction into university teaching has been only recently gaining an increasing interest. Our integration of science and arts has hitherto led to involvement of undergraduate students in scientific and artistic activities. Also it includes composing of music inspired by science and pursuing excellence in science and music in joint projects. We envisage these achievements as an incipient stage of the process, and are looking forward to their development in the future.

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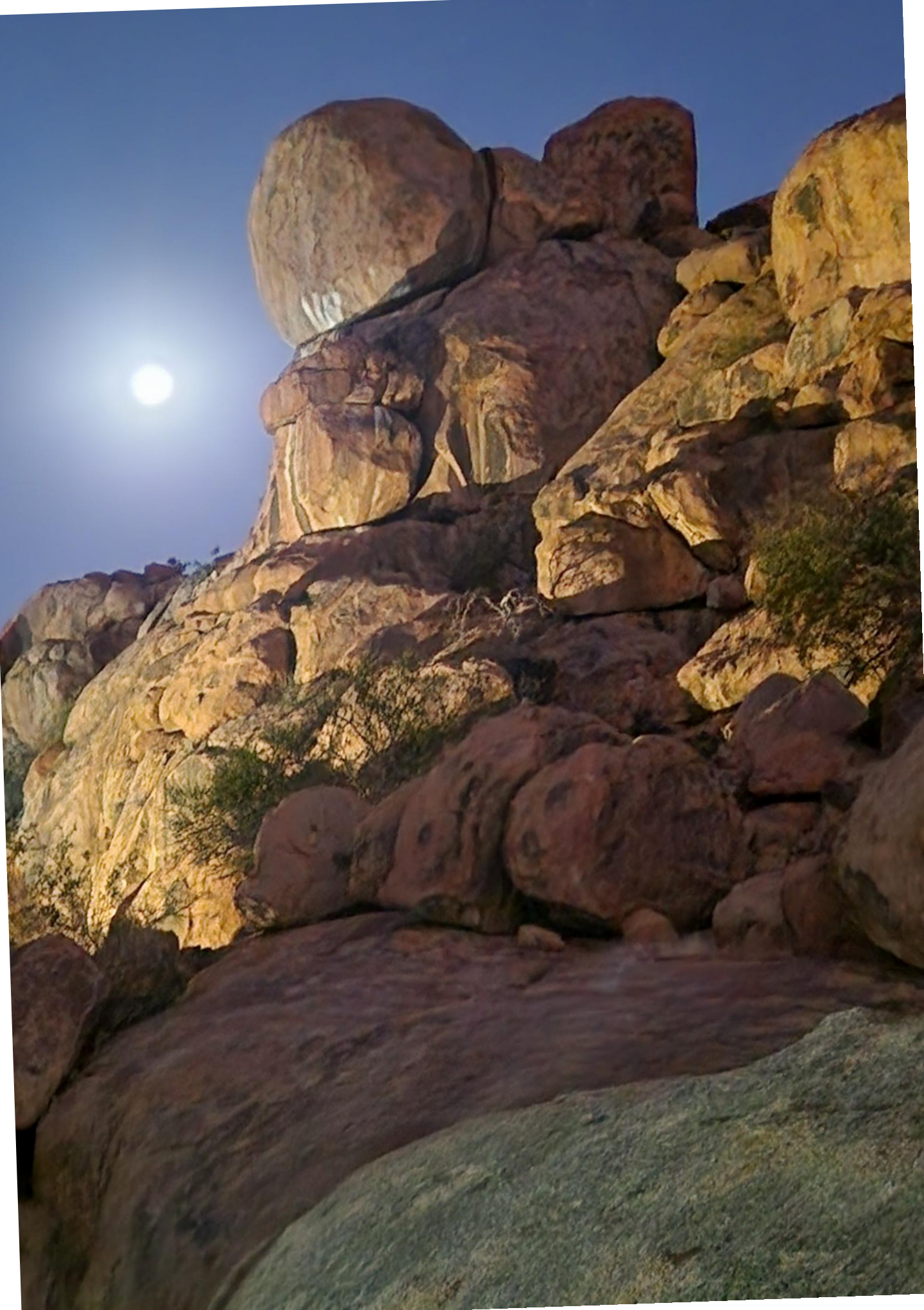

Research

Charging and Discharging a Capacitor: A Case Study in Solving Differential Equations with Separable Variables

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Abstract:
This article looks at the mathematical modelling of an electrical circuit that involves charging and discharging a capacitor. It is a great way to understand and solve first-order differential equations with separable variables. First, how to solve separable differential equations is explained. Second, a detailed derivation of the associated differential equations for discharging and charging are presented, followed by analytical solutions. This study shows how important mathematical ideas are used in solving real-world problems, especially in electrical engineering.

Keywords: Differential equations; Separable variables; Charging a Capacitor; Discharging a Capacitor

1. Introduction

Differential equations are very important tools for modelling lots of different physical problems. In electrical engineering, one example of this is the discharging of a capacitor through a resistor. This problem can be solved by using a first-order differential equation. Another similar example of this is charging a capacitor. This article uses these examples to show how differential equations with separable variables can be used to model and analyse dynamic systems.

2. Theoretical background: Separable differential equations

2.1. *Definition*

A first-order ordinary differential equation is called *separable* if it can be expressed in the form

$$y'(x) = f(x)g(y). \tag{1}$$

This means the derivative of y with respect to x can be written as a product of a function solely of x and a function solely of y.

2.2. *Algorithm for solving separable differential equations*

Separable differential equations can be solved in four steps.

Step 1. In the differential equation $y' = f(x)g(y)$ rewrite y' as $\frac{\mathrm{d}y}{\mathrm{d}x}$ and get

$$\frac{\mathrm{d}y}{\mathrm{d}x} = f(x)g(y). \tag{2}$$

Step 2. This allows us to separate the variables. Use algebra to change the equation to get all y-related terms with $\mathrm{d}y$ on one side and all x-related terms with $\mathrm{d}x$ on the other side of the equation:

$$\frac{\mathrm{d}y}{g(y)} = f(x)\,\mathrm{d}x. \tag{3}$$

Step 3. Integrating both sides of the equation independently yields:

$$\int \frac{1}{g(y)}\,\mathrm{d}y = \int f(x)\,\mathrm{d}x. \tag{4}$$

Solve these integrals and, if possible, express y explicitly in terms of x.

Step 4. Use any given initial conditions to find the value of the constant of integration.

This technique is a simple way to solve many first-order ordinary differential equations. These equations are found in physical, biological and engineering applications. Separable equations are useful when modelling how a system changes over time gives a product of functions of the dependent and independent variables. As we said, this feature lets us separate the variables and then integrate both sides of the equation. Many pure mathematical examples can be found in (Boyce, 2017).

3. Example: Discharging a capacitor

Imagine a capacitor with capacitance C that is initially charged to a voltage U_0. It is then connected to a resistor with resistance R (see Figure 1). Our goal is to find the function $U_C(t)$ that describes how the voltage across this capacitor changes over time.

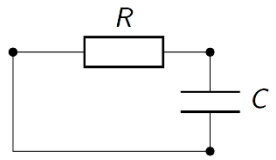

Figure 1. Discharging circuit diagram.

3.1. Required pre-knowledge

Tackling this problem requires some prior knowledge (Nilsson, 2023; Serway, 2019).

- Ohm's Law: The voltage across the resistor, U_R, equals the product RI (where I is the current)

$$U_R = RI \tag{5}$$

- Capacitor Relationship: The current I through a capacitor is related to the rate of change of voltage across it by

$$I = C\frac{\mathrm{d}U_C}{\mathrm{d}t} \tag{6}$$

- Second Kirchhoff's Law (Voltage Law): For a closed loop, the sum of voltages is zero. In our discharging circuit (with no external voltage source in the loop during discharge), this means

$$U_R + U_C = 0 \tag{7}$$

3.2. Modelling the RC circuit

We start with Kirchhoff's Law:

$$U_R + U_C = 0. \tag{8}$$

Substituting Ohm's Law yields:

$$RI + U_C = 0. \tag{9}$$

Substituting the capacitor relationship yields:

$$RC\frac{\mathrm{d}U_C}{\mathrm{d}t} + U_C = 0. \tag{10}$$

This is our (first-order linear homogeneous) differential equation describing the voltage across the capacitor during discharge.

3.3. Solving the discharging differential equation

First, we rearrange the equation to isolate the derivative term. This is a separable differential equation.

$$\frac{\mathrm{d}U_C}{\mathrm{d}t} = -\frac{1}{RC}U_C \tag{11}$$

We group terms involving U_C on one side and terms involving t on the other.

$$\frac{\mathrm{d}U_C}{U_C} = -\frac{1}{RC}\,\mathrm{d}t \tag{12}$$

Next, we integrate both sides. This yields:

$$\log|U_C| = -\frac{1}{RC}t + K, \tag{13}$$

where K is the constant of integration. Let us denote this constant as $\log(D)$ for convenience (and assuming $U_C > 0$):

$$\log(U_C) = -\frac{1}{RC}t + \log(D). \tag{14}$$

To solve this equation for U_C, we exponentiate both sides (we compute an antilogarithm).

$$U_C(t) = D\mathrm{e}^{-\frac{1}{RC}t} \tag{15}$$

Now, we apply the initial condition: at $t = 0$ s, the voltage is U_0. So, $U_C(0) = U_0$. This means $U_0 = D\mathrm{e}^0$ and therefore $D = U_0$.

Finally, the function describing the voltage across the discharging capacitor is:

$$U_C(t) = U_0\mathrm{e}^{-\frac{1}{RC}t}. \tag{16}$$

3.4. Graphs for discharging

The last obtained equation shows an exponential decay of voltage. The product RC is known as the "time constant" of the circuit, often denoted by τ. It determines how quickly the capacitor discharges. As you can see in Figure 2, if $RC = 7$ s, the voltage drops to a certain level. If RC is larger, say 10 s, the discharge is slower. Similarly, if you look at the second row in Figure 1, you can see that the initial voltage U_0 affects the whole curve. If U_0 is higher, the discharge starts from a higher voltage, but the shape of the decay, which is determined by RC, stays the same.

