Culture Systems of Isolated Pancreatic Islets with Extracellular Matrix Biomimetics as Tissue-Engineered Constructs of the Pancreas

Victor I Sevastianov (viksev@yandex.ru)
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova
https://orcid.org/0000-0003-1995-3373

Natalia V Baranova
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova

Lyudmila A Kirsanova
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova

Anna S Ponomareva
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova

Eugene A Nemets
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova

Sergey V Gautier
FSBI Academician V I Shumakov Federal Research Center of Transplantology and Artificial Organs: FGBU Federal'nyj naucnyj centr transplantologii i iskusstvennyh organov imeni akademika V I Sumakova

---

Research Article

Keywords: pancreatic islets, culture, biopolymer scaffolds, decellularization of pancreas, tissue-engineered structure, insulin secretion

Posted Date: March 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-263825/v1

Abstract

The creation of a tissue-engineered structure of the pancreas based on isolated pancreatic islets is hindered by problems associated with maintaining their viability and insulin-producing function. Both biopolymer and tissue-specific scaffolds can contribute to the preservation of the structure and function of pancreatic islets *in vitro* and *in vivo*. Comparative morphofunctional analysis *in vitro* of two different types of tissue-engineered structures of the pancreas, which represent culture systems of isolated islets with biomimetics of an extracellular matrix - a biopolymer collagen-containing scaffold and a tissue-specific scaffold obtained as a result of pancreatic decellularization, - was performed. The results showed that the use of scaffolds in the creation of a tissue-engineered design of the pancreas contributes not only to the preservation of the viability of the islets, but also to the prolongation of their insulin-producing functions, compared to the monoculture of the islets *in vitro*. A significant increase was found in the basal and stimulated (under glucose load) insulin concentrations in the tissue of engineered structures studied, at the same time the advantage of using a tissue-specific scaffold compared to a biopolymer collagen-containing scaffold was shown. We think that these studies will become a platform for creating a tissue-engineered design of the human pancreas for treatment of type 1 diabetes mellitus.

Introduction

In the last decade, as an alternative to traditional surgical methods of treatment, there was an active development of technologies of tissue engineering and regenerative medicine (TERM) to restore the structure and functions of damaged tissues/organs. These technologies are aimed at creating tissue-engineered constructs (TECs), including a tissue-engineered construct of the pancreas (TECP), performing an insulin-producing function [1, 2]. The relevance of the search for new methods of treating type 1 diabetes mellitus is beyond doubt. The incidence of type 1 diabetes mellitus in the world is increasing annually [3] and the enhancement of the traditional method of treatment with insulin therapy does not save patients from developing severe complications [4].

Currently, for the treatment of severe cases of type 1 diabetes, donor pancreas transplantation [5] or transplantation of pancreatic islets isolated from the pancreas of posthumous donors [6] are used. Transplantation of allogenic islets, as an alternative to pancreas transplantation, is able to provide patients with insulin independence for a certain period without subjecting patients to serious surgical intervention [7].

However, a significant drawback of pancreatic islet transplantation is the low functional activity of islets, due to the activity of a number of damaging factors during the isolation procedure, such as ischemia, oxidative stress, and possible cytotoxic effect of enzymes. The isolated islets not only lose vascularization and innervation, yet also connections with the extracellular matrix, which play an important role in maintaining the viability of the islets [8].

This problem can be solved by the development of the TECP, often referenced in publications as a bioartificial pancreas, formed on the basis of pancreatic islets or other insulin-producing cellular components [9] and scaffolds [10, 11] which contribute to the preservation of the structure and function of islets *in vitro* and *in vivo*. The advantage of using the islets as a cellular component lies in the accumulated secretion of hormones and specific biologically active endogenous polypeptides by all types of islet cells [1]. The obtainment of the viable functionally active islets where β-cells constitute the main cell population is a defining step in the development of the TECP. Recently, the prospects for obtaining insulin-producing cells (IPCs) from stem cells or progenitor cells have been studied [12]. Stem cells are capable of self-renewal and differentiation into various types of cells, including pancreatic differentiation. Attempts have been made to obtain the IPCs by differentiating stem cells of various origins, including embryonic stem cells, mesenchymal stem cells, into endocrine cells of pancreas, including β-cells [13]. There was an effort to obtain the IPCs from cells that are ontogenetically close to β-cells: acini and duct cells by dedifferentiating cells into a progenitor state with a subsequent stimulation of repeated differentiation into β-cells [14]. However, in the process of constructing the TECP, it is necessary to take into account that not only β-cells, but also other types of islet cells participate in its overall functionality. This is the advantage of creating bioartificial pancreas based on islets [1].

The choice of a scaffold, which in optimal cases acts as a carrier of cells, a scaffold, and a nutrient medium, is the second key problem in the creation of a TEC [10, 11]. Optimally, scaffolds should imitate the extracellular matrix (ECM) functions [11, 15], providing the necessary conditions for the formation of three-dimensional biological structures of a TEC *in vitro* (in a bioreactor) or *in vivo* (*in situ*, if possible) [16–19]. When developing scaffolds, the pore size and total porosity of the scaffold are of a great importance to create the necessary conditions for neovascularization and neoinnervation of bioartificial structures. Thus, the islets on a porous scaffold with microcells (pore size 40 μm) injected to mice with experimental type 1 diabetes mellitus ensured the viability of the β-cell population and the restoration of glucose levels (six of eight mice returned to stable normoglycemia with an average time to remission of 6.2 ± 3.2 days) compared to the transplantation of the islets suspension (only two out of seven mice returned to stable normoglycemia) [20].

It was shown that pancreas islets of an adult rat cultured in a porous polyglycolic scaffold were more than 2 times viable and secreted 4 times more insulin than control pancreatic islets cultured under standard conditions for 15 days [21].

In experiments with culturing of human islets in the presence of polylactoglycolide scaffold [22], the level of insulin secretion turned out to be higher than in similar islets cultured in suspension.

To create bioartificial tissues, including bioartificial pancreas, it is preferable to use injectable forms of bioresorbable hydrogel scaffolds obtained from the ECM components [10, 18]. These include three-dimensional one-, two- and multi-component non-crosslinked or cross-linked (structured) hydrogel scaffolds from collagen, gelatin, hyaluronic acid, etc., the so-called biomimetics of ECM, imitating, to a certain degree, its structure and bioactive properties. In the composition of bioartificial tissues/organs,

such hydrogel biopolymer mimetics create a microenvironment close to ECM for cell cultures, providing adhesion, proliferation, differentiation of cells, as well as their synthesis of a tissue-specific ECM.

The biopolymer microheterogeneous collagen-containing hydrogel [23] contains the main components of ECM of tissues of animal origin (peptides of partially hydrolyzed collagen, glycoproteins and uronic acids) as well as biologically active agents of ECM, such as growth factors necessary for the vital activity of cells and the synthesis of exogenous uronic acids, proteoglycans and collagen. In a series of works *in vitro* and *in vivo* [17, 18, 24, 25], the effectiveness of using the BMCH as a scaffold in cellular-engineered structures for the regeneration of damaged articular cartilage, liver and pancreas was proved.

