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In vitro models of the endocrine pancreas

Modeli *in vitro* endokrine trebušne slinavke

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Abstract

Ambitions to develop artificial tissue substitutes, combined with the need to study underlying mechanisms of disease under controlled conditions, shortcomings of animal models, as well as ethical constraints, were the main driving forces behind the development of advanced *in vitro* models. These are defined as alternative experimental systems made by leveraging recent advances in tissue engineering and additive manufacturing that mimic tissue or organ level physiology *in vitro*. Simple *in vitro* models are already being used in many applications, however, due to their many drawbacks, they incompletely mimic dynamic responses of native tissues. In order to construct functionally more relevant *in vitro* models, cells need to be grown in three-dimensional (3D) environments or bioscaffolds. Generally, bioscaffolds must recapitulate the microarchitecture, hierarchical structure, physical properties, and composition of native tissues. Nonetheless, progress towards building more complex models is hindered primarily by the diffusion of gases and nutrients into the constructs´ interior. Currently, 3D printing presents the most promising solution for the production of advanced bioscaffolds, which resolve the above-mentioned limitations. In addition to the technique´s ability to simultaneously use multiple biocompatible materials, 3D printing enables material deposition with micrometer spatial resolution under cell-friendly conditions. The development of a functional *in vitro* pancreas model is governed by the desire to study diabetes aetiology and is one of the main goals of the *in vitro* modelling domain, which to date remains unfulfilled. Despite having some drawbacks, the tissue slice method presents the gold standard for basic and translational studies of the pancreas, while the currently most advanced 3D fabricated *in vitro* pancreas models mimic only basic functions of the organ. The purpose of this review is to provide an overview of *in vitro* models with a focus on *in vitro* models of the endocrine pancreas. We will highlight different model types and fundamental elements which need to be considered when constructing a model. Emphasis will be placed on more complex 3D fabricated *in vitro* models, tissue slices, bioscaffold material properties, and the use of 3D printing for the fabrication of advanced bioscaffolds. We believe that the simultaneous development of advanced materials, micro-manufacturing technologies, and advanced cell culture methods presents a very promising approach towards the construction of a functional *in vitro* pancreas model.

Izvleček

Želja po razvoju tkivnih nadomestkov, potrebe po preučevanju mehanizmov bolezni v kontroliranih pogojih, pomanjkljivosti živalskih modelov in etične omejitve so bile povod za razvoj področja naprednih modelov *in vitro*. Te definiramo kot alternativne eksperimentalne sisteme, ki z uporabo modernih tehnik tkivnega inženirstva in aditivne proizvodnje posnemajo strukturo in funkcionalnost tkiv ali organov *in vitro*. Enostavni modeli *in vitro* se že uporabljajo v številne namene, vendar zaradi mnogih pomanjkljivosti ne posnemajo dovolj dinamičnih odzivov izvornih tkiv. Za izgradnjo funkcionalno kompleksnejših modelov *in vitro* je celice potrebno gojiti v tridimenzionalnem (3D) okolju ali bioloških nosilcih. Splošno morajo biološki nosilci posnemati mikroarhitekturo, hierarhično strukturo, fizikalne lastnosti in sestavo izvornega tkiva. Toda napredek k izgradnji kompleksnejših modelov omejuje predvsem difuzija plinov in hranil v notra-

njost konstrukta. Tehnika 3D tiska je trenutno najobetavnejša rešitev za proizvodnjo naprednih bioloških nosilcev, ki odpravljajo te pomanjkljivosti. 3D tisk poleg zmožnosti sočasne uporabe več biološko kompatibilnih materialov omogoča nalaganje materiala z mikrometrsko prostorsko ločljivostjo pri pogojih, primernih celicam. Vse od zasnove področja modelov *in vitro* je zaradi želje po preučevanju nastanka sladkorne bolezni razvoj funkcionalnega modela trebušne slinavke eden od osrednjih, a še nedoseženih ciljev. Zlati standard za bazične in translacijske raziskave trebušne slinavke pa je kljub nekaterim pomanjkljivostim metoda tkivne rezine, medtem ko trenutni najnaprednejši 3D zgrajeni modeli trebušne slinavke *in vitro* posnemajo le osnovne funkcije organa. Namen tega članka je predstavitev modelov *in vitro* s poudarkom na modelih trebušne slinavke. Predstavil bo tipe modelov in ključne elemente, ki jih je treba pri izgradnji upoštevati. Poudarek bo na kompleksnejših 3D zgrajenih modelih *in vitro* in tkivnih rezinah, materialnih lastnostih bioloških nosilcev ter tehniki 3D tiska za izgradnjo naprednih bioloških nosilcev. Menimo, da je sočasni razvoj znanosti o materialih, mikroproizvodni tehnologiji in celičnih kulturah izjemno obetavna pot k izgradnji funkcionalnega modela trebušne slinavke *in vitro*.