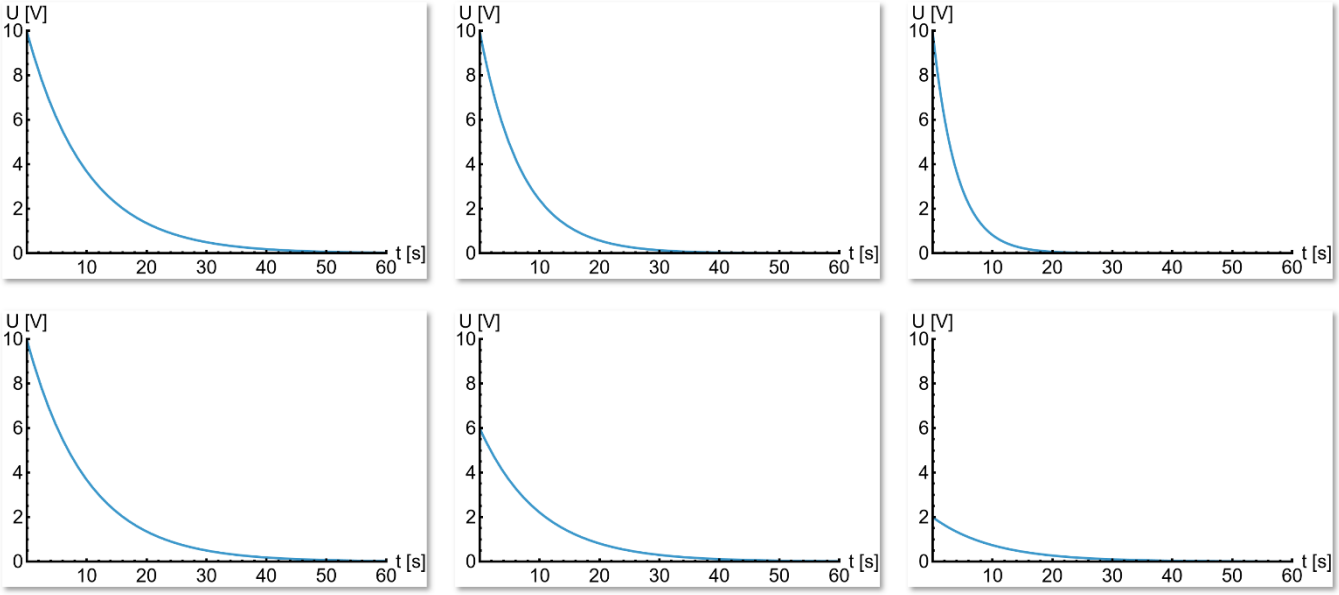

Figure 2. Discharging a capacitor. The first row shows how voltage decreases with different values of RC (10 s, 7 s, and 4 s with $U_0 = 10$ V) and the second row shows how voltage changes with different values of initial voltage U_0 (10 V, 6 V, and 2 V with $RC = 10$ s).

4. Example continues: Charging a capacitor

We revisit our RC circuit, but this time, we will look at charging a capacitor.

Imagine a capacitor with capacitance C, initially having zero voltage: $U_C(0) = 0$ V. At the time $t = 0$ s, we connect it to a resistor with resistance R and a DC voltage source with a constant voltage U_{source} (see Figure 3).

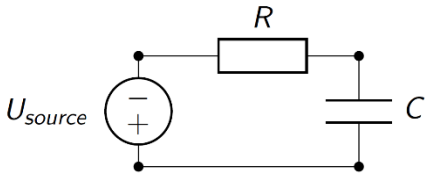

Figure 3. Charging circuit diagram.

We want to find the function $U_C(t)$ that describes how the voltage across the capacitor changes over time as it charges.

4.1. What is different from the discharging case?

- Ohm's Law $(U_R = RI)$ still holds.
- The capacitor relationship $\left(I = C\frac{\mathrm{d}U_C}{\mathrm{d}t}\right)$ still holds.
- However, Kirchhoff's Second Law now includes the voltage source:

$$U_R + U_C = U_{\text{source}}. \tag{17}$$

4.2. Modelling the charging RC circuit

We start with the Eq. (17) - modified Kirchhoff's Law.
Like in the discharging case, we use Ohm's law and substitute the capacitor relationship. We get

$$RC\frac{\mathrm{d}U_C}{\mathrm{d}t} + U_C = U_{\text{source}}. \tag{18}$$

4.3. Solving the charging differential equation

This is again a separable differential equation.
We can rearrange it as before (U_C on one side and t on the other side of the equation)

$$\frac{\mathrm{d}U_C}{U_{\text{source}}-U_C} = \frac{1}{RC}\,\mathrm{d}t. \tag{19}$$

Integrating both sides yields

$$-\log|U_{\text{source}} - U_C| = \frac{1}{RC}t - \log(D). \tag{20}$$

Next, we multiply both sides by -1, exponentiate both sides, and express U_C explicitly

$$U_C = U_{\text{source}} - D\mathrm{e}^{-\frac{1}{RC}t}. \tag{21}$$

By applying the initial condition $U_C(0) = 0$ V it follows $D = U_{\text{source}}$ and therefore the final solution for charging a capacitor follows

$$U_C = U_{\text{source}}\left(1 - \mathrm{e}^{-\frac{1}{RC}t}\right). \tag{22}$$

4.4. Graphs for charging

The last obtained equation shows that the voltage across the capacitor increases from 0 and exponentially approaches the source voltage U_{source} (see Figure 4).

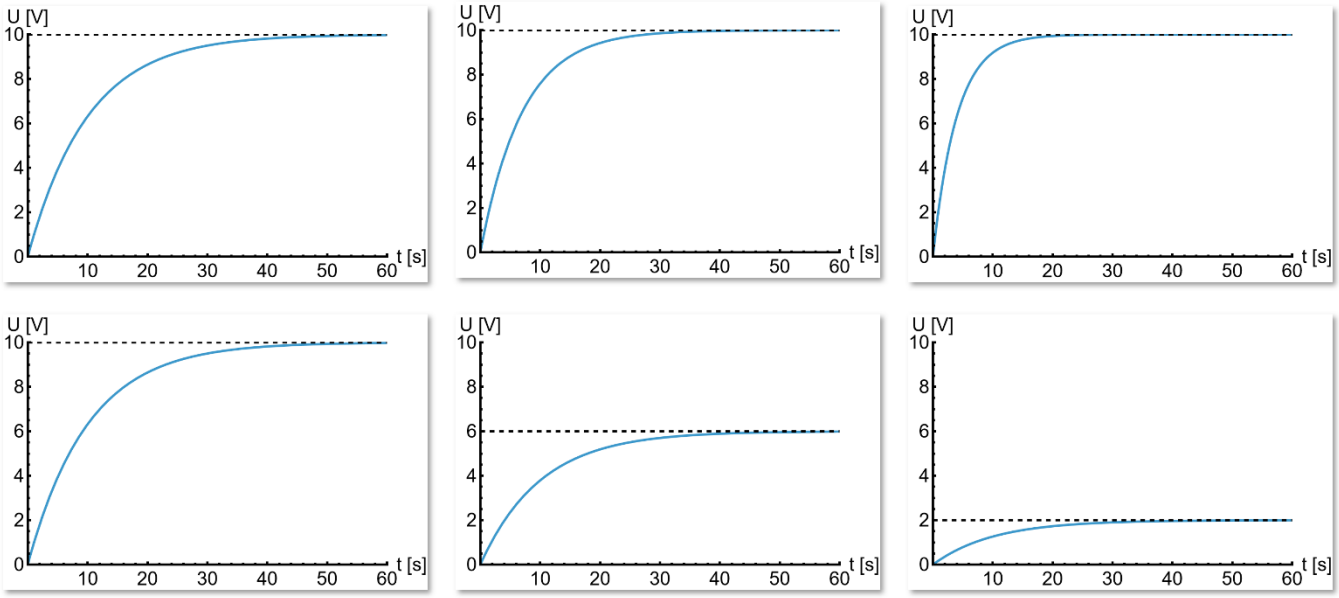

Figure 4. Charging a capacitor. The first row shows a rise of voltage with different values of RC (10 s, 7 s, and 4 s with $U_0 =$ 10 V), and the second row shows different values of source voltage U_0 (10 V, 6 V, and 2 V with RC = 10 s). The dashed line shows the target voltage, U_{source}.

Again, the time constant RC determines how quickly this charging process happens. The dashed line shows the target voltage, U_{source}. You can see that for smaller RC values (e.g., $RC = 4$ s), the capacitor charges faster towards U_{source} than for larger RC values (e.g.,

$RC = 10$ s). The different values of U_{source} simply set the maximum voltage the capacitor is trying to reach.

5. Some broader applications of separable differential equations

Separable differential equations are useful for much more than just RC circuits. You can use them to model many natural phenomena. Here are some examples. You can find even more examples in books and articles (e.g., Boyce, 2017; Braun, 1993; or Kreyszig, 2011).

5.1. Radioactive decay

The rate of decay of a radioactive substance $\frac{\mathrm{d}N(t)}{\mathrm{d}t}$ is proportional to the current amount of substance $N(t)$:

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = -k\,N(t). \tag{23}$$

The solution is

$$N(t) = N_0\,\mathrm{e}^{-kt}, \tag{24}$$

where N_0 is the initial amount and k is the decay constant.

5.1. Newton's law of cooling

The rate of change of temperature of an object $\frac{\mathrm{d}T(t)}{\mathrm{d}t}$ is proportional to the difference between its temperature $T(t)$ and the temperature of its surroundings $T_{\text{surroundings}}$:

$$\frac{\mathrm{d}T(t)}{\mathrm{d}t} = -k\left(T(t) - T_{\text{surroundings}}\right). \tag{25}$$

The solution is

$$T(t) = T_{\text{surroundings}} + \left(T_0 - T_{\text{surroundings}}\right)\mathrm{e}^{-kt}, \tag{26}$$

where T_0 is the initial temperature.