Thus, biomimetic bioresorbable 3D scaffolds are universal platforms for the creation of bioartificial pancreas, ensuring the vital activity of islet cells during the formation of the TECP and their subsequent implantation.

In recent years, the development of cell and tissue-engineered constructs based on tissue-specific scaffolds made from decellularized tissues, preserving the biochemical, spatial and vascular relationships of the native ECM, with the most complete removal of DNA, cellular material, and cellular surface antigens, has begun [26–29].

For decellularization, physical, chemical, enzymatic or mechanical processing of tissues was used [30–32], with the choice depending on the density and thickness of the original tissue, the number of cells, and the content of proteins and lipids. The presence of DNA in the decellularized material can cause an adverse immune response and the appearance of adverse reactions during the subsequent recellularization of the scaffold [33]. On the contrary, the presence in the decellularized material of scaffold proteins containing components of tissue structures (glycoproteins of extracellular substance, structural proteins of intercellular contacts, and cell attachment factors) makes it possible to optimize the conditions for prolonged vital activity of attached cells [32].

Among physical methods of decellularization, cyclic repetition of freezing and thawing and the method of osmotic shock have become widespread. Chemical decellularization involves incubation of tissues in solutions of detergents of various compositions. There are some studies devoted to production of decellularized scaffolds from pancreatic tissue with their subsequent successful recellularization [30, 33, 34]. It has been shown that the three-dimensional structure of native ECM components determines the topographic location of pancreatic endocrine cells, which affects the survival rate and secretory activity of islets. Thus, in case of recellularization of the islets of decellularized pancreas, an increase in insulin secretion was observed as compared with a monoculture of islets [33]. At the same time, on an experimental model of diabetes mellitus it was shown that the recellularized pancreatic tissue is able to control the blood glucose level in mice [34].

Thus, the main tasks in creating bioartificial pancreas remain to determine the optimal conditions for the isolation and culturing of islets and the choice of a scaffold that provides the best conditions for maintaining the functional activity of islets in the TECP.

The aim of this work was to carry out a comparative *in vitro* morphofunctional analysis of two tissue-engineered pancreatic structures, being culture systems of isolated pancreatic islets with extracellular matrix biomimetics.

Materials And Methods

Experimental animals

The studies were carried out on sexually mature male Wistar rats (180–220 g.). All manipulations with animals were carried out in accordance with the ARRIVE guidelines and the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Biopolymer microheterogeneous collagen-containing hydrogel (the BMCH scaffold)

As a biopolymer hydrogel mimetic of ECM, the injectable form of the BMCH (trademark SpheroGEL®, manufactured by "BIOMIR service" JSC, Russia) was chosen [23]. The BMCH is a γ-radiation crosslinking scleral collagen type I of animal origin 350 ± 100 μm in size, dispersed in a homogeneous hydrogel solution containing low and high molecular weight components of an ECM [23–25].

At room temperature, the sterile (γ-sterilization, 1.5 Mrad) syringe form of the BMCH scaffold has the form of a transparent, granular, viscous gel, elastic modulus 1170 ± 12Pa, viscosity modulus 62.9 ± 7.9Pa and pH = 6.80 ± 0.05. Visualization of the heterogeneous component of the BMCH scaffold by cryo scanning probe nanotomography revealed a porous structure of microparticles with a pore size of 2–4 μm [24], which is a positive property in the processes of neovascularization and neoinervation of bioartificial structures based on it. The time for complete resorption of the BMCH scaffold depends on the place of implantation and varies from 1 to 9 months.

Tissue-specific scaffold from decellularized rat pancreas

A finely dispersed decellularized pancreas (DP) tissue-specific scaffold (a DP scaffold) with preservation of the architectonics and basic composition of the original ECM of pancreases was obtained by the method of physicochemical decellularization of the rat pancreas, focusing on the known methods of decellularization of various parenchymal organs [29–33]. For decellularization, a subtotally removed rat pancreas was used, which was manually crushed using eye scissors to a fragment size of no more than 1x2 mm. The pancreas fragments were processed at room temperature under continuous stirring in a MultiBio RS-24 rotary system (BioSan, Latvia).

We used 0.1% sodium dodecyl sulfate (SDS) solution and phosphate-buffered saline (PBS) of high and low ionic strength (osmotic shock method), followed by thorough washing of SDS residues from decellularized fragments of pancreatic tissue in three changes of PBS containing an antibiotic (ampicillin, 10 μg/ml) and antimycotic (amphotericin, 1.5 μg/ml).

Then DP scaffold samples were introduced into cryovials, frozen, sterilized (γ-sterilization, 1.5 Mrad) and stored at 4–6°C.

To determine the immunogenicity of the decellularized material by the residual amount of nuclear material in the DP scaffold, DNA isolation and fluorescent staining were performed. DNA isolation from DP scaffold samples was performed using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. For the quantitative determination of DNA according to the protocol, a fluorescent dye ™Picogreen Quant - iT (Invitrogen, USA) was used.

The cytotoxicity of the DP scaffold *in vitro* was assessed under aseptic conditions by direct contact on fibroblast cultures of L929 [35]. The culture was visually assessed using an Eclipse TS 100 inverted microscope (Nikon, Japan). The metabolic activity of fibroblasts after contact with the samples was assessed after 24 h using the vital dye prestoBlue™ Cell Viability Reagent (Invitrogen™, USA) according to the manufacturer's protocol.

The functional properties of the DP scaffold with respect to its ability to maintain cell adhesion and proliferation were studied after 10 days of culturing L929 mouse fibroblasts on a scaffold. Fibroblast viability was assessed by fluorescent staining with acridine orange and propidium iodide (AO/PI).

Pancreatic islets isolation and identification

Islets were isolated from the pancreas of a mature rat based on the traditional method [36] using type I collagenase (activity 100–110 U/ml, Sigma, USA) with some modifications in order to increase the viability and the number of isolated islets.

Thus, the enzyme solution was injected into the pancreatic tissue by successive intraparenchymal injections. The extended pancreatic tissue was not cut, but carefully divided into the resulting lobules. The purification of the islets after isolation in the Ficoll density gradient was replaced by centrifugation in the Hanks solution under the previously established regimes [37].

Dithizone staining was used to identify the islets. 0.2–0.4 ml of islet suspension were added to a Petri dish, 0.1–0.2 ml of dithizone solution were added and incubated for 20–30 min at 37°C.

Culturing of pancreatic islets in the presence of biopolymer hydrogel and tissue-specific scaffolds

A comparative analysis of the morphological state, secretory capacity, and functional activity of the islets during culturing with scaffolds was carried out by studying the following culture systems: islets cultured in suspension (monoculture), islets cultured with the BMCH scaffold, and islets cultured with the DP scaffold.