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1 Introduction

The main purpose of the majority of biomedical research is to decipher the origin and molecular mechanisms of human diseases with the goal of developing new or better preventive, diagnostic, and therapeutic approaches. For obvious reasons, basic research alone cannot be performed on humans, and animal models differ too much anatomically, physiologically, and genetically from humans, so they often do not mimic critical aspects of human healthy or pathologically altered tissues and organs, especially not with the desired molecular resolution (1). The development of alternative models that mimic human anatomy and physiology *in vitro* is therefore urgently needed. In parallel, the ultimate goal of the field of tissue engineering (TE) is the production of functional tissues and organs *in vitro* that could be used as substitutes to repair or replace the damaged and diseased human body parts (2-4). Individual TE research studies focus mainly on the development of individual milestones of this process in order to achieve the ultimate goal (e.g. development of advanced materials, production methods and cellular resources, etc.) (5,6) and on the construction of simple *in vitro* models that mimic the basic functions of tissues and organs *in vivo*. In order to achieve the principles of 3Rs – replacement, reduction and refinement in the face of ethical limitations and shortcomings of animal models and the goals of tissue engineering to study the mechanisms of disease in controlled conditions, they led to the development of complex *in vitro* models. The field of advanced *in vitro* models lies at the intersection of TE, regenerative medicine, pathophysiology, advanced materials science, and additive manufacturing and focuses on mimicking the three-dimensional (3D) structure and functionality of tissues or organs *in vitro*.

2 *In vitro* models

2.1 Simple *in vitro* models

Simple two-dimensional (2D) *in vitro* models are already being used for a number of purposes (Figure 1), such as the development and testing of new therapeutical substances and for pharmacological and toxicological studies. For this purpose, transformed or immortal commercially available cell lines are most often used, but during prolonged cultivation (increase in the number of cell divisions), they usually begin to differ genetically, epigenetically and physiologically from primary cells. For the construction of *in vitro* models, commercial cell lines are, therefore, the worst choice (7,8). In contrast, isolated primary cell cultures are the best candidates for use in *in vitro* models, as they best represent functional units of the native tissue. The construction of *in vitro* models based on primary cell cultures is limited mainly due to the difficult availability and isolation of cells that, in addition, have difficulty proliferating in standard 2D cell cultures and have a short lifespan (i.e., Hayflick limit) (9). Recently, these problems have been overcome by using induced pluripotent stem cells (iPSCs), which can be obtained from somatic cells by a dedifferentiation process (10). iPSCs have the ability to self-regenerate and differentiate into many mature cell types, making them extremely interesting for use in *in vitro* models where we study the onset and the course of disease development. The reason for the rare use of iPSCs is that the control of differentiation into mature cell types is extremely complex, and in addition, immature cell phenotypes are always present in cell culture (11,12).

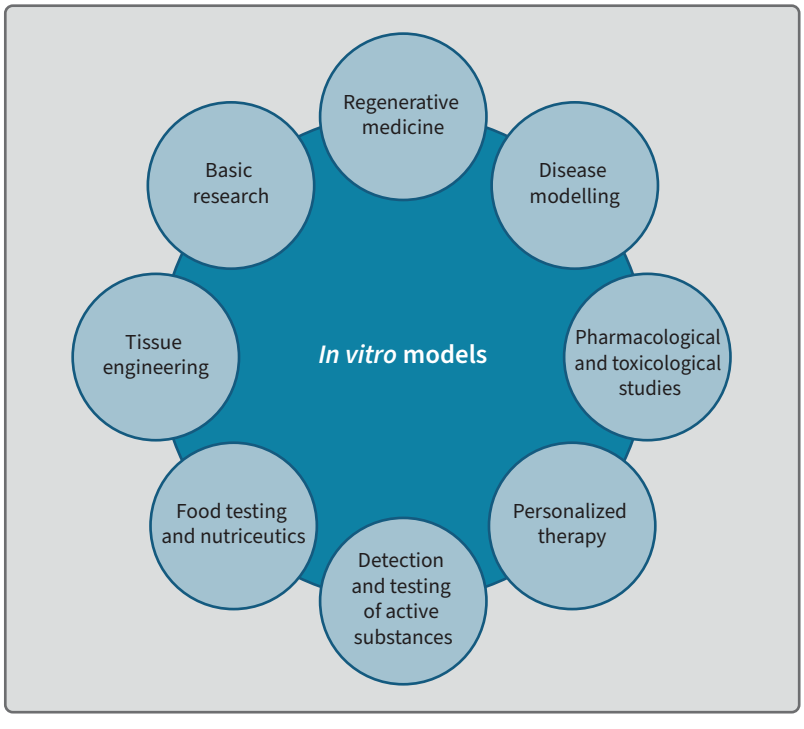

Figure 1: Areas, where *in vitro* models are used.