5.1. Population growth (unlimited resources)

In an environment with unlimited resources, the rate of population growth $\frac{\mathrm{d}N(t)}{\mathrm{d}t}$ can be proportional to the current population size $N(t)$:

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = k\,N(t). \tag{27}$$

This leads to exponential growth:

$$N(t) = N_0\,\mathrm{e}^{kt}, \tag{28}$$

where N_0 is the initial population size.

5.1. Logistic growth (limited resources)

A more realistic population model considers a maximum carrying capacity $N_{\max}$. The growth rate is then

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = k\,N(t)\left(N_{\max} - N(t)\right). \tag{29}$$

The solution is

$$N(t) = \frac{N_{\max}}{1 + \left(\frac{N_{\max} - N_0}{N_0}\right) e^{-N_{\max} k t}} \tag{30}$$

where N_0 is the initial population size.
The solution shows that growth is slowing as it approaches the limit which is $N_{\max}$.

5. Conclusion

We have shown how we can define separable differential equations and how to solve them. We used this to model and analyse how a capacitor charges and discharges in a Resistor-Capacitor circuit. This way we derived the exponential decay and rise of voltage, respectively. Finally, we saw that these mathematical patterns are basic and appear in many different areas, from radioactive decay to population dynamics.
The key takeaway is the power of identifying a mathematical structure — like separable differential equations — and then use a method to find solutions that can be used to predict how things will behave in lots of different physical and biological systems.

Conflicts of interest: The author declares no conflict of interest.

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Research

Gender Identity and Social Perception in Balinese Performing Arts: Cross-Dressing Practices and Their Impact on Artists' Mental Health and Social Acceptance

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Abstract:
Cross-dressing in performance traditions is a global phenomenon that reflects complex intersections between gender, identity, and social norms. In Balinese performing arts, cross-gender performance are both longstanding convention and where social identity is negotiated. This study explores two such roles in Calonarang ritual performance: the comedic Liku and the antagonistic Matah Gede. Using qualitative methods—including semi-structured interviews with performers and analysis of textual and recorded performances—the research explores the sociocultural framing of these roles. Matah Gede is typically performed by heterosexual men of high social standing, often priests, whose cross-gender portrayals are ritually sanctioned and widely accepted. In contrast, Liku is often performed by queer-identifying individuals of varied social status. These performers describe the role as both a form of artistic expression and a socially sanctioned outlet for gender fluidity, with some noting its positive impact on their mental well-being.
The study argues that gender in Balinese performance is not merely representational but performative, ritualized, and relational—capable of both reinforcing and subverting social norms. Beyond Bali, these findings contribute to global gender studies by showing how traditional performance can provide culturally legitimate spaces for queer expression and emotional resilience in conservative contexts. This research thus enriches broader conversations around identity, embodiment, and the mental health dimensions of performance.

Keywords: Gender identity; Social perception; Balinese performing arts; Cross-dressing; Mental health

1. Introduction

Cross-dressing in performance has been a recurring and complex phenomenon across global cultural histories. Far from being merely theatrical or comedic, it offers valuable insight into how gender roles are socially constructed, challenged, and negotiated. In many traditions, cross-gender performance is not only accepted but institutionalized, reflecting deeper cultural logics surrounding identity, representation, and social norms.

Historically, theatrical practices have often relied on gender crossing as a means of fulfilling performative needs, especially in societies that restricted participation based on sex. Cross-dressing which can be defined in its simplest form as "the act of wearing items of clothing not commonly associated with one's sex" (Ben-Iheanacho et al., 2023, p. 390). In Elizabethan England, for instance, the prohibition of female actors led to all roles, including those of women, being portrayed by men (di Laurea, 2023). Similarly, Japanese Kabuki theatre institutionalized the role of *onnagata*, male actors trained to perform highly stylized female characters (Isaka, 2023). These cases demonstrate how cross-dressing in performance can emerge from social constraints while simultaneously cultivating its own aesthetic traditions and symbolic meanings.

In Southeast Asia, analogous practices have also been part of long-standing cultural repertoires. In the Lengger Lanang tradition of Banyumas, for example, male dancers perform female roles with femininity not only tolerated but celebrated (Hartanto, 2016). Such forms suggest that gender, when performed through the arts, can be rendered fluid, liminal, and deeply symbolic—an idea that resonates particularly in the context of Balinese performing arts.

Historically, Balinese performing arts during the era of the kingdoms were marked by gender exclusivity. Classical genres such as Gambuh, Topeng, and Arja were originally performed exclusively by men, even when the narratives included female characters (see Bandem, 1983; Dibia, 1992; Saba, 2002; Prasetyo, 2018). Significant shifts began to emerge in the early 20th century, as women increasingly participated as dancers and performers. Female roles that had previously been portrayed by men were gradually assumed by women, signifying a transformation in gender representation on the performance stage. Over time, even roles traditionally considered the exclusive domain of men—such as puppeteers and mask dancers—began to be recognized as artistic professions accessible to women (see Coldiron et al., 2015; Goodlander, 2010, 2012, 2016; Singarsa et al., 2024).

In Bali, cross-gender performance holds a distinctive position, situated at the intersection of ritual, community life, and artistic expression. Rather than functioning solely within the domain of entertainment, performance in Bali often serves spiritual and ceremonial functions, embedding gendered enactments within a broader cosmological and ethical framework. Here, gender is not only portrayed but also dynamically negotiated—performed in ways that reflect both cultural continuity and personal expression. These performances challenge binary understandings of gender by foregrounding flexibility, ambiguity, and transformation.

This study focuses on the gender dynamics within Balinese performing arts by examining specific instances of cross-gender performance, especially within the context of ritual drama and dance. Two prominent figures are central to this analysis: the comedic Liku character and the antagonistic Matah Gede in Calonarang performances. Both roles are traditionally performed by men embodying female personas, and their portrayals often carry complex affective, social, and symbolic weight. The Liku, often humorous and grotesque, navigates the tension between ridicule and familiarity, while the Matah Gede, embodying witch-like power, destabilizes conventional female archetypes.

While such practices are culturally embedded, they also provoke critical questions about how artists who embody these roles are perceived in contemporary Balinese society. As Balinese culture continues to evolve within a globalized and increasingly conservative socio-political environment, performers who regularly cross gender boundaries may face stigma, misrecognition, or psychological strain. This study therefore considers not only the aesthetic and symbolic dimensions of gender performance but also its social and mental health implications for the artists involved.

By tracing the historical, cultural, and psychological layers of gender-crossing performance in Bali, this research aims to contribute to broader discourses on gender performativity, cultural resilience, and the role of the arts in mediating social acceptance and identity formation. It invites a rethinking of how traditional art forms both reflect and shape contemporary understandings of gender and selfhood in an increasingly plural yet contested cultural landscape.

2. Methodology

This study employs a qualitative research approach that integrates performer interviews with textual and performance analysis to explore the intersections of gender, identity, and cultural practice in Balinese performing arts. Data collections were conducted by the authors between 2024 and early 2025 in Bali through semi structured interviews in the Balinese and Indonesian language.

Primary data were collected through in-depth interviews with dancers who have undergone extensive training and are widely recognized for their portrayals of the characters Liku and Matah Gede, primarily in the Gianyar and Denpasar regencies. These performers were selected not only for their technical expertise but also for their embodied knowledge of cross-gender roles within Balinese performance traditions. While the Matah Gede dancers are heterosexual, most of the Liku dancers identified as queer and requested anonymity in this research. The ages of the interlocutors ranges from 20 to 55 years old.

In addition to the interviews, the research includes close analysis of recorded performances to examine how gender expression is constructed and communicated on stage. This analysis is further supported by an extensive review of relevant literature, including scholarly works on Balinese dance, gender theory, and ritual performance. Together, these methods provide a contextual and interpretive understanding of how performers negotiate social identity through artistic practice, and how cross-gender roles operate within the broader socio-cultural landscape of Bali.

3. Results and Discussions

3.1 Cross-Gender Embodiment in Calonarang: Ritual Authority and Symbolic Liminality

Among Bali's most renowned ritual dance-dramas, Calonarang dramatizes the legend of Ni Calonarang, a powerful widow and practitioner of witchcraft who unleashes destruction upon the kingdom of Daha (Bandem & deBoer, 1995). Central to this performance is the figure of Matah Gede, a terrifying manifestation of Calonarang's supernatural forces. The name Matah Gede, meaning "half-cooked" or "not fully formed" (see also Wirawan, 2019; Pratita, 2020) captures the liminality of the character—occupying a space between the human and the supernatural, the domestic and the chaotic, the gendered and the indeterminate.

Portraying Matah Gede requires not only mastery of dance technique but also an ability to navigate formal linguistic registers, as the character often speaks in kawi (Old Javanese) poetic verse. Traditionally performed by senior male artists, often those with ritual or spiritual authority, the role reflects a broader cultural logic where certain spiritual and aesthetic responsibilities are encoded through gendered expectations. Despite the character being female, no documented instances of women performing Matah Gede exist—highlighting a culturally sanctioned convention of cross-gender ritual embodiment. This exclusivity is not arbitrary. Within Balinese performance cosmology, embodying Matah Gede is not merely an act of representation but one of invocation. The performer is believed to temporarily channel a supernatural force that must be contained, respected, and ritually managed. As such, the performer must be someone deemed spiritually "capable"—a status often aligned with male practitioners such as *balian* (traditional healers) or individuals with a priestly lineage. In this context, the act of cross-gender performance is not only legitimate but essential: it embodies both symbolic inversion and spiritual responsibility.

What emerges here is a ritualized construction of femininity—an idea of the female not simply as a gendered subject but as a force, a symbolic embodiment of power, danger, and transformation. Ironically, this potent representation of female power must be enacted by

male bodies, perhaps as a form of symbolic containment. Thus, the cross-gender portrayal of Matah Gede reveals a layered discourse: femininity as a site of power, mediated through masculinity for the sake of cultural, ritual, and social stability.