Freshly isolated islets were resuspended in DMEM/F12 medium (1:1) with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM Hepes, and 50 mg/ml gentamicin, and approximately equal number of islets (n = 300 ± 25) were added to three 25 cm^2 culture flasks. Islets cultured in monoculture (flask 1) served as control.

In flasks 2 and 3, 0.2–0.3 ml of the BMCH scaffold and 0.2–0.3 ml of a suspension of the DP scaffold with an average size of microfragments of 500 ± 45 μm, respectively, were added.

All culture systems were incubated under standard conditions at 37°C in a CO_2 incubator in a humidified atmosphere containing 5% CO_2.

Daily monitoring and photographing of cultured islets (control and experimental) were carried out using an inverted microscope Nikon Eclipse TS 100 (Nikon, Japan) equipped with a digital camera.

Determination of the viability of pancreatic islets in various culture systems using fluorescent staining

To determine the viability of islets in different culture systems, fluorescent staining with acridine orange and propidium iodide (AO/PI) was performed. 0.2–0.4 ml of the islet suspension were placed in a Petri dish, 0.02–0.03 ml of the working solution were added and incubated in the dark at room temperature for 15–17 minutes. The result was evaluated using a Nikon Eclipse 50i fluorescent microscope (Nikon, Japan).

Morphological analysis

Samples of the original rat pancreas, the DP scaffold, as well as islets, freshly isolated and cultured in different culture systems, were subjected to a morphological study using routine histological and specific immunohistochemical staining methods.

Connective tissue (total collagen) was detected in the DP scaffold samples by Masson's method. Elastic fibers in DP scaffold samples were detected by orsein staining according to the Unna-Tentzer method.

For a qualitative assessment of the completeness of the removal of cellular (nuclear) material from decellularized samples, fluorescent staining with DAPI (Sigma, USA) was used.

The identification of the islet β-cells in the native pancreatic tissue and isolated islets, the determination of type I and IV collagen in the DP scaffold samples were carried out by immunohistochemistry according to the classical method with horseradish peroxidase using rabbit anti-insulin antibody (Sigma, USA), rabbit anti-collagen I antibody and rabbit anti-collagen IV antibody (Abcam, UK). The reaction was visualized using the Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Abcam, UK) following the manufacturer's protocol. Analysis and photography of the obtained histological preparations were carried out using a Nikon Eclipse 50i microscope (Nikon, Japan) equipped with a digital camera.

The insulin-producing function of pancreatic islets in various culture systems

To determine the basal insulin concentration at different times, the growth medium was removed from the flasks containing pancreatic islets in monoculture or with scaffolds and replaced with a fresh portion

of a low glucose medium (2.8 mmol/L). After 60 minutes of incubation under the same conditions, samples of the culture medium were taken and frozen (-23°C) for ELISA.

To determine the concentration of insulin under the influence of the traditional stimulator of insulin secretion, glucose, a separate experiment was carried out on the culturing of islets in monoculture and in the presence of scaffolds. On the third day of incubation, the growth medium was replaced with a fresh portion of the low glucose medium (2.8 mmol/L). After a 60-minute incubation under the same conditions, samples of the culture medium were taken and frozen (-23°C). The growth medium was then removed from these flasks and replaced with a fresh high glucose medium (25 mmol/L). After 60 minutes of incubation under the indicated conditions, samples of the growth medium were also taken and frozen (-23°C) for ELISA.

For a quantitative analysis of insulin concentration in the growth medium, we used a kit for enzyme immunoassay Rat Insulin ELISA Kit (Invitrogen, USA) and a Spark 10M microplate reader (Tecan Trading AG, Switzerland) with Spark Control ™ Magellan V1.2.20 software.

The obtained data were statistically processed using Microsoft Excel 2007. The results of the quantitative ELISA method were calculated using a linear calibration curve. All results are presented as mean ± standard deviation. Differences were considered significant at $p < 0.05$.

Results

Tissue Specific Scaffold (DP Scaffold)

Morphological examination of the decellularized pancreas showed that the architectonics of the stroma were preserved, showing an openwork fibrous structure resembling a mesh network. The surviving cells and individual cell nuclei were not found in the samples. Specific staining with DAPI confirmed the absence of cell nuclei and fragments of nuclear material in the scaffold (Fig. 1B) compared to the native pancreas (Fig. 1A). Masson staining of the samples allowed visualization of intact collagen fibers in the resulting scaffold (Fig. 1C), and orsein staining also revealed elastic fibers (Fig. 1D). Antibody immunohistochemical staining confirmed the presence of type I (Fig. 1E) and type IV collagens (Fig. 1F) in decellularized samples.

A comparative quantitative analysis of the DNA content in a sample of the original rat pancreas tissue (1354.8 ± 168.7 ng) and decellularized tissue 1.3 ± 0.3 ng showed that as a result of decellularization, no more than 0.1% of DNA remained in the tissue, which indicates a low immunogenicity (relative to the residual amount of DNA) of the obtained tissue-specific DP of the scaffold.

The metabolic activity of fibroblasts cultures of L929 after contact with DP scaffold was 96.25 ± 1.69% relative to the negative control sample (growth medium), which indicates that DP scaffolds had no cytotoxic effect.

The matrix properties of the DP scaffold were assessed on the 10th day of incubation of L929 mouse fibroblasts with the scaffold. Intensive cell colonization of the scaffold DP was observed; viable fibroblasts were located not only on the surface of the DP scaffold, but also as actively penetrated into its deep layers (Fig. 2).

Morphological study of pancreatic islets cultured in monoculture

A preliminary morphological study confirmed the classic picture of the structure of the rat pancreas: islets were clearly visualized among the exocrine tissue. Freshly isolated islets were generally rounded or oval, sometimes with a slight roughness formed by remnants of the surrounding exocrine tissue. The islets remained intact, indicating that the macrostructure of the islets was not damaged during the isolation process. Staining with dithizone gave the pancreatic islets a terracotta-red color, while the acinar cells remained unstained. The islets stained with dithizone were counted: about 235 ± 12 islets were contained in 1 ml of the islet suspension, and in general, we managed to isolate at least 800–900 islets from one rat pancreas with almost 100% survival.

Islets cultured in monoculture (control) retained their original external characteristics during the first three days of incubation (Fig. 3A). Intravital staining with AO/PI confirmed their viability. The histological examination showed that the islets at this stage retained their characteristic structure with a predominance of insulin-positive β-cells (Fig. 3B).

During the next 3 days of culturing, most of the islets in monoculture underwent destructive changes. The outlines of the islets became irregular, with cavities and signs of fragmentation observed in some islets (Fig. 3C). Fluorescent staining revealed the appearance of dead cells (red fluorescence) in the surviving islets, along with living cells (green fluorescence).