Regardless of the source and type of cells, we regard as simple *in vitro* models the standard 2D single cell cultures (consisting of one type of cells grown in cell vessels) and their upgraded variants, such as 2D cocultures, 2D cultures grown in containers coated with extracellular matrix (ECM) components, and cultures grown on Transwell® plates. These are simple 2D cell cocultures grown on plates that mimic more complex three-dimensional (3D) intercellular signalling (13,14). In fact, such 2D *in vitro* models have many drawbacks. In addition to the fact that cell vessels differ statistically significantly from the native tissue in structure, mechanical properties, topography and composition, these cells cannot communicate with each other auto- and paracrineally in all spatial dimensions. Also, cells are unevenly exposed to concentration gradients of oxygen, nutrients, and biologically active molecules, leading to the fact that cells grown in 2D cultures do not show the correct morphology and often do not express the appropriate phenotype long enough. Therefore, they do not

allow the performance of more complex and time-consuming experiments. As a result, simple *in vitro* models do not mimic the key characteristics and functions of the native tissue (15,16). In contrast to 2D cultures, cells grown in 3D cultures establish complex interactions with the ECM and neighbouring cells in three spatial dimensions, thus better mimicking the biochemistry and mechanics of the original microenvironment. Therefore, in order to build functionally more complex models, *in vitro* cells must be grown in a 3D environment or on 3D cell scaffolds, i.e., bioscaffolds that mimic the original ECM in which cells grow, differentiate, proliferate, and communicate in all spatial dimensions (17).

2.2 Advanced *in vitro* models

Advanced *in vitro* models are defined as synthetic alternative experimental systems based on living human cells that mimic the physiology of a tissue or organ *in vitro* using modern TE approaches and micro-additive manufacturing. Within the framework of this definition, the aim is not to perfectly mimic the complex architecture and function of a tissue or organ, but rather the model reproduces, at least representatively, only the key functions that we want to mimic *in vitro*. Advanced 3D *in vitro* models are particularly useful when conventional 2D cell cultures do not replicate the dynamic responses of native tissues well enough (16,18). The basic elements (Figure 2) that must be considered in the construction of the *in vitro* model are the source and type of cells (9), physicochemical stimuli (19) and biologically active molecules (biochemical stimuli) (20,21), which promote the desired cellular phenotype.

The simplest 3D *in vitro* models include spheroids and organoids grown in special cell vessels to which cells do not adhere. With appropriate biochemical stimuli, cells organize themselves into a simple spherical 3D shape. iPSCs are the most commonly used. As the name suggests, *ex vivo* organoids mimic the basic hierarchical structure and physiology of organs, while spheroids do not have a clearly defined internal structure and cellular organization (22). Organoids are mainly used for basic *in vitro* research dealing with organogenesis and the course of the disease (23). In addition to the fact that organoids differ significantly in size and shape, the main limitation of both models is size, as cells within the 3D construct rapidly necrotize due to limited diffusion (24). To construct larger and more complex 3D constructs, it is extremely important to build cellular bioscaffolds with adequate porosity, facilitating the access of oxygen and nutrients to the cells (17,25).

When constructing advanced 3D *in vitro* models, it is, therefore, necessary to additionally select appropriate building blocks for the production of cellular scaffolds with appropriate structural and mechanical properties. The nano-, micro- and macro-properties of *in vitro* models should be adapted to mimic the characteristics of the native or diseased tissue. Emphasis should also be placed on mimicking the mechanical conditions in which cells grow. Therefore, suitable biocompatible materials with appropriate mechanical properties must be selected. An important part of modelling also involves mimicking concentration gradients of nutrients, gases, pH, and metabolites (9).