While the portrayal of Matah Gede is ritually sanctioned and socially honored, performers still navigate intense emotional and psychological demands. The role requires not only technical skill but also a capacity to internalize and convey symbolic terror, power, and otherness—often leading to a heightened state of affective engagement. For many senior performers, this embodiment affirms their spiritual authority and cultural value, yet it can also induce emotional strain due to the gravity and complexity of the character. Although performers of Matah Gede may not face the same social stigma as those portraying Liku, they still engage in cross-gender embodiment that challenges personal and public understandings of identity, often in ways that affect their inner emotional life and social self-concept.

3.2 Arja and the Evolution of Cross-Gender Performance

While Calonarang performances reveal how cross-gender performance is negotiated through ritual authority and spiritual symbolism, the case of Arja illustrates a more accessible, community-based context in which cross-dressing practices shape performers' social standing and personal identity. Here, the impact of gendered performance extends beyond ritual significance, touching directly on questions of mental health, identity expression, and public reception—especially among performers whose gender identities diverge from heteronormative expectations.

Outside the explicitly ritual sphere of Calonarang, Balinese traditional theatre also provides fertile ground for analyzing gender performance. Arja, a form of Balinese dance-opera that emerged in the early 19th century, originally excluded women from the stage, leading male performers to assume all roles (see Desiari, 2017; Sadguna, 2018; Desiari & Suratni, 2022). This early mode of cross-dressing was driven more by practical and religious constraints than by aesthetic preference. However, as women gradually entered the stage in the mid-20th century, Arja underwent significant gender realignments.

Dibia mentioned that, during the 1960s and 1970s, the Arja scene witnessed a notable shift: female performers began portraying heroic male leads, while male actors were relegated to secondary or comedic roles (Dibia, 1992). This reversal of normative gender hierarchy onstage revealed the genre's flexibility in accommodating and subverting social expectations. Rather than stabilizing gender binaries, Arja allowed for their creative reconfiguration.

In response to waning popularity in the late 1980s, a new format emerged: Arja Muani, an all-male troupe that foregrounded cross-dressing as a central comedic device (Saba, 2002). Here, the performative act of gender became a deliberate aesthetic and humorous strategy. Male performers donning female attire—often exaggerated in style and affect—revitalized audience interest by infusing traditional narratives with fresh visual spectacle and comic timing. This evolution marked a shift from necessity to theatrical choice, with cross-dressing recast as a mode of satire and social commentary.

In this recontextualization, gender performance became a key site of artistic identity and public engagement. For some performers, particularly those whose gender identity or sexual orientation diverged from heteronormative frameworks, Arja Muani offered a culturally permissible arena for expression. Rather than transgressing social norms outright, cross-dressing within Arja became a strategic practice: it allowed performers to negotiate personal identities under the protective guise of tradition and entertainment.

3.3 The Role of Liku: Comedy, Queerness, and Psychosocial Negotiation

One figure emblematic of this negotiation is the character of Liku, a flamboyant and often grotesque female role typically performed by men. Within Arja troupes—both mixed-gender and all-male—Liku has become a recurring and recognizable figure, associated with humor, satire, and social inversion. Often adorned with exaggerated makeup, high-pitched voice, and gestural excess, Liku appears to mock femininity; yet within this performance lies a more complex set of social, emotional, and psychological functions—particularly for performers who use the role as a channel for identity expression and emotional coping within a culturally sanctioned space.

For queer performers in particular, the Liku role operates as a culturally sanctioned outlet for expressing gender variance. In everyday Balinese society, where heteronormative expectations remain strong, individuals who do not conform to traditional gender roles often face subtle marginalization or exclusion. However, within the codified space of performance and ritual frameworks, these same individuals may achieve visibility, acclaim, and even reverence. For many, this opportunity to perform Liku provides more than social validation—it becomes a psychosocial buffer against the mental stress of everyday marginalization, offering performers emotional relief, pride, and a sense of stability.

The social legitimacy of performing Liku does not lie solely in its comedic appeal but in its affective resonance. Audiences may laugh at Liku, but they also applaud, recognize, and celebrate the performer's artistry. This dual response—laughter and approval—creates a powerful psychosocial loop: the stage becomes a space where social norms are both enforced and subverted, where marginal identities are momentarily centered through humor, talent, and spectacle. This circulation of laughter and recognition functions as a form of community care—replacing shame with shared joy, and allowing performers to experience psychological affirmation within the bounds of tradition.

3.4 Performance as Psychosocial Survival and Cultural Carework

For many performers, particularly those identifying as queer or gender non-conforming, embodying Liku is more than an artistic act—it is a crucial form of mental health support (see also Wedastra & Lesmana, 2015). Cross-dressing in performance offers a rare avenue for self-expression and emotional validation in a cultural context where such expressions are often constrained. The theatrical space becomes a psychosocial refuge, allowing performers to explore aspects of their identity that may otherwise be repressed in daily life. Ethnographic interviews reveal that the experience of playing Liku often provides a profound sense of psychological relief. Performers describe the role as affirming, cathartic, and empowering, offering them a temporary yet vital sense of visibility and self-worth. Being seen and applauded—even within the safety of character—acts as a proxy for deeper social recognition, helping to mitigate feelings of marginalization and emotional distress. In this sense, the performance becomes a form of emotional survival—a protective mechanism that contributes directly to mental well-being.

This dynamic illustrates a form of psychosocial negotiation, wherein performers operate within traditional cultural limits while simultaneously pushing against them. The stage serves as a liminal space where identity is both concealed and revealed, where stigma is suspended, and where personal authenticity meets communal validation. Cross-dressing, in this context, is not merely theatrical—it is a therapeutic act. It allows performers to channel internal struggles into artistic form, offering an emotionally sustaining experience that supports their broader mental health and sense of social belonging.

Thus, these performances constitute a form of cultural carework: affective labor that fosters resilience, fosters empathy, and nurtures the emotional health of individuals who often live at the margins of dominant gender ideologies. Through the character of Liku, performers can process and navigate the complexities of identity within a socially sanctioned space, thereby enhancing both psychological coping and cultural continuity.

3.5 Gender Performance, Symbolic Order, and Social Reintegration

The broader cultural significance of cross-dressing roles such as Liku and Matah Gede lies in their capacity to mediate between personal identity and communal values. In Balinese cosmology, performance is a ritualized expression of social and spiritual order, and gender is enacted through codified gestures, costumes, and vocal stylizations. Within this performative structure, gender becomes fluid, symbolic, and negotiable.

While roles such as Liku may be seen as humorous or transgressive, they carry substantial symbolic weight. Cross-dressing destabilizes fixed binaries by allowing male performers to embody traits associated with femininity—vulnerability, expressiveness, emotionality—within a space that shields them from direct censure. The structured nature of Balinese performance traditions legitimizes this enactment, rendering gender variance temporarily acceptable. This enables performers to experience moments of psychological

freedom and emotional release, reinforcing the role of performance as a space of mental health affirmation.

Though these performances are temporally bounded, their impact is long-lasting. Performers often recall the affective power of embodying Liku as a turning point in their emotional journey—where laughter, applause, and artistic success translated into greater self-acceptance. Importantly, audiences too become participants in this psychosocial exchange, extending recognition and appreciation that may not occur in everyday life.

Moreover, these cross-gender performances facilitate social reintegration for those who may otherwise be excluded. Artists whose gender identities or expressions deviate from the norm are reabsorbed into the cultural community through their roles, not by suppressing their difference, but by channeling it into ritual and artistic significance. Their gender variance is not erased—it is aestheticized and ritualized, thereby gaining symbolic and social value. Through the medium of performance, they access a form of social acceptance that supports not only their professional legitimacy but also their emotional and psychological well-being.

In this way, Balinese cross-gender performance traditions do more than preserve aesthetic and ritual heritage—they offer a resilient cultural mechanism through which individuals can sustain their mental health, assert their identities, and be meaningfully recognized by their communities. Performance becomes a vital therapeutic space—a cultural site where marginalized voices are given form, function, and the possibility of healing.

4. Conclusions

Cross-gender roles in Balinese performing arts reflect the culture's inherent flexibility and adaptability in articulating gender expression, particularly within ritualized performance contexts. Far from being peripheral or comedic devices, roles such as Matah Gede and Liku serve as key nodes in the sociocultural and spiritual matrix of Balinese life. These roles exemplify how performance traditions not only reflect but actively shape evolving understandings of gender, identity, and belonging.

The character of Matah Gede, a fearsome female antagonist within the Calonarang ritual, is traditionally performed by older male dancers who often possess high social or ritual status, such as priests or *balian* (healers). Although the role represents a female figure, it is imbued with spiritual force and symbolic gravitas—attributes culturally associated with male ritual expertise. The portrayal of Matah Gede by male performers is not only accepted but ritually authorized and socially honored. This indicates that gender fluidity, when aligned with religious function and aesthetic convention, becomes a legitimate and even revered practice within Balinese performance culture.

Conversely, the character of Liku in Arja provides an alternative but equally significant space for negotiating gender expression—particularly for performers whose identities diverge from heteronormative frameworks. While Balinese society continues to be shaped by patriarchal and binary gender norms, Liku allows queer-identifying or non-binary individuals to participate in cultural life through sanctioned channels of performance. In such cases, gender expression is not only permitted but applauded, provided it remains embedded within the established boundaries of theatrical convention. For many performers, the opportunity to portray Liku offers not merely artistic fulfillment, but a vital form of psychosocial sustenance—enabling the negotiation of private identity and public recognition in a socially acceptable form.

In illuminating how gender is rendered performative, fluid, and symbolically charged on the Balinese stage, this study contributes to broader conversations on the intersections of performance, identity, and mental health. It reveals that traditional forms are not static relics, but dynamic, living frameworks through which individuals articulate belonging, negotiate social norms, and sustain psychological well-being. By offering structured yet expressive spaces for cross-gender embodiment, these performance traditions provide not only cultural continuity but also critical avenues for emotional resilience and mental health support—particularly for performers navigating marginalization or identity-based stress. In doing so, they reimagine the possibilities of selfhood, visibility, and community within a rapidly evolving cultural landscape.