Morphological state of pancreatic islets cultured with extracellular matrix biomimetics

During culturing with the BMCH scaffold, after the first day, a significant part of the islets has attached to the surface of the scaffold microparticles, while the rest continued to float, with all the islets remaining intact without signs of structure degradation throughout the entire period (10 days of observation) (Fig. 4A) compared to the islet monoculture. Fluorescent staining, carried out for a period of 10 days of incubation, confirmed the viability of most of the islets preserved by this time (about 70.0%) (Fig. 4B). After 10 days of incubation, there was a decrease in the number of the viable islets, which by 14 days was no more than 45–50% of the islets preserved by this date.

Histological examination showed that the islets represented compact formations consisting of medium-sized light polygonal cells with fine-grained cytoplasm and a well-defined spherical nucleus (Fig. 4C). Upon immunohistochemical staining with antibodies to insulin, most of the cells in the islets were found to be immunopositive β-cells with an abundant specific granularity in the cytoplasm (Fig. 4D).

Islets cultured with the DP scaffold did not adhere until 5 days of incubation. Subsequently, at least half of the cultured islets were attached to the scaffold surface (Fig. 5A). Fluorescent staining performed on the 14th day of incubation confirmed the viability of the majority of the remaining islets – 85.0% and 70.0% (Fig. 5B and 5C), respectively, which is significantly higher than the viability of the islets cultured with the BMCH scaffold at the same time.

Insulin-producing function of pancreatic islets cultured in monoculture and with extracellular matrix biomimetics

The insulin-producing function of the islets *in vitro* within the TECP was determined in the culture medium by basal insulin concentration and by insulin concentration upon stimulation with glucose.

A comparative analysis of the secretory capacity and functional activity of the TECP was performed relative to islet monoculture.

Secretory activity of the islets was determined at the first, second, third, sixth, eighth and tenth days of culturing. On the first day, the insulin concentration during the culturing of the TECP with the BMCH or DP scaffold was higher, respectively, by 26.2% and 48.7%, compared to the secretory activity of the islets in monoculture, on the third day of incubation – by 62.1% and 102.9%, respectively (Fig. 6A). The revealed difference in the concentration of the hormone in the control and experimental groups at these periods can be explained by the positive effect of scaffolds on the secretory capacity of the islets. On the sixth day, an even greater difference (249.6% and 373.6%) was observed between insulin concentrations during the TECP culturing and control. This correlates with morphological data on destructive changes occurring in the islets after three days of culturing in suspension (see Fig. 3C).

On the 8-10th day of incubation, the remaining islets in the monoculture were not found, therefore the culture medium was not examined. At the same time, when culturing the TECP based on the BMCH or DP scaffolds, the insulin concentration remained practically constant: 93.7 μIE/mL and 126.9 μIE / mL, respectively, while the insulin secretion level of the islets in the TECP with the DP scaffold was 35.5% higher (Fig. 6A).

The functional activity of the islets during culturing with the selected scaffolds was confirmed by the results of the analysis of samples of the culture medium taken on the third day of incubation before and after stimulation with a hyperglycemic glucose level of 4.5 g/L (25 mmol/L). The values of insulin concentrations after stimulation with glucose on the third day of incubation in the samples of the islets in suspension increased by 20.8%, while for the TECP with the BMCH scaffold it increased by 33.0%, and based on the DP scaffold - by 50.7% (Fig. 6B).

The sum of the results obtained gives us grounds to say that the culture systems of pancreatic islets with the BMCH and DP scaffolds represent tissue-engineered structures of the pancreas.

The relative nature of the quantitative assessment of the functional activity of cultured islets in monoculture and in the presence of ECM mimetics should be noted, since it is impossible to reproduce

conditions similar to a native pancreas for insulin-producing cells in *in vitro* model experiments.

The given indices of viability and functional activity of islets in the TECP are comparable with the data on culturing of pancreatic islets with biopolymer collagen-containing scaffolds [2] and scaffolds from decellularized pancreas [33].

For comparison, functional activity was determined through the stimulation index as the ratio of insulin concentration under glucose load to basal insulin concentration. Thus, the viability of human islets cultured in collagen-containing microcapsules [8] on the 6th day of incubation was 78.3% ± 1.6% compared to 85% of the viability of rat islets cultured for the same period with the BMCH scaffold. According to Napierala H. [27], the viability of rat islets cultured with decellularized rat pancreas was 81.96% – 84.5% as early as on the 1st day of incubation with the stimulation index of 1.2, while for the TECP based on the DP scaffold at this time, almost 100% safety of viable rat islets with a stimulation index of 1.5 was observed. On the contrary, it was shown [38] that the stimulation indices of human islets cultured in monoculture and islets cultured with the DP scaffold were practically the same, that is, no positive effect of tissue-specific scaffold on the functional activity of the islets was revealed. It should be noted that these studies were carried out using a tissue-specific scaffold obtained as a result of decellularization of a complete pancreas, whereas in this work, the DP scaffold represents finely dispersed fragments of pancreatic tissue.

Conclusion

In two tissue-engineered pancreatic constructs studied *in vitro*, which are culture systems of isolated pancreatic islets with extracellular matrix biomimetics, not only a better preservation of the islets was observed compared to monoculture under standard conditions, but also a prolongation of their insulin-producing function.

The advantage of using a tissue-specific DP scaffold in comparison with the BMCH for creating a bioartificial pancreas was revealed. However, it should be noted that much less labor and time were required to create a bioartificial pancreas based on the BMCH scaffold.

The islets cultured in suspension underwent destructive changes after 3 days of incubation, which was confirmed by methods of routine histological staining and fluorescence.

In the study of the functional ability *in vitro*, it was found that as early as the first day of culturing, the basal insulin concentration in the islet monoculture was lower by 26.2% and 48.7% lower compared to the islets in the TECP composition with the BMCH and DP scaffolds, respectively.

Nevertheless, a monoculture of islets can be maintained under standard incubation conditions without significant loss of morphological and functional properties of the islets from 3 to 5 days with a certain application, for example, for use:

• in studies of biological safety (immunogenicity) on an experimental model of subcutaneous implantation *in vivo*;

• in studies for research of functional efficacy *in vivo* on an experimental streptozotocin model of diabetes mellitus;

• as a model for testing hypoglycemic drugs.

Note that the shelf life and expiration date of laboratory samples of experimental models of bioartificial pancreas are no more than 48 hours in the temperature range from + 4 °C to + 8 °C and relative humidity of 30-50%.

The results obtained are the basis for research of the creation of the TECP – a bio-artificial human pancreas based on the BMCH scaffold or tissue-specific scaffold from the decellularized human pancreas.

On one hand, from the point of view of early clinical application, the development of human allogeneic TECP based on a tissue-specific scaffold from decellularized prostate tissue and islets of posthumous donors is promising [26].

However, when creating a tissue-specific scaffold from allogeneic pancreas, it is impossible to avoid the same problem of lack of donor organs, as in transplantation of pancreas from posthumous donors, which necessitates the development of protocols for decellularization of fragments of xenogenic pancreas, for example, of a pig or other farm animals.