3 Pancreas

Ever since the development of the *in vitro* model, one of the primary goals has been to develop a functional *in vitro* model of pancreas, and in particular the islets

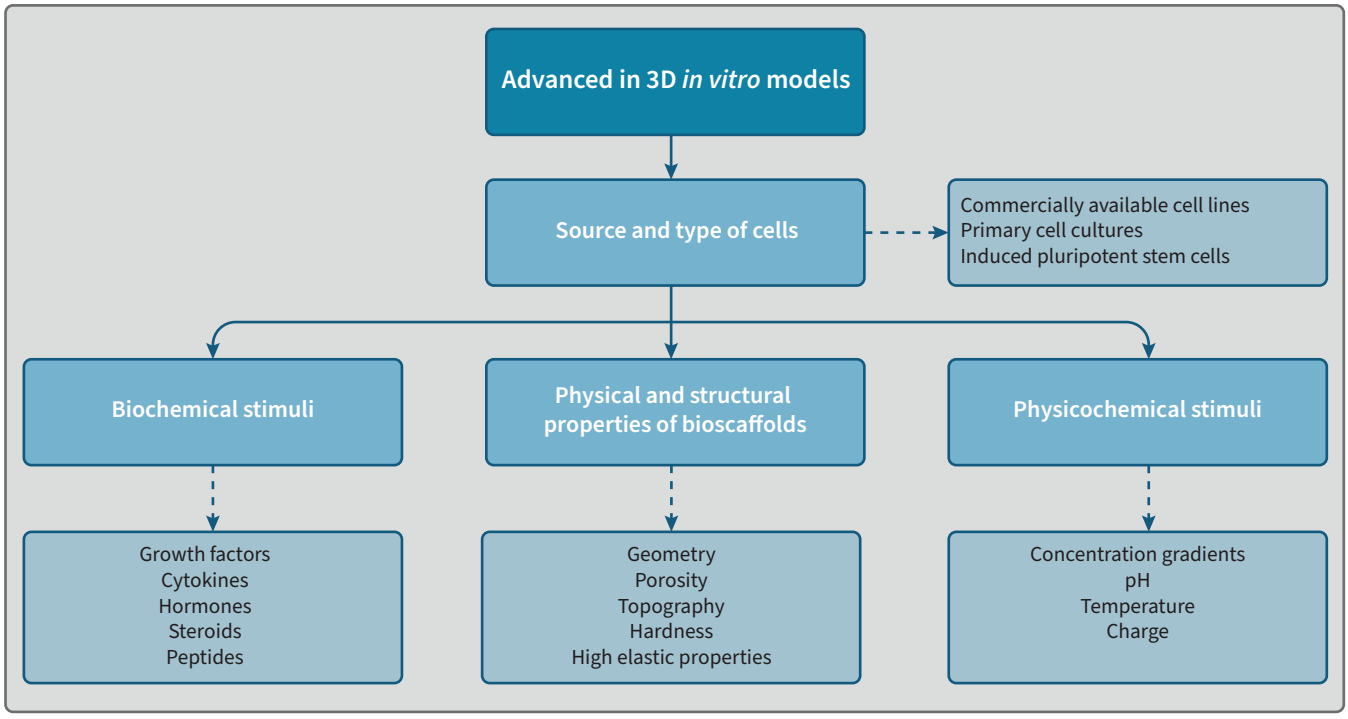

Figure 2: Key elements to consider when building advanced 3D *in vitro* models. After selecting the appropriate source and cell type, important biochemical and physicochemical stimuli must be mimicked in vivo which ensure the maintenance of the desired cellular phenotype. It is also essential that 3D bioscaffolds mimic the key physical and structural properties of the original ECM.

of Langerhans, due to the desire to study the course of type 1 and 2 diabetes (lat. Diabetes mellitus, DM). The normal functioning of the human body depends on the precise regulation of blood glucose levels. The pancreas, more precisely the beta cells located in the islets of Langerhans, play a central role in maintaining the dynamic balance of glucose. These secrete insulin when the concentration of energy-rich molecules in the blood (especially glucose) and certain hormones (e.g. GIP and GLP-1 incretins) increases and under the influence of neurotransmitters (e.g. CCK and acetylcholine) (26,27). The pancreas is a retroperitoneal organ about 14-18 cm in size, consisting of three main anatomical parts: the head, body and tail. It is a gland that is structurally and functionally divided into a larger (~ 95%), exocrine portion, consisting mainly of ductal and acinar cells, and a smaller (~ 5%), endocrine portion, consisting of islets of Langerhans. Acinar cells secrete digestive enzymes indirectly into the duodenum, which are responsible for breaking down proteins, lipids and carbohydrates. Ductal cells, on the other hand, secrete fluid rich in bicarbonate anions (28). Endocrine tissues in humans are represented by about a million endocrine microorganisms, 50-500 µm in size, called islets of Langerhans. Each islet (even in different species) contains about 1,000 cells. The most numerous are beta cells (50-60%), which secrete insulin, followed by alpha cells (35-40%), which secrete glucagon, and delta cells (10-15%), which secrete somatostatin. Less represented are PP or gamma cells that secrete pancreatic polypeptide and epsilon cells that secrete ghrelin (29). DM types 1 and 2 are considered to be among the most