Conflicts of Interest: The authors declare no conflict of interest.

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Cripto-Positive Extracellular Vesicles as a Novel Anti-Migratory Strategy for Cancer Therapy

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13TH 2025 SOCRATIC LECTURES

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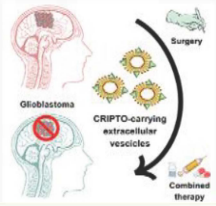
INTRODUCTION

Extracellular vesicles (EVs) are lipid bilayer structures that transport bioactive molecules and can modulate recipient cell behavior (Kalluri and LeBleu, 2020). Therefore, EVs are key modulators of cell communication in both physiological an pathological processes (Mantile et al., 2020). Tumor-derived EVs modulate cancer cell proliferation, migration, and metastasis,and are able to selectively recognize cancer cells, offering potential for targeted therapies (Liguori and Kralj-Iglič, 2023). Glioblastoma (GB) is a rare but extremely aggressive brain tumor that significantly impacts patient outcomes, affecting both duration and quality of life (Liguori, 2024). We have first isolated large EVs (lEVs) from NTERA2 teratocarcinoma cells, demostrating their ability in reducing migration of GB cells without inducing proliferation and chemoresistance. This anti-migratory effect is linked to membrane-associated CRIPTO, a key regulator in development and tumorigenesis (Mantile et al., 2022).

WORKING HYPOTHESIS



CRIPTO-carrying EVs might be used in GB therapy to contrast tumor invasiveness and improve patient prognosis (patent PCT/IB2023/053735)

EXPERIMENTAL DESIGN

1. Isolation of EVs from NTERA2 cells → Differential Ultracentrifugation (dUC)

2. EV characterization → Western blotting; Electron microscopy; Myriade

3. Functional Assays → Cell cultures; Ex vivo (organoids); In vivo (mouse models)

Figure 1. Isolation of EVs from NTERA2 cell line

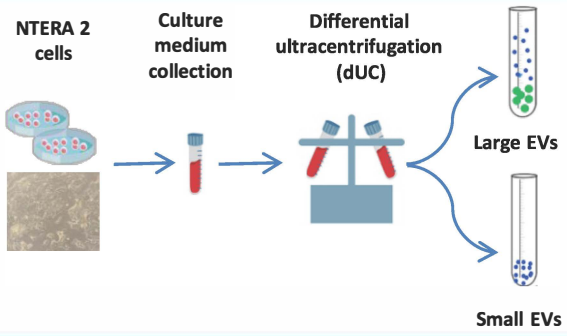
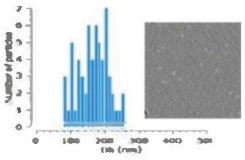


Figure 2. Large EV characterization

Western Blotting

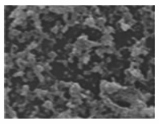
L: cell lysate; Sup: supernatant

Interferometric light microscope Myriade



Concentration	Median diameter
1,01e+ 11 part/ml	211,50 nm

Scanning Electron Microscopy



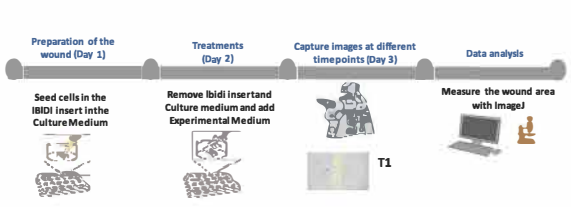
RESULTS: First, we separated lEV and sEV preparations by dUC (Fig. 1). Next, we focused on lEVs performing Western blotting with both positive and negative EV markers, Myriade analysis to assess particle concentration and size and electron microscopy (Fig. 2). Then, we performed preclinical cell culture studies to determine the effect of different EV doses on GB (U87) cell migration by wound healing assays. Finally, we investigated the effect of EVs on the viability of normal cells using XTT assays (Fig. 3).

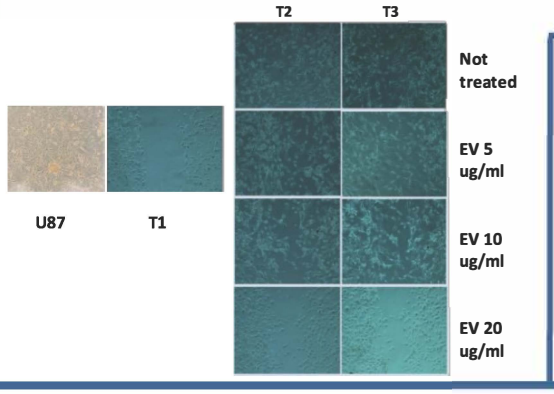
CONCLUSIONS: We identified 10ug/ml as the optimal EV dose able to cause a 50% inhibition of GB cell migration without affecting the viability of normal cells.

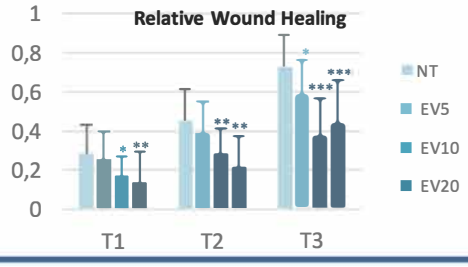
FUTURE PLANS: Performing preclinical assays both ex vivo on GB organoids and in vivo using GB orhotopic graft mouse models.

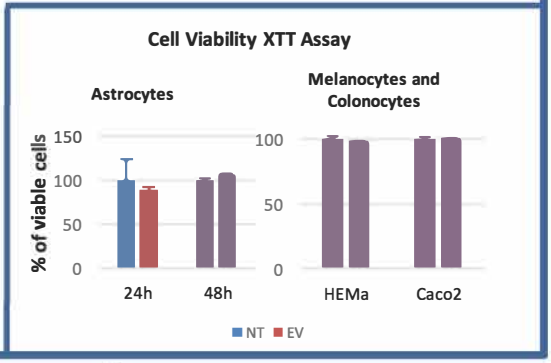
Figure 3. Functional assays

Wound Healing Assay









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The "*Good Evening Science*" Project is a New Platform for Communicating Complex Social and Scientific Issues to the General Public, Merging Science with the Role of the Library

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OUTLINE

Due to the pace of life in modern society, the development of various disciplines, and not least, the progress of science, we have decided to organize monthly forum under the title *"Good Evening Science"*. In discussions with various experts on everyday topics, we will shed light on their regularities and look for answers to various questions in a relaxed atmosphere. The event is organized in cooperation with the Library Domžale, where our talks take place.

ACTIVITY

In the second season, we dealt with interesting and current topics, met extraordinary personalities who are active in both the academic and applied fields. At the end of the event, a popular article is always published in the local newspaper, informing citizens and conveying the basic message of the event. The event was well received by the residents of the region. After the main description of the topic and the introduction of the guests, the moderator (M. Jeran) leads a 90-minute discussion and enriches the event with various anecdotes. The event attracts many visitors every month who follow the discussion and ask various questions. Questions from the general public are very welcomed as they usually further support some scientific content in practice (**Figure 1**).

Figure 1: Highlights of the event *"When the uninvited guest of dementia opens the door of memory"*. Memory is more than just a database – it is what defines us, connects us to others and shapes our past, present and future. It also has a symbolic and emotional value. We study it in science, represent it in art, but sometimes we don't understand it enough in society. In this talk, we explored its value and utility from different angles. In everyday life, we often take it for granted until we begin to lose it – in ourselves or in the people close to us.

Figure 2: Highlights of the last event "*Life is a great gift: From cancer diagnosis to realisation*". The meeting invited us to reflect deeply on life, trials and the inner strength of a person. In the face of a diagnosis, the world often comes to a standstill, but it is precisely from pain that the greatest insights and gratitude for life itself can arise. With soft music, the gathering provides an opportunity for honest stories, inspiration and connecting with the power of the human experience (**a**, **b**). Some also refer to illness as a "special gift". In the moment of suffering we often cannot understand this, but with time many look back with different eyes. We asked ourselves: What does cancer teach us about life?

CONCLUSION

An important task for every scientist is to communicate the passion for their work and its importance to society. This includes communicating effectively with other experts and people outside their field, as well as with the general public. Full halls and increasing interest in such content encourage us to continue our work.

This research was supported by the **Domžale Library**. Special thanks to Ms. **Barbara Zupanc Oberwalder**, MSc and *her team*. Thanks also to *Slamnik* and *Modre novice* newspaper for help and support. M. J. thanks the Slovenian Research Agency for scientific knowledge through core funding no. P1-0045. ***Without these self-sacrificing people this project could not exist***.

AI-DRIVEN DRUG DISCOVERY AND PERSONALIZED IMMUNOTHERAPY IN CANCER TREATMENT

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ABSTRACT

AI is revolutionising our understanding of cancer immunotherapy. This aids in identifying drug targets, developing improved treatments, and tailoring patient care accordingly. AI can identify patterns that traditional methods cannot, by analysing big data such as gene expression, single-cell sequencing, and medical scans. By utilising deep learning and associated generative models, researchers can predict drug activity, identify immune system targets, and suggest combinations. Non-invasive imaging and the tracking of tumour response to treatment are made possible by AI. Additionally, while AI still faces hurdles in terms of data availability and ethical considerations, it has the potential to revolutionise cancer care by enhancing efficiency, intelligence, and effectiveness (Olawade et al., 2025; Derraz et al., 2024).

WHY AI MATTERS IN IMMUNOTHERAPY

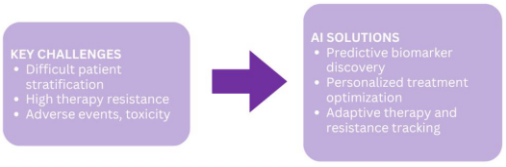

Figure 1: AI tackles key immunotherapy challenges by improving patient selection, reducing resistance, and managing toxicity.