Positive results of assessing biological safety of the TECP and their destruction products *in vitro* and *in vivo* with an emphasis on studies of their immunogenicity and immunotoxicity will serve as proof of the possibility of using tissue-specific scaffolds from decellularized xenogenic tissues. The final stage of preclinical studies of a human TECP based on a scaffold of xenogenic pancreatic tissue and allogeneic insulin-producing cells obtained from donor pancreas will prove the functional efficiency of a bioartificial pancreas in an experimental model of diabetes mellitus.

It is presumed that a human TECP as a biomedical cell product can be used in clinical practice to compensate for the function of the damaged pancreas. Based on the available experimental data [39], it can be assumed that, in addition to the direct antidiabetic effect, the TECP implantation will have a stimulating effect on the processes of restoration of the pool of actively functioning β-cells of the recipient.

Abbreviations

3D: Three-dimensional; AO: Acridine Orange; BMCH: Biopolymer Microheterogeneous Collagen-containing Hydrogel; DAPI: 4',6-диамидино-2-фенилиндол; DMEM: Dulbecco's Modified Eagle's medium; DP: Decellularized Pancreas; ECM: Extracellular Matrix; IPCs: Insulin-Producing Cells; MSCs:

mesenchymal stem cells; PBS: Phosphate-Buffered Saline; PI: propidium iodide; SDS: Sodium Dodecyl Sulfate; TECs: Tissue-Engineered Constructs; TECP: Tissue-engineered Construct of the Pancreas; TERM: Tissue Engineering and Regenerative Medicine

Declarations

Acknowledgements

Not applicable.

Authors' contributions

VS and SG designed the study, wrote and edited the manuscript. NB, LK, AP and EN conducted experiments, participated in data analysis and interpretation. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kumar K, Joisher H, Ganguly A. Polymeric scaffolds for pancreatic tissue engineering: a review. Rev Diabet Stud. 2018. doi:10.1900/RDS.2017.14.334.
2. Lemos NE, de Almeida Brondani L, Dieter C, Rheinheimer J, Boucas AP, Bauermann Leitao C, Crispim D, Bauer AC. Use of additives, scaffolds and extracellular matrix components for improvement of

human pancreatic islet outcomes in vitro: a systematic review. Islets. 2017. doi:10.1080/19382014.2017.1335842.

3. Creusot RJ, Battaglia M, Roncarolo MG, Fathman CG. Concise review: cell-based therapies and other non-traditional approacher for type 1 diabetes. Stem Cells. 2016. doi:10.1002/stem.2290.
4. Ehlers MR. Strategies for clinical trials in type 1 diabetes. J Autoimmun. 2016; doi:96, 10.1016/j.jaut.2016.03.008.
5. Dean PG, Kukla A, Stegall MD, Kudva YC. Pancreas transplantation. BMJ. 2017; doi:j1321, 10.1136/bmj.j1321.
6. Shapiro AM, Pokrywczynska M, Ricordi C. Clinical pancreatic islet transplantation.
7. Nat Rev E. 2017; doi:10.1038/nrendo.2016.178.
8. Maffi P, Secchi A. Clinical results of islet transplantation. Pharmacol Res. 2015. doi:10.1016/j.phrs.2015.04.010.
9. Llacua LA, Faas MM, de Vos P. Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. Diabetologia. 2018. doi:10.1007/s00125-017-4524-8.
10. Sumi S. Regenerative medicine for insulin deficiency; creation of pancreatic islets and bioartificial pancreas. J Hepatobiliary Pancreat Sci. 2011. doi:10.1007/s00534-010-0303-3.
11. Fisher SA, Tam RY, Shoichet MS. Tissue mimetics: engineered hydrogel matrices provide biomimetic environments for cell growth. Tissue Eng. 2014. doi:10.1089/ten.tea.2013.0765.
12. Amer LD, Mahoney MJ, Bryant SJ. Tissue engineering approaches to cell-based type 1 diabetes therapy. Tissue engineering. 2014. doi:10.1089/ten.TEB.2013.0462.
13. Aguayo-Mazzucat C, Bonner-Weir S. Stem cell therapy for type 1 diabetes mellitus. Nat Rev. 2010. doi:10.1038/nrendo.2009.274.
14. Li Y, Zhao LJ, Xia FZ, Li YX, Lu YL. Transdifferentiation of hepatic oval cells into pancreatic islet beta-cells. Front Biosci (Landmark Ed). 2012. doi:10.2741/4060.
15. Minami K, Seino S. Pancreatic acinar-to-beta cell transdifferentiation in vitro. Front Biosci. 2008. doi:10.2741/3119.
16. Abualhassan N, Sapozhnikov L, Pawlick RL, Kahana M, Pepper AR, Brun A, Gala-Lopez B, Kin T, Mitrani E, Shapiro AM. Lung-derived microscaffolds facilitate diabetes reversal after mouse and human intraperitoneal islet transplantation. PLoS One. 2016. doi:10.1371/journal.pone.0156053.
17. Zhang X, Xie Y, Koh CG, Lee L. A novel 3-D model for cell culture and tissue engineering. Biomed Microdevices. 2009. doi 10.1007/s10544-009-9294-8.
18. Sevastianov VI. Technologies of tissue engineering and regenerative medicine. Russian Journal of Transplantology Artificial Organs. 2014. doi:10.15825/1995-1191-2014-3-93-108. (In Russ).
19. Sevastianov VI, Basok YB, Grigor'ev AM, Kirsanova LA, Vasilets VN. Formation of Tissue-Engineered Construct of Human Cartilage Tissue in a Flow-Through Bioreactor. Bull Exp Biol Med. 2017. doi:10.1007/s10517-017-3971-z.