important disorders in the functioning of the endocrine portion of the pancreas. Diabetes is a metabolic disease characterized by the inability to regulate blood glucose homeostasis. In particular, (auto) immune-mediated type 1 diabetes is caused by an absolute lack of insulin due to the destruction of beta cells (30). DM type 2 is a complex polygenic disease that is also influenced by a number of environmental and epigenetic factors. It is caused by a reduced sensitivity of the target tissues to insulin with initial compensatory increase but relative insufficiency of insulin secretion. This ultimately leads to secretory dysfunction, beta cell failure, and absolute insulin deficiency (31-33). Both types of disease can lead to acute and chronic complications, including cardiovascular problems, renal failure, neurological damage, vision loss, and generally increased patient mortality (34,35). The most basic research on the origin and development of diabetes uses a number of different animal models (predominantly rodents, especially mice) (36). Despite many similarities, there are many structural and physiological differences in the islets of Langerhans between humans and rodents, leading to differences in functional linkage between cells and finally to differences in the complex dynamics of insulin secretion (26,28). This means that the results obtained in animal models cannot always be reliably transferred to humans in all respects.

4 *In vitro* models of the pancreas

The development of a functional human model of an *in vitro* pancreas would mark a breakthrough in basic diabetes research and lead to the development of new therapeutical substances for the treatment of diabetes. At the same time, it would greatly reduce the need to use animal models. Despite many years of developing *in vitro* models, there are many problems remaining in the area of the pancreas. The first problem in the cultivation of *in vitro* pancreatic cells arises with the demanding process of isolation and purification of viable pancreatic islets and cells within the islet. Islet of Langerhans cells are no longer capable of auto- and paracrine communication soon after isolation, when they lose homo- and heterotypic intercellular contacts. In addition, they lose critical ECM contacts and basement membrane contacts, which ultimately leads to reduced viability and loss of cell function. The additionally high metabolic requirements and size of the islets limit the availability and access of oxygen and nutrients to the cells inside the islet. Due to limited diffusion, necrotic cell death begins rapidly. All this contributes to the fact that current 2D *in vitro* pancreatic cell models do not mimic the critical dynamics of insulin secretion when stimulated with glucose (37). 2D substrates thus restrict the growth of pancreatic cells, prevent the formation of complex 3D *in vivo* morphology, and do not mimic ECM cell-type contacts that are critical for normal endocrine cell function. Even more in favour of the importance of the 3D environment for the differentiation, growth and functionality of the islets of Langerhans is the fact that recently a direct link between the morphology of the pancreatic islet and endocrine differentiation was confirmed. In the differentiation of endocrine progenitor cells, the alpha cells that develop first migrate to the outside of the islet and form a mantle, while the beta cells that form later remain in the nucleus of the islet. Such temporal and spatial proportionality leads to the typical spherical 3D architecture of the human pancreatic islet (alpha cell mantle and beta cell nucleus), which is essential for the normal functioning of the islet (38).

4.1 3D Biomimetic scaffolds mimic the ECM of the native tissue

In the most elementary sense, the ECM is a natural biologically active cellular scaffold composed of structural (collagen, laminins, fibronectins) and signalling proteins, polysaccharides, glycoproteins, proteoglycans, biologically active molecules, electrolytes, and water (39). In *in vivo* conditions, the ECM creates a complex 3D framework by providing mechanical support to cells and, in addition to intercellular communication in all spatial dimensions, enables key cellular processes such as adhesion, migration, proliferation, and differentiation (40). ECM plays a key role in the microenvironment of pancreatic cells (41), so it is extremely important to mimic the basic properties of the original ECM using 3D cellular scaffolds in the construction of an advanced *in vitro* model of the pancreas (42,43).