PERSONALIZED IMMUNOTHERAPY

AI enables real-time treatment customization and biomarker discovery.

Key advances include:

- **Novel Resistance Mechanisms Identified**: Explainable AI (XAI) revealed TREM2⁺ macrophages as key players in resistance to anti-CTLA-4 therapy, leading to the launch of five targeted clinical trials (Derraz et al., 2024).
- **Efficient Biomarker Discovery**: Integrating single-cell RNA sequencing with AI algorithms has reduced the cost of biomarker discovery by 60%, while preserving high accuracy and drastically cutting analysis time (Olawade et al., 2025).
- **Real-Time Treatment Adaptation**: Bayesian network models identified IL-10 upregulation in 73% of CAR-T therapy relapses, allowing for timely therapeutic interventions and improved patient outcomes (Derraz et al., 2024).

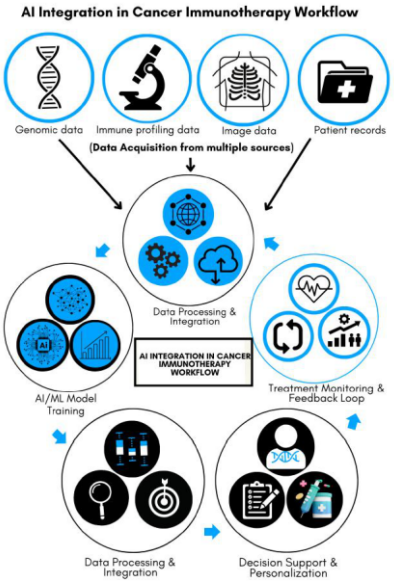

Figure 2: AI integration in cancer immunotherapy workflow (Olawade et al., 2025).

EMERGING TECHNOLOGIES

Radiomics + PET imaging: non-invasive tumor phenotyping (McGale et al., 2024b)
Transcriptomics: ML enhances RNAseq interpretation, splicing, and cell-type mapping (Gui et al., 2023b)
Quantum computing: simulating 1 million immune cell interactions in real-time
Federated learning: data privacy preserved across 47 institutions (CFLI project)

AI IN DRUG DISCOVERY AND DEVELOPMENT

AI drastically reduces drug development time and cost.

Breakthroughs include:

- **Generative AI**: 12,000 neoantigens predicted in 48h, 14% immunogenic (Li et al., 2023)
- **Reinforcement learning**: +47% response in TNBC with optimized ICI + CDK4/6 combos (Prelaj et al., 2024)
- **Graph Neural Networks**: 92% specificity in predicting irAEs (McGale et al., 2024a)

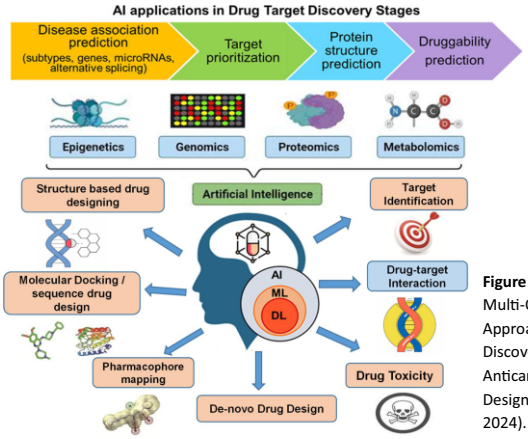

Figure 3: AI-Driven Multi-Omics Approach for Target Discovery and Anticancer Drug Design (Garg et al., 2024).

Applications:

- Target identification from multi-omics (Olawade et al., 2025)
- Binding site prediction for ICIs (PD-1, CTLA-4) (Ogunmola & Aloba, 2025)
- Molecular docking using AlphaFold (Ogunmola & Aloba, 2025)

AI AND TUMOR MICROENVIRONMENT (TME)

Integration of multi-modal data allows AI to understand and reshape the immune landscape.

- Deep learning models predict ICI response with 85% accuracy (Gui et al., 2023a)
- AI-driven TME analysis improved TNBC response from 28% → 41% (Prelaj et al., 2024)
- Liquid biopsy + AI → +32% accuracy in NSCLC survival forecasting (Garg et al., 2024)

CHALLENGES AND ETHICAL CONSIDERATIONS

Despite rapid advances, data heterogeneity remains a major hurdle, with 68% of AI models failing external validation due to batch effects and center-specific protocols (Gui et al., 2023a). Initiatives like ML-SPORE and CFLI promote standardized, privacy-preserving data sharing. Yet, population bias persists—African genomic underrepresentation leads to 2.1-fold higher prediction errors (Derraz et al., 2024). Transparency is key; SHAP analysis is now vital for model interpretability. Regulatory progress lags, with no AI tools yet approved for immunotherapy companion diagnostics. However, quantum simulations of immune responses and adaptive AI-driven trials like DECODE-NIVO signal a transformative future for personalized oncology.

CONCLUSION

Oncology is being revolutionized by the combination of AI and immunotherapy, thanks to three primary mechanisms: accurate formulation of drugs, management of resistance (via dynamic modeling), and discovery of accessible markers. While there are still major issues with data quality, population bias, and regulatory frameworks, AI-powered immunotherapy has shifted from an experimental approach to a clinical necessity.
Synergy may be further developed through the use of federated learning, quantum computing and adaptive clinical trial designs. The end goal is not to replace clinician judgment with AI, but to provide the necessary tools for creating effective cancer treatments that are personalized and successful.

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Pursuing Assessment of Extracellular Particles in Diluted Blood by Interferometric Light Microscopy and UV-vis Spectrophotometry

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Methods

Study included 3 samples from human blood, Blood was taken into an evacuated tube with trisodium citrate anticoagulant and processed fresh. Blood was diluted with physiological saline solution. Plasma was obtained by centrifugation of blood and diluted with physiological solution. Diluted blood was centrifuged to sediment erythrocytes and larger segments. Number density and hydrodynamic diameter of Eps were assessed by Videodrop (Myriadelab, Paris, France) interferometric light microscope. Absorption of UV and visual light at 260 and 280 nm was assessed by the Nanodrop spectrophotometer (Thermofischer Scientific, Waltham, MA, USA). The absorbance ratio A280/A260 indicates the protein/nucleic acid content of the sample.

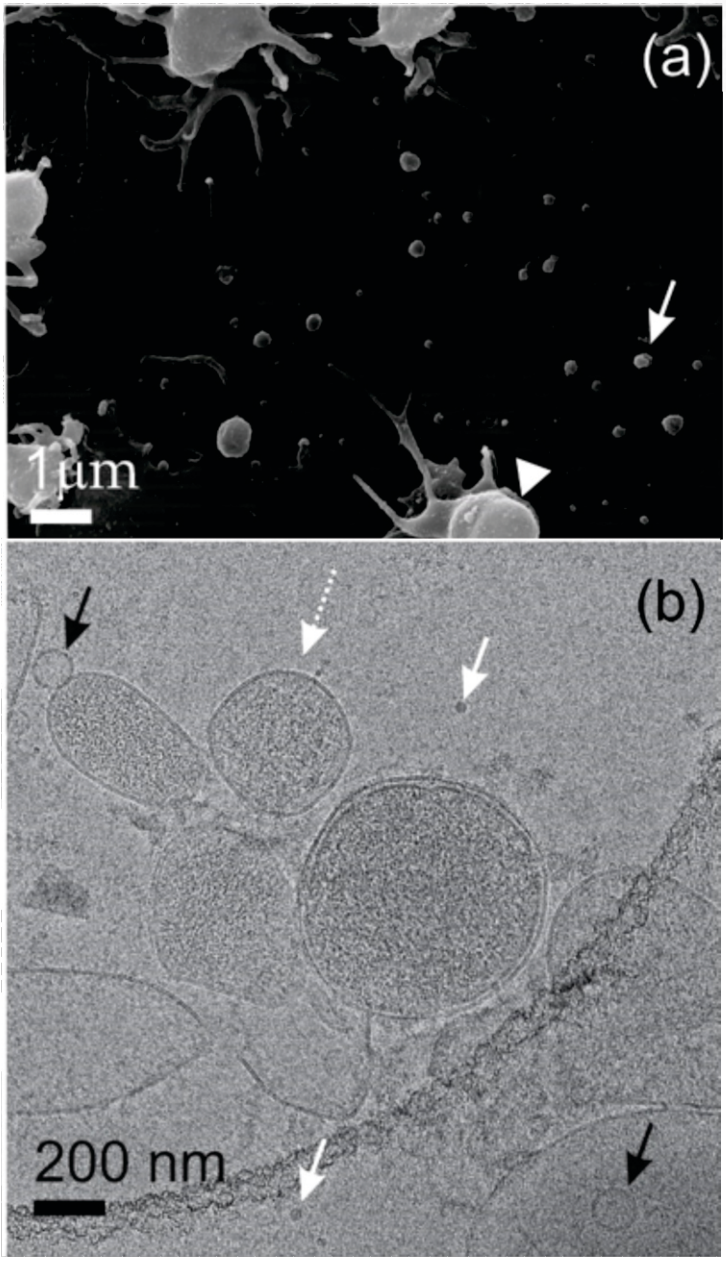

Figure 1. (a): EPs in canine plasma as observed by the Scanning Electron Microscope; white triangle points to a platelet, white arrow points to an EP; (b): EPs as observed by the Cryogenic Transmission Electron Microscope; dashed white arrow points to a large vesicle, black arrows point to smaller vesicles, white arrows point to molecular complexes. From Korenjak B, et al., Cells, 2024; 13(24):2054. https://doi.org/10.3390/cells13242054

Outline

Harvesting of EPs from body fluids and their assessment is challenging as processing significantly affects the samples. A recently developed technique Interferometric Light Microscopy (ILM) enabled determination of the number density n and the hydrodynamic diameter D_h of EPs (sized 80 nm to 500 nm) in samples which may contain larger particles (cells) and smaller particles (lipoproteins). In order to produce an observable signal, a threshold saturation of light is reached by appropriate dilution of samples with physiological solution thereby **avoiding isolation** of EPs prior to assessment. Figure 3 shows n, D_h and A280/A260 in dependence on the centripetal acceleration of the centrifuge rotor Xg, where g is the gravity acceleration of the Earth, and on the dilution factor R.
It is indicated that centrifugation up to cca 1000g does not affect n, D_h or A280/A260. In contrast, dilution is important for n and D_h. Too strong dilution induces aggregation of particles resulting in lower n and higher D_h (Figure 4). We estimated the optimal dilution of blood 10x and optimal settings of the centrifuge: room temperature, time 10 minutes and centripetal acceleration of the centrifuge rotor 500g.