20. Coronel MM, Stabler CL. Engineering a local microenvironment for pancreatic islet replacement. Curr Opin Biotechnol. 2013. doi:10.1016/j.copbio.2013.05.004.
21. Buitinga M, Assen F, Hanegraaf M, Wieringa P, Hilderink J, Moroni L, et al. Micro-fabricated scaffolds lead to efficient remission of diabetes in mice. Biomaterials. 2017.
22. doi: 10.1016/j.biomaterials.2017.03.031.
23. Chun S, Huang Y, Xie WJ, Hou Y, Huang RP, Song YM, et al. Adhesive growth of pancreatic islet cells on a polyglycolic acid fibrous scaffold. Transplant Proc. 2008; doi:10.1016/j.transproceed.2008.02.088.
24. Daoud JT, Petropavlovskaia MS, Patapas JM, Degrandpre CE, Diraddo RW, Rosenberg L, Tabrizian M. Long-term in vitro human pancreatic islet culture using three dimensional microfabricated scaffolds. Biomaterials. 2011. doi:10.1016/j.biomaterials.2010.10.036.
25. Patent RU. 2433828.
26. Sevastianov VI. Cell-engineered constructs in tissue engineering and regenerative medicine. Russian Journal of Transplantology Artificial Organs. 2015. doi:10.15825/1995-1191-2015-2-127-130. (In Russ).
27. Surguchenko VA, Ponomareva AS, Kirsanova LA, Skaleckij NN, Sevastianov VI. The cell-engineered construct of cartilage on the basis of biopolymer hydrogel matrix and human adipose tissue-derived mesenchymal stromal cells (in vitro study). J Biomed Mater Res Part A. 2015. doi:10.1002/jbm.a.35197.
28. Ponomareva AS, Kirsanova LA, Baranova NV, Surguchenko VA, Bubentsova GN, Basok YB, et al. Decellularization of donor pancreatic fragment to obtain a tissue-specific matrix scaffold. Russian Journal of Transplantology Artificial Organs. 2020. doi:10.15825/1995-1191-2020-1-123-133.
29. Napierala H, Hillebrandt K-H, Haep N, Tang P, Tintemann M, Gassner J, et al. Engineering an endocrine neo-pancreas by repopulation of a decellularized rat pancreas with islets of Langerhans. Sci Rep. 2017. doi:10.1038/srep41777.
30. Mirmalek-Sani SH, Orlando G, McQuilling J, Pareta R, Mack D, Salvatori M, et al. Porcine pancreas extracellular matrix as a platform for endocrine pancreas bioengineering. Biomaterials. 2013. doi:10.1016/j.biomaterials.2013.03.054.
31. Shirakigawa N, Ijima H. Decellularized tissue engineering. Advances in Biomaterials for Biomedical Applications. Springer Nature Singapore Pte Ltd. 2017. doi:10.1007/978-981-10-3328-5_5.
32. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011. doi:10.1016/j.biomaterials.2011.01.057.
33. Keane TJ, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. Biomaterials. 2012.
34. doi:1771–1781, 10.1016/j.biomaterials.2011.10.054.
35. Goh SK, Bertera S, Olsen P, Candiello J, Halfter W, Uechi G, et al. Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. Biomaterials. 2013.

doi:10.1016/j.biomaterials.2013.05.066.

36. Guruswamy Damodaran R, Vermette P. Decellularized pancreas as a native extracellular matrix scaffold for pancreatic islet seeding and culture. J Tissue Eng Regen Med. 2018. doi:10.1002/term.2655.
37. Wu D, Wan J, Huang Y, Guo Y, Xu T, Zhu M, et al. 3D culture of MIN-6 cells on decellularized pancreatic scaffold: in vitro and in vivo study. Biomed Res Int. 2015. doi:10.1155/2015/43264.
38. ISO 10993-5:2009. Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.
39. London NJ, Swift SM, Clayton HA. Isolation, culture and functional evaluation of islets of Langerhans. Diabetes Metab. 1998; 200-7. PMID: 9690051.
40. Kirsanova LA, Baranova NV, Bubentsova GN, Sevastianov VI. Morphological analysis of isolated rat pancreatic islets cultured under standard culture technique and with biopolymer microstructured collagen-containing hydrogel. Russian Journal of Transplantology Artificial Organs. 2017. doi:10.15825/1995-1191-2017-2-90-97. (In Russ).
41. Peloso A, Urbani L, Cravedi P, Katari R, Maghsoudlou P, Fallas ME, et al. The human pancreas as a source of protolerogenic extracellular matrix scaffold for a new-generation bioartificial endocrine pancreas. Ann Surg. 2016. doi:10.1097/SLA.0000000000001364.
42. Skaletskaya GN, Skaletskiy NN, Kirsanova LA, Bubentsova GN, Volkova EA, Sevastyanov VI. Experimental implantation of tissue-engineering pancreatic construct. Russian Journal of Transplantology Artificial Organs. 2019. doi:10.15825/1995-1191-2019-2-104-111. (In Russ).