In general, cellular scaffolds must replicate the complex 3D microarchitecture, hierarchical structure, and ECM composition of the native tissue to be mimicked *in vitro*. The basic building blocks, 3D structure, and composition of the pancreatic ECM are best mimicked by scaffolds constructed of natural polymers (primarily polysaccharides). These form hydrogels and have a positive effect on the growth and viability of pancreatic cells (44). Therefore, to build advanced *in vitro* models of the pancreas (islets of Langerhans), great effort is invested in the development of 3D biomimetic cellular scaffolds that mimic the basic building blocks of the original microenvironment (ECM) with which pancreatic cells are surrounded. A key criterion in the construction of the scaffold is the choice of the appropriate material. It must be based on the mechanical properties of the tissue, as the surface of the material plays an important role in directing the growth and development of cells, and it is also the central interface for intercellular interactions (45). The pancreas is a nonlinear visco-elastic soft tissue with low shear modulus (46). Therefore, natural polymer hydrogels, which have a high water content and exhibit similar structural and mechanical properties as pancreatic ECM, are the best materials for building cellular scaffolds (47). In addition to biological compatibility, the basic properties that must be taken into account in the construction of the bioscaffold are the structure and elementary building blocks of the scaffold. Topographic, material, and physical characteristics of all size classes (e.g. macro-, nano-, and microtopography, macro-, and microporosity) are structural stimuli that guide cell behaviour. The choice of biocompatible materials must be based on the fact that both the micro- and macro-properties of the scaffolds mimic the characteristics of the native or pathologically altered pancreatic tissue. The surface properties of scaffolds (e.g. roughness), their mechanical properties, microstructures (e.g. pore size and shape), and other characteristics (e.g. swelling and biodegradability of scaffolds) significantly affect the growth and phenotype of the cell (48). Pancreatic cells grown *in vitro* on 3D cellular scaffolds were able to differentiate into physiologically appropriate tissue, and at the same time, their morphology differed significantly from cells grown in 2D cell cultures. Compared to 2D substrates, 3D scaffolds made of polysaccharides also promoted better adhesion, proliferation, and survival of cells (49-51).

Although materials of natural origin represent a good choice for the construction of 3D biomimetic scaffolds, they cannot mimic the complexity of the ECM of the native tissue down to the last detail. Recently developed decellularized

extracellular matrix (dECM) techniques promise the possibility of building dECMs cellular scaffolds that almost completely mimic the complexity of the native tissue. DECM cell scaffolds can be obtained from a variety of tissues by a decellularization method, which typically involves the lysis and removal of cells from the tissue by perfusion with deionized water or detergents (52,53). Thus, only tissue-specific ECM remains after the process, which typically involves macromolecules such as collagens, laminins, fibronectin, elastin, and other tissue-specific glycosaminoglycans, cytokines, and growth factors. Recently, a group of scientists demonstrated that such dECM-specific scaffolds also significantly affect the function of pancreatic cells. Using the decellularization method, they successfully prepared cellular scaffolds from the pancreas with a unique composition, physical structure, and biological activity. iPSC cells grown on specific pancreatic scaffolds spontaneously differentiated into cells, similar to pancreatic cells, which also began to express corresponding genes (54). DECM prepared from pancreatic tissue has also been successfully used for 3D printing of biologically mimetic cellular scaffolds. Such 3D printed dECM scaffolds promoted the differentiation of iPSCs against the pancreatic phenotype while maintaining the long-term viability and function of isolated islets of Langerhans (55). The biggest problem with the use of dECM remains the fact that during the decellularization process, the highly specific spatial arrangement of proteins and other molecules is disrupted. Therefore, the current goal in development remains to find a balance between the complete removal of cellular components and the preservation of small vessels (capillaries) and other tissue structures. Toxic effects were also observed on cells grown on dECM scaffolds, most likely due to residual detergents used during the process (56).

Currently, the biggest limitation in the construction of 3D scaffolds that support the growth of pancreatic cells in the long run is the inadequate mechanical properties of materials, especially hydrogels. Various crosslinking techniques (e.g. ionic crosslinking), inorganic/organic additives (cellulose fibres, various nanoparticles) (57,58), or other synthetic materials (polycaprolactone) are used to improve the mechanical stability of hydrogels. These improve the mechanical properties of the scaffold or provide a suitable basic framework (57,59). An additional problem is the fact that it is extremely difficult to adjust the aforementioned key characteristics of scaffolds (porosity, topography, mechanical properties, rate of decay and water uptake, rheological properties of materials) to fully mimic the properties of the native tissue. The development of hydrogel formulations, on the basis of which it would be possible to produce long-term stable cellular scaffolds and to which the desired properties could be additionally arbitrarily adjusted (e.g. visco-elastic, mechanical, surface), would mean remarkable progress in the construction of advanced *in vitro* models (60).

5 3D printing for building *in vitro* models

Despite all the technological advances in TE, the development of physiologically relevant tissues, especially the pancreas, remains difficult. Due to the limitation in the diffusion of oxygen and nutrients, the building of constructs larger than 200 μm is extremely problematic (25). Progress towards building larger tissue constructs is hampered by the fact that vascular functionality must be incorporated into the scaffold to ensure and improve the transfer of oxygen and nutrients to the