Results

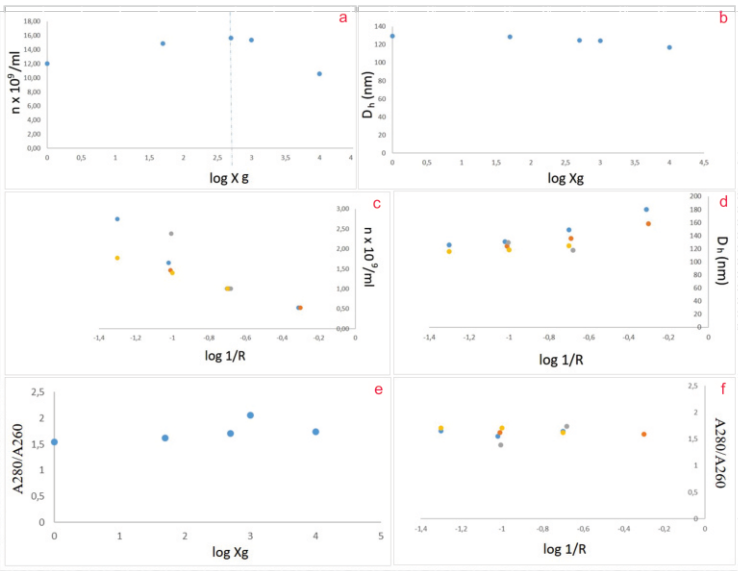

Figure 2. (a): Number density of EPs in 10x diluted blood in dependence on the centripetal acceleration of the centrifuge rotor Xg; (b): hydrodynamic diameter of EPs D_h in dependence on the centripetal acceleration of the centrifuge rotor Xg; (c): number density of EPs in dependence on the dilution of blood 1/R; (d): hydrodynamic diameter of EPs D_h in dependence on the dilution of blood 1/R; (e): absorbance ratio A280/A260 in dependence on the centripetal acceleration of the centrifuge rotor Xg; (f): absorbance ratio A280/A260 in dependence on the centripetal acceleration of the dilution of blood 1/R.

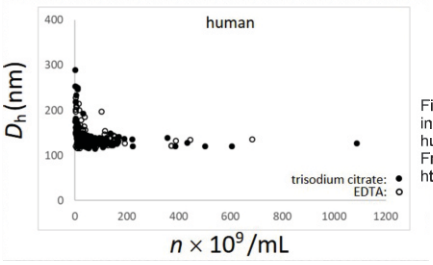

Figure 3. (a): Hydrodynamic diameter of EPs D_h in dependence on the number density of EPs in human plasma exhibiting the swarm effect. From (Korenjak B, et al., Cells, 2024, 13, 2054. https://doi.org/10.3390/cells13242054)

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An Introduction to Hydroponic Farming and Its Advantages

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OUTLINE

Hydroponic farming is a method in which plants are grown in water instead of soil. The roots of the plants are supported by an inert medium such as rock wool or coconut coir and immersed directly in nutrient solution, supplying all the essential elements for healthy growth. The word hydroponics is derived from the words “hydro”, meaning water, and “ponics”, meaning labour. The water is the labour that does all the necessary hard work.

THE MAIN ADVANTAGES OF HYDROPONIC FARMING

No soil needed Physical and chemical factors such as pollution and deforestation are the main reasons of land degradation. Conventional agriculture accelerates this process through the use of farming equipment used and the intensive use of fertilizers and pesticides. Since hydroponics does not rely on soil, it offers an alternative can help to slow land degradation.

Conserves water Hydroponics uses less water than conventional farming because the water is recycled repeatedly in recirculation systems. Proper filtration and treatment keep the water usable for longer periods of time. In contrast, outdoor farming often results in significant water loss through evaporation and runoff.

Higher yields in a smaller area Pests and contaminants are rarely an issue in hydroponics due to the controlled and fully or partially enclosed environment. Plants benefit from optimal nutrient and climate conditions, which accelerates their growth. Additionally, space utilization is maximized and even increased as many hydroponic systems allow for vertical farming.

Figure 1: Growing lettuce in a hydroponic system.

Predictability and seasonality Most crops only grow in certain climates and at certain times of the year, requiring additional effort to import food from around the world to meet demand. In contrast, plants in closed hydroponic systems grow all year round, regardless of weather and climate conditions. This eliminates the problem of harvest predictability, as indoor cultivation is not dependent on climatic factors such as frost and drought.

Shorter supply chain Hydroponic cultivation helps to overcome the challenges of the supply chain. In conventional agriculture, food has to travel long distances after harvest before it ends up on store shelves. Hydroponic farming offers the opportunity to grow fresh food that can be delivered to the consumer fast, ensuring maximum freshness and reducing transport and storage needs.

CONCLUSION

Although hydroponic farming offers many advantages, it also has some drawbacks, such as high initial set-up costs, ongoing power consumption, high maintenance and monitoring requirements and the need for specialized knowledge.

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Expectation, Exposure and Reality: A Nocebo-Based Perspective on Wind Turbine Syndrome

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OUTLINE

Both in Slovenia and internationally, we are observing the activities of a small but vocal group that is speaking out against wind energy. These people try to alarm the public with unfounded claims, especially about alleged health risks. Their arguments often centre on the infrasound generated by wind turbines, which they claim causes health problems for local residents. What they may not realise is that by spreading such inaccurate and often misleading information, they are inadvertently contributing to actual discomfort or even health problems in those who believe them. Health complaints voiced by people who strongly oppose wind energy and convinced of its negative effects are often better explained by the nocebo effect. This psychological phenomenon occurs when negative expectations – such as the belief that infrasound is harmful – lead to actual physical symptoms in those who hold such beliefs. In contrast, others who are not influenced by these messages do not experience such effects. Research has shown that people who expect negative effects from environmental factors are more likely to interpret physical sensations as confirmation of their fears. Experimental studies confirm this and show that the mere suggestion of the harmfulness of infrasound can significantly influence a person's subjective well-being – regardless of the actual exposure. As we are concerned about the unnecessary psychological and health burdens caused by the nocebo effect, this contribution aims to highlight its existence and powerful influence. In the wrong hands, the nocebo effect can become a powerful psychological weapon.

INFRASOUND AND EMF

Infrasound is a natural and everyday part of our acoustic environment. It refers to sound waves with frequencies below 20 Hz, which are generally below the human hearing threshold. Almost all sound emissions, whether natural or artificial, contain infrasound components, whereby the intensity of these sounds is decisive.
In wind turbines, infrasound is generated by the rotation of the rotor blades. When the rotor blades move, they generate turbulent air currents. When a blade passes in front of the tower, this air flow is briefly interrupted and an infrasound pulse is generated (Kegel et al., 2025).
However, wind turbines are not unique in this respect – many other everyday sources such as road traffic, the wind itself, household appliances such as fridges and electric motors emit infrasound at a higher intensity. For example, the level of infrasound measured inside a car with the rear windows open, or even fully open, is much higher than that generated by a wind turbine. In addition, the infrasound emitted by wind turbines is far below the threshold of human perception and is not considered harmful to health (Fachagentur Wind und Solar, ND).

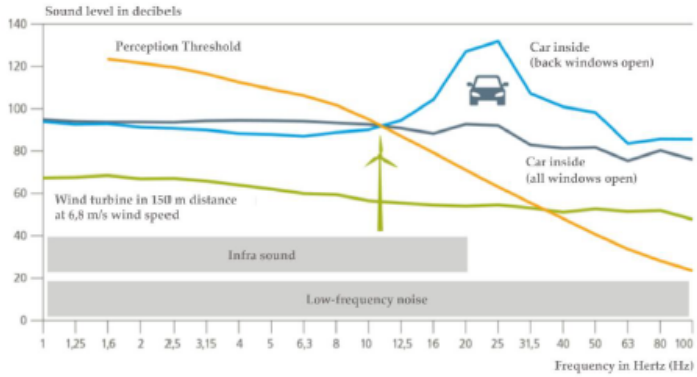

Figure 1: The other sound sources are significantly louder than the wind turbine (Fachagentur wind und solar, n. d).

Electromagnetic fields (EMFs), on the other hand, are caused by the interaction of electric and magnetic fields that arise when electrical voltages and currents change. These fields occur not only in industrial plants, but also in a variety of everyday electronic devices such as televisions, hair dryers and even wind turbines. EMFs are characterised by their frequency and magnetic flux density and can be measured with special instruments, such as Hall sensors (Kegel et al., 2025).
With regard to wind turbines, studies have shown that EMFs are only be detected in the immediate vicinity of the turbines themselves. Measurements in the vicinity of the turbines show that the EMFs quickly drop to background levels within a few metres. The electromagnetic fields in the vicinity of wind turbines – within about 2 to 3 metres – are comparable to the EMFs emitted by normal household appliances, or even weaker. Therefore, exposure to EMF from wind turbines does not pose a significant health risk to residents living near wind farms (Knopper et al., 2014).

WHAT IS NOCEBO EFFECT?