Figures

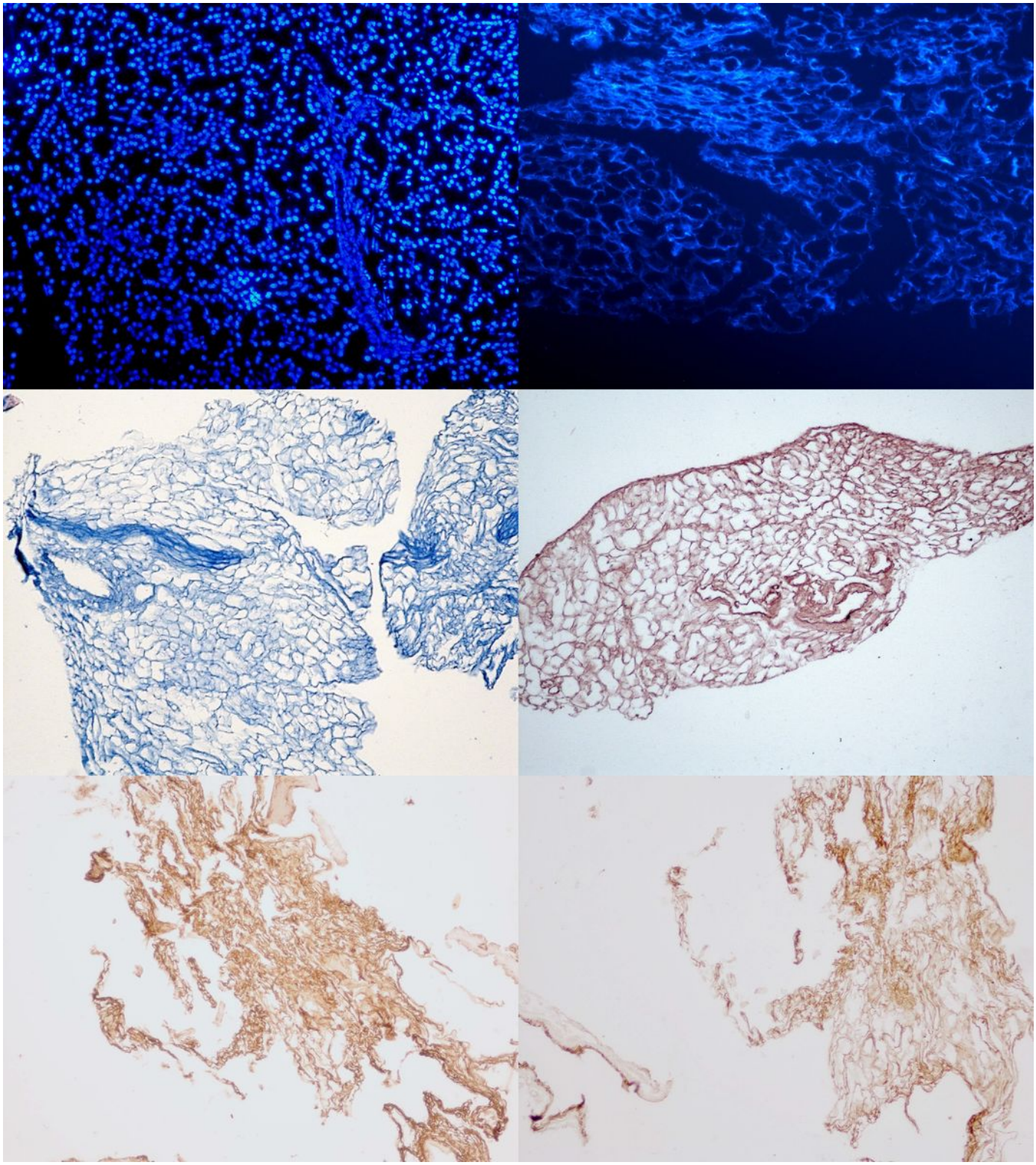


Figure 1

Histological structure of native and decellularized rat pancreas tissue. (Top Left panel) A – rat native pancreas, fluorescent staining of cell nucleus with DAPI. x 100; (Top Right panel)B – fluorescent staining of decellularized rat pancreas (DP scaffold) with DAPI. x 200; (Middle Left panel) C – Masson's trichrome staining. x 200; (Middle Right panel)D – Unna-Tentser's staining reveals preservation of elastic fibers, x

200; (Bottom Left panel) E – immunohistochemical staining of the DP scaffold to collagen type I. x 100; (Bottom Right panel)F – immunohistochemical staining of the DP scaffold to collagen type IV. x 100.

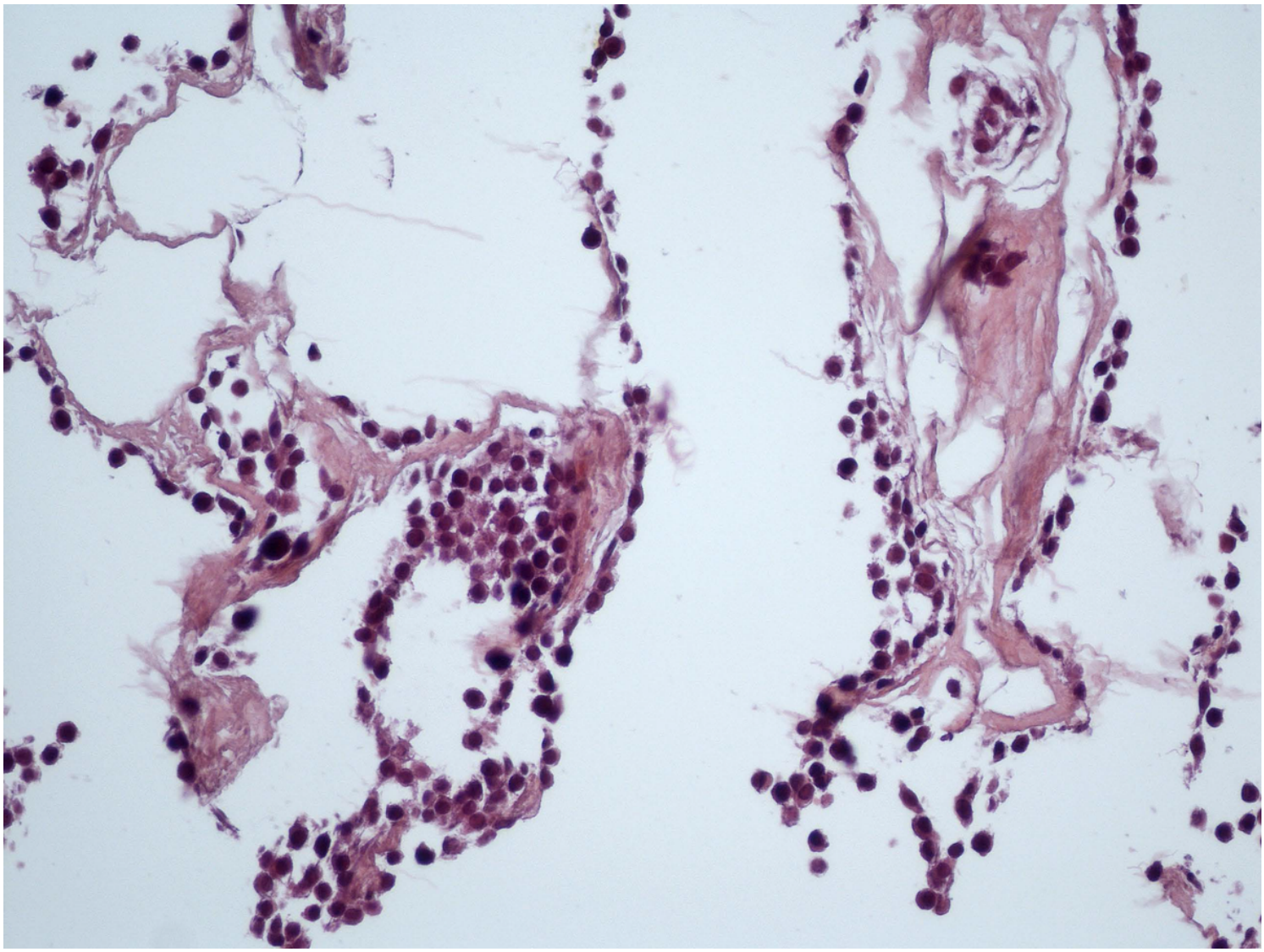


Figure 2

Adhered fibroblasts L929 on decellularized rat pancreatic tissue (DP scaffold) after 10 days of culturing. H&E staining, x 200.

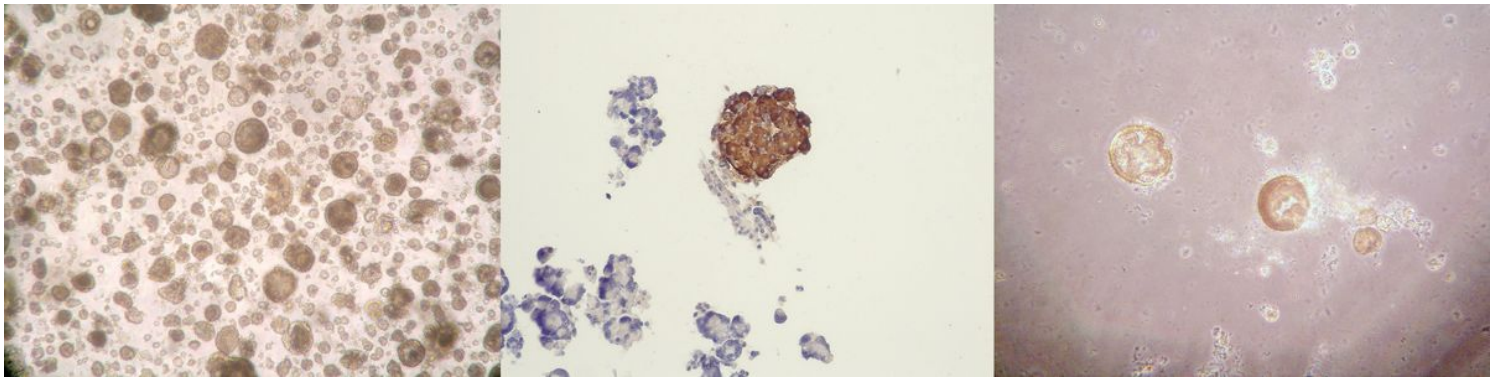


Figure 3

Monoculture of isolated rat pancreatic islets. (Left panel) A – isolated islets, Days 3 of culturing inverted microscope. x 100; (Middle panel) B – isolated islets Days 3 of culturing, staining with anti-insulin antibodies. x 200. (Right panel)C – destructive changes in islets, Day 7 of culturing of culturing, inverted microscope. x 200;