cells while promoting the removal of their waste products. Construction of 3D vascular networks within tissue constructs plays a key role in the long-term survival and maintenance of cell viability in *in vitro* models (61,62). To date, researchers have not been able to develop cellular scaffolds to build *in vitro* soft tissue models that demonstrate adequate resolution, structural integrity, and required biocompatibility at the same time (63). Several TE approaches (64-67) have been developed to address these problems, but most are limited to inducing *in vitro* angiogenesis. In addition to the fact that such approaches require long-term incubation for vessel formation as well as the use of costly growth factors (e.g. vascular endothelial growth factor, VEGF), their main drawback is limited repeatability and the inability to spatially control the distribution of blood vessels within the construct, which often does not allow perfusion at all. In recent years, additive approaches such as 3D printing have been used more frequently to produce cellular scaffolds based on biocompatible materials, advanced *in vitro* models, and tissue constructs with blood vessels included (68). In doing so, various additive production techniques are developed and used simultaneously (e.g. lithographic techniques, i.e., ink-jet printing, microextrusion methods) to build 3D cellular scaffolds that better mimic the architecture, biochemistry, and functionality of native tissues. Individual techniques show certain advantages and disadvantages (52). Among them, 3D bioprinting represents a new and most promising method used, which is expected to revolutionize the field of building advanced *in vitro* models. In addition to the ability to simultaneously use a wide range of biocompatible materials and exceptional application versatility (69,70), the 3D biological printer allows the loading of material with a micrometre spatial resolution (71) under cell-friendly conditions such as low shear forces (72,73). This gives the 3D printer a great advantage over other conventional techniques for the preparation of cellular scaffolds, which are often limited by the control of the 3D shape, the spatial arrangement of individual components of the material and thus the local distribution of the density of material and cells (74).

5.1 Core/Shell 3D printing technique for building advanced *in vitro* models

As already mentioned, for the development of larger and physiologically more complex models *in vitro*, it is extremely important to build cellular scaffolds that allow the smooth flow of cellular medium or even mimic the basic functionality of the vessel lumen. Probably the best and simplest approach to building a 3D printed *in vitro* flow model is to include a connected network of hollow channels inside the tissue scaffold. The construction of such a bioscaffold would reduce the likelihood of the formation of necrotic areas in the construct and also solve many other already mentioned shortcomings of the existing models (73). Endothelial cells can be further populated into the hollow canals of the scaffolds, thus gaining the ability to mimic synthetic vessels in 3D tissue models (75). In addition to the already mentioned facilitated diffusion of oxygen, nutrient inflow, and uninterrupted removal of CO_2 and metabolites, such lumens of the channels can also fulfil other important physiological tasks depending on the specifics of *in vitro* cultured tissue. *In vitro* endocrine tissue models allow secretion collection and assessment of the secretory function of the construct, and exocrine tissues allow secretion and its collection for quantification. In order to build an

advanced 3D *in vitro* model of the entire pancreas, which would include both endocrine and exocrine portions, in addition to the lumens where endocrine cells release their secretions, ducts for the release of enzymes must be additionally included in the biological scaffold, because their activation could lead to autodigestion or even trigger *in vitro* pancreatitis of cultured tissue.

Recently, a new version of the method of 3D printing of hollow channels (i.e., core/shell printing) (76-79) has attracted a lot of attention, as it promises fast, simple, and repeatable construction of stable cellular scaffolds with built-in flow channels. Using a coaxial nozzle allows so-called core/shell printing, 3D printing of two materials at the same time, one being extruded as a core filament (the core) and the other as a shell around it (the shell). The technique opens the possibility that by choosing biological materials with different mechanical properties, the harder material structurally supports the softer material during and after printing. If to construct the scaffold a material is used that can be chemically crosslinked (e.g. alginate) and it is extruded as a shell while at the same time the crosslinking agent (e.g. $CaCl_2$) is extruded into the core, then it is possible to construct a stable bioscaffold with hollow filaments in a single process step (80). Coaxial printing has already been used to build scaffolds with solid (81), so-called core/shell filaments (82), and hollow filaments (83). However, the materials used have not yet been optimized for simultaneous cellular viability and even mechanical robustness of the constructs. Despite all the advantages of both ordinary 3D printing and so-called core/shell printing, the development of appropriate material formulations (ink) that simultaneously demonstrate all the necessary properties suitable for 3D printing and meet all biological requirements still presents a great challenge (84).

6 Tissue slice as a model of the pancreas *in vitro*

Due to the many limitations and the complexity of building good 3D models of the pancreas, because this organ has a complex structure and function (28,85), isolated endocrine cells, especially beta cells, isolated acinar cells, isolated islets of Langerhans, and isolated ducts and acinuses are still mostly used for basic and translation studies of the pancreas (86,87). The results of these studies are often complemented by various *in vivo* measurements at the level of the whole organism (88). An important step towards the best possible 3D model for pancreatic tissue, which is alternative in many respects and partly complements the development of 3D models with the help of various media and 3D printing, has recently been developed and the method of pancreatic tissue slices is increasingly being used (86,89).