It is noteworthy that the reports of symptoms attributed to wind turbines gained prominence following the publication of a self-published book by Pierpont (2009). This book theorised that the infrasound generated by wind turbines causes a variety of symptoms in people living near wind farms and proposed a biological mechanism to explain the occurrence of these symptoms. Although such claims are not supported by solid scientific evidence, the idea that infrasound from wind turbines poses a health risk has since gained traction in public discourse and in the media, particularly via the internet (Leventhall, 2013). The dissemination of health warnings suggesting that environmental exposure is dangerous can trigger the onset of symptoms through the so-called nocebo effect alone – even if the exposure is physiologically harmless (Crichton et al., 2014a). A nocebo response to a harmless environmental exposure occurs when people expect symptoms due to the exposure and therefore become more vigilant and more likely to report symptoms related to perceived health risks (Pennebaker, 1994; Petrie et al., 2005). Both epidemiological and experimental evidence suggests that the reporting of symptoms in the vicinity of wind farms is not due to harmful physiological effects of infrasound, but rather to negative expectations and the misattribution of common symptoms (Chapman et al., 2013; Crichton et al., 2014b). As there is growing evidence that fears and negative expectations can contribute to symptoms attributed to infrasound from wind turbines, a simple but potentially effective intervention could be to explain the nocebo effect to those affected. This could help to reduce fears and change expectations.

NOCEBO EFFECT STUDY RESULTS

In their 2015 study, Crichton and Petrie investigated whether the explanation of the nocebo effect could reduce the symptoms triggered by negative expectations associated with infrasound from wind turbines – expectations that are often shaped by media and information from the internet. The study involved 66 healthy individuals from the local population. Each participant took part in two 14-minute sessions during which they were exposed to sound recordings containing both audible wind turbine noise and infrasound (9 Hz, 50.4 dB). Before the first session, all participants watched a video with negative information about the health effects of infrasound. They were then randomly assigned to one of two groups: The group with the nocebo explanation received a scientifically based explanation after the first session, suggesting that the symptoms could be due to a psychological mechanism – the nocebo effect. The group with the biological explanation received an explanation that suggested a physiological basis for the symptoms (e.g. effects on the inner ear and vestibular system). Both groups reported increased symptoms and worsening mood after the first session. However, in the group with the nocebo explanation, symptoms and mood returned to baseline levels during the second session. The group with the biological explanations, on the other hand, showed persistent or even increased symptoms and negative feelings. The study confirms that negative expectations shaped by media information can trigger real physical symptoms in otherwise healthy individuals – a hallmark of the nocebo effect. At the same time, it shows that a thoughtful and empathetic explanation of this mechanism can help to reduce anxiety and alleviate symptoms. The authors emphasise that it is generally not enough to simply deny physiological effects in order to alleviate anxiety. The key lies in a meaningful, neutral and non-stigmatising explanation that enables sufferers to understand their symptoms without arousing feelings of guilt or ridicule. The results support the use of psychological approaches as a complementary tool in the treatment of symptoms related to environmental factors such as wind turbines (Crichton & Petrie, 2015).

CONCLUSION

This article, supported by empirical findings, clearly shows how the fear of the alleged health effects of infrasound and noise from wind turbines can arise – not through physiological damage, but through psychological mechanisms such as the nocebo effect. The results of the study by Crichton and Petrie (2015) show that negative expectations, especially those fuelled by reporting from citizens' initiatives and misinformation, can lead to otherwise healthy people experiencing real symptoms. The evidence is clear: addressing symptom reports related to wind turbines requires more than denial – it requires transparent, evidence-based public communication that explains the role of expectations, perceptions and misinformation in creating health concerns. Therefore, those who publicly speak out against wind energy and spread unverified claims must be held more accountable. The dissemination of inaccurate or misleading information about health risks – without a scientific basis – not only undermines public understanding, but can also contribute directly to mental and physical health problems. Raising awareness of factual, peer-reviewed data and educating the public about the nocebo effect are essential steps to reduce unnecessary fears and build an informed, resilient attitude towards renewable energy infrastructure.

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A Pioneering Cultural and Charitable Initiative as a Form of Social Solidarity and Support for Children With Cancer in Slovenia: A Bridge between Science, Medicine And Art

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OUTLINE

Solidarity and social support are key to improving the quality of life of children with cancer and their families. Childhood cancer is not only a medical challenge, but also an emotional, social and financial burden for the whole family. During the illness, children and their parents need not only medical support, but also emotional, social and financial support. A community that rallies together in good faith contributes to a sense of security, acceptance and hope. Such support can provide access to better care, rehabilitation and psychosocial programmes designed specifically for young cancer patients. Through solidarity, we create a more compassionate and just society where no child with cancer – and no family – has to walk the journey alone.

ACTIVITY

The project was initiated by Jolanda Ravnikar, who has been a volunteer at the Institute of Oncology Ljubljana for 15 years. She first launched the project, which also includes the "*Pesem zate – od srca do srca (Engl. A song for you – From hart to hart)*" charity concert, in 2017. Since then, it has united hearts every year with one goal: to raise money for projects and organizations that support children with cancer and their families. Over the years, this highly successful initiative has raised considerable funds for various causes, including the Foundation for the Support of Children with Cancer and Blood Disorders, the Association of Friends of Youth Ljubljana Moste-Polje and the Heroes of the 3rd Floor (from the Department of Paediatric Oncology and Haematology, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia).

The donations collected so far have been used to purchase infusion chemotherapy pumps, which are extremely important for the treatment of children. In addition to the pumps, various medical devices for diagnostics and therapeutic aids were also purchased, which are essential for successful treatment. Aids for the daily care of patients are also necessary to ensure humane accommodation and care during treatment. Many children and young people come with their parents or guardians from other parts of the country, so they need to be accommodated appropriately in the treatment area.

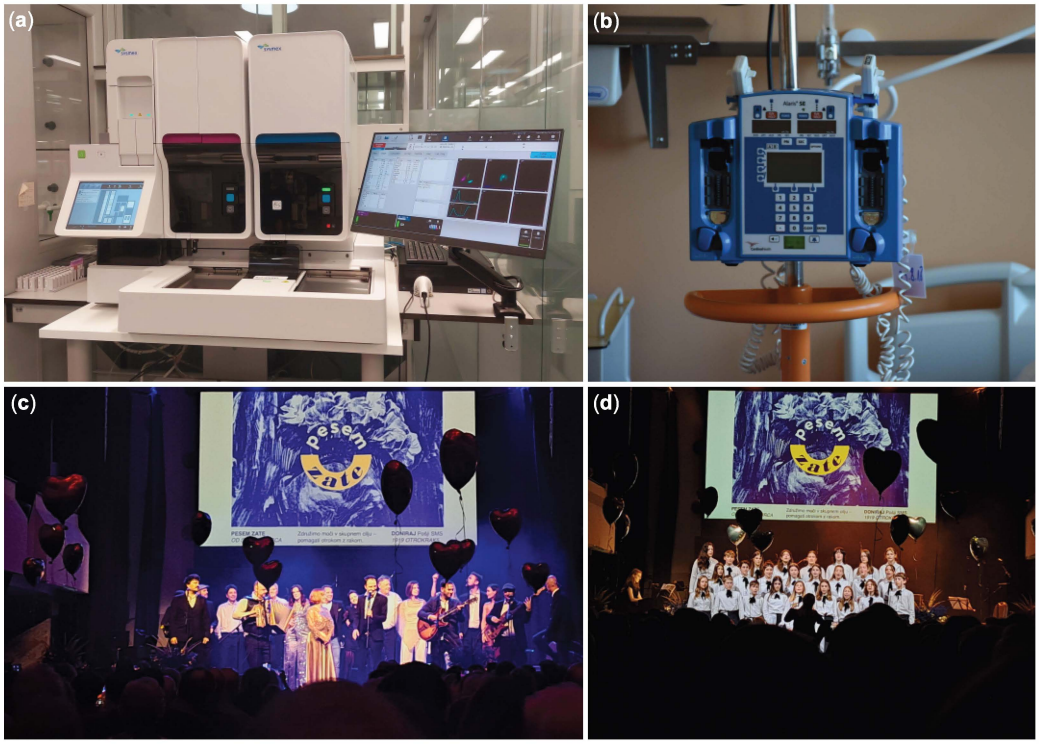

Figure 1: The donations from the *"A song for you"* concert were used to purchase (a) a haematological analysis device for the Clinical Institute for Special Laboratory Diagnostics at the Children's Hospital and (b) infusion chemotherapy pumps for the Department of Paediatric Oncology and Haematology at the University Children's Hospital. Highlights of the *" A song for you"* concert: (c) the assembled artists and (d) the youth choir of Glasbena Matica Ljubljana. Together we can help these children and their families to get through the most difficult time and provide them with the best possible care and support. Everyone's help is crucial to the success of this mission.

The concert is traditionally performed by numerous well-known Slovenian musicians and artists. Children's choirs and artistic and musical groups of medical staff were also performed.

Photos: (a) and (b) by Jolanda Ravnikar, "Pesem zate" Facebook page & https://pesemzate.si/; (c) and (d) Marko Jeran.

CONCLUSION

The *"A song for you"* concert is not only a cultural event, but also a socially committed initiative that combines art and humanity to support children with cancer. Behind the musical performances lies a deeper message – an encouragement to community, solidarity and active compassion. The initiative, led by Jolanda Ravnikar and her organizing team, shows how art can serve as a bridge between the individual and the common good. Through small contributions from individuals, a strong foundation of support is created for those facing life's most difficult challenges. *" A song for you"* reminds us that each of us has the power to contribute to change – through action, participation and music. In this context, the concert becomes a symbol of hope and a powerful reminder that true connection has the potential to create a better future.

A song for you – From heart to heart, would not be possible without the extraordinary support of dear people, partners and sponsors. A big thank you to the Festival hall Ljubljana, the Ravnikar initiative, Eventim, Fužine nursing home, Birografika Bora and our sponsors and partners: Gostišče Jezero, Gostišče Portal, Gostilna Rogovilc, Dubočica, Pivnica Kratofil BTC, Vina Belak, Klet Brda, Cvetličarna Butik IN, Slaščičarna kavarna VIKI, Berryshka, Tiskarna Cicero, Hiša Ančka Šenčur, Studio Brane Petrovič, Balonmania, Istenič, Vino Kranjc, Vina Koper, Secret frizerski salon, FOTA - COP, Storck and L-tek, Ltd.