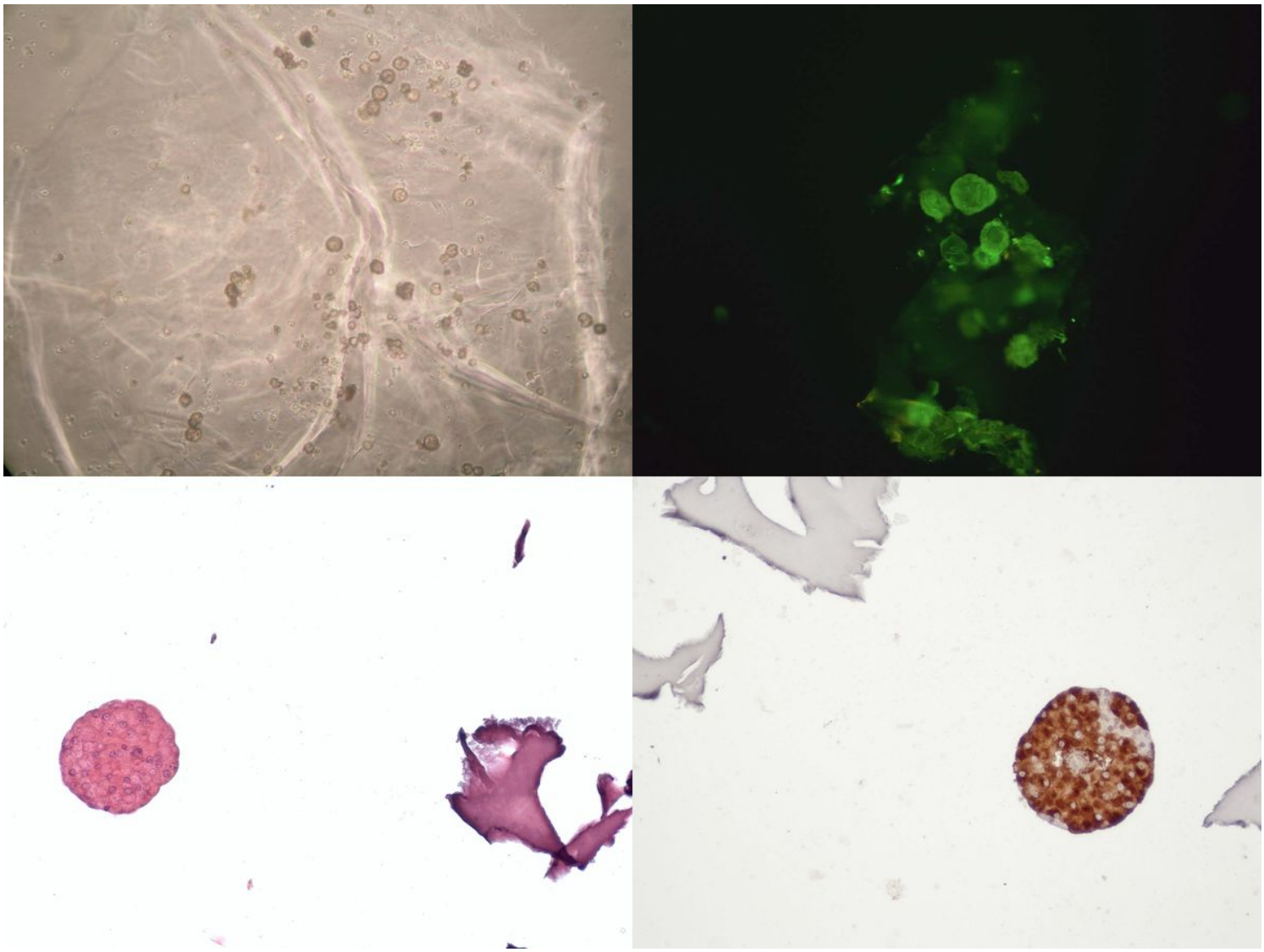


Figure 4

Rat pancreatic islets during culturing with the BMCH scaffold. (Top Left panel) A – inverted microscope, Day 10 of culturing. x 100; (Top Right panel) B – fluorescent staining with acridine orange and propidium-iodide, Day 10 of culturing. x 100; (Bottom Left panel) C – Day 7 of culturing, H&E staining, x 200; (Bottom Right panel) D – Day 7 of culturing, staining with anti-insulin antibodies. x 200.

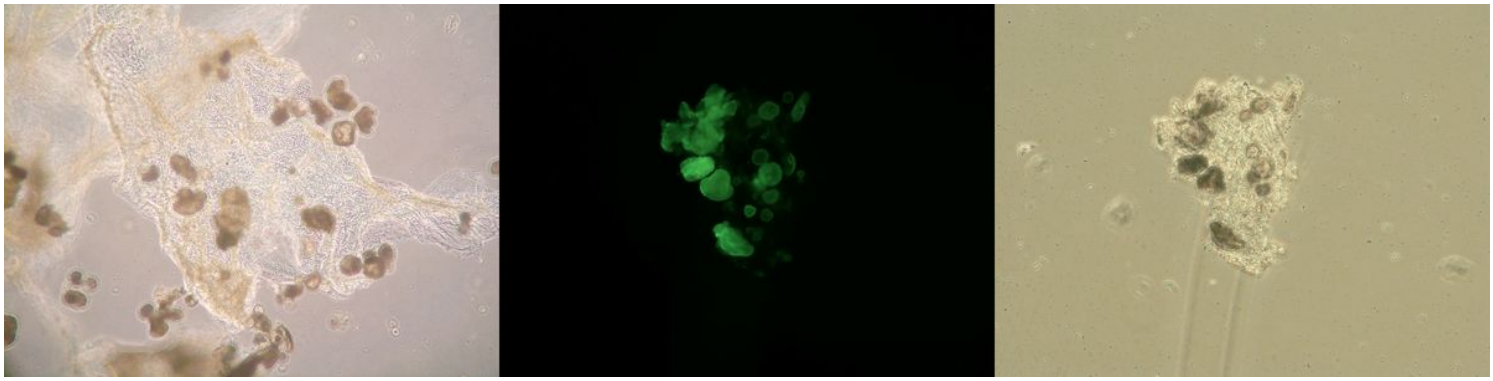