In this method, the pancreatic tissue of various types of model organisms (most often mice, possibly rats or pigs) or humans is cut into tissue slices about 100 micrometres thick. In contrast to the isolation of cells and islets, no enzymes are used, but only minimal mechanical stress due to cutting. Tissue slices contain intact islets of Langerhans, cut islets of Langerhans, large areas of intact acinuses, and long sections of ducts of different orders of magnitude. One of the most important properties of the tissue slices thus obtained is that the cells within the islets of Langerhans and within the acinuses are interconnected by various intercellular contacts while preserving the paracrine interaction within the endocrine portion, within the exocrine portion and between the two portions. To a greater extent than

in the isolation of islets and acinuses, the vessels, basement membrane, connective envelopes, immune cells, and other elements of the mesenchyme are also preserved, and thus also the 3D structure of the tissue (86,87,90,91). Acute pancreatic slice is thus a special form of primary cell culture that can be used for at least 24–48 hours without special additional scaffolds (87,92).

In combination with electrophysiological measurements, intracellular calcium ion concentration measurements, secretion measurements, and various morphological measurements, the tissue slice method has been shown to be at least equivalent, in terms of results and repeatability, to cell and islet or acinus isolation methods (93-99). In many respects, however, the tissue slice allows for more physiological data, especially when it comes to assessing communication between different cells (37,100-102). An additional important advantage of the tissue slice method is that it is well compatible with many different model organisms with fluorescently labelled cells that interest us, and at the same time also with diabetes models that lead to the decay of most beta cells (e.g. streptozotocin model) and so they are not compatible with the isolation of cells or islets, as in these cases too little isolate is obtained (103,104). The results of morphological and functional measurements in the slice are becoming the gold standard in this field, as well as an important reference for measurements in other 3D models.

Finally, we should emphasize that tissue slices have recently been used in combination with special scaffolds that extend the lifespan of the *ex vivo* slice and its usefulness for studies by at least one week (87). When using human tissue, it is anticipated that scaffolds will need to allow tissue transfer for a duration of several days so that it can be used in studies in those parts of the world where there is no local source of human tissue or for measurements in specialized laboratories using methods that are not available at the location of the source of human tissue. We are assuming that a larger or smaller part of the tissue from the slice can in the future also be used to build 3D models using the already mentioned advanced materials and techniques of combining cells with these materials (60).

7 A glance into the future

Despite remarkable advances in both the broader interdisciplinary domain of tissue engineering and the narrower field of *in vitro* pancreas models, both fields still face many problems that need to be overcome. Currently, the most advanced 3D *in vitro* models mimic only the basic functions of individual tissues and organs, so despite the shortcomings, tissue slices are used as the gold standard for performing basic studies (especially with the pancreas). A step in the right direction means the development of two seemingly unrelated fields of microfluidics and the production of computer microchips. The so-called organs-on-a-chip are cell cultures grown on advanced microfluidic devices (105). Microchip manufacturing techniques (e.g. soft lithography) (106) can produce high-precision flow scaffolds that better mimic physicochemical (concentration gradients of gases and nutrients), structural (nano-topology), and biochemical stimuli (concentration gradients of biologically active molecules) of the tissues that are being mimicked. In addition, the cells in such devices are not grown in a static environment of cell vessels or 3D bioscaffolds, but are continuously exposed to the flow of cellular medium. Medium perfusion mimics key physicochemical

stimuli (formation of shear forces and concentration gradients) to which cells are exposed in the source environment. At the same time, it facilitates the diffusion of oxygen and nutrients and enables the removal of waste products (107). The cells in the islet of Langerhans grown in such a microfluidic environment have shown, for example, morphology that is more correct and an improved viability compared to static cultures. In addition, when stimulated with glucose, beta cells maintained adequate insulin secretion dynamics for a longer time (108,109). Future research in the field of organs-on-a-chip will focus on the functional connection of individual organs and on the development of the so-called body-on-a-chip and additional coupling of the system with microsensors. This will not only provide insight into the functioning and responses of individual cells, but also into complex signalling and communication between different tissues and organs in real time. This will, among other things, open up a number of new possibilities in pharmacological and toxicological studies, and enable more advanced and targeted development of therapeutical substances, as well as the development of disease models affecting several organs at the same time (110). Current developments suggest that the simultaneous development of (1) advanced materials with dynamically adaptable mechanical, physicochemical, and structural properties, (2) more accurate micro-additive production methods to build more complex bioscaffolds (3), as well as the use of new cellular resources and (4) optimization of cell culture conditions will lead to the construction of advanced *in vitro* models that will fully and representatively mimic the hierarchical structure and functionality of tissues or organs *in vivo*.

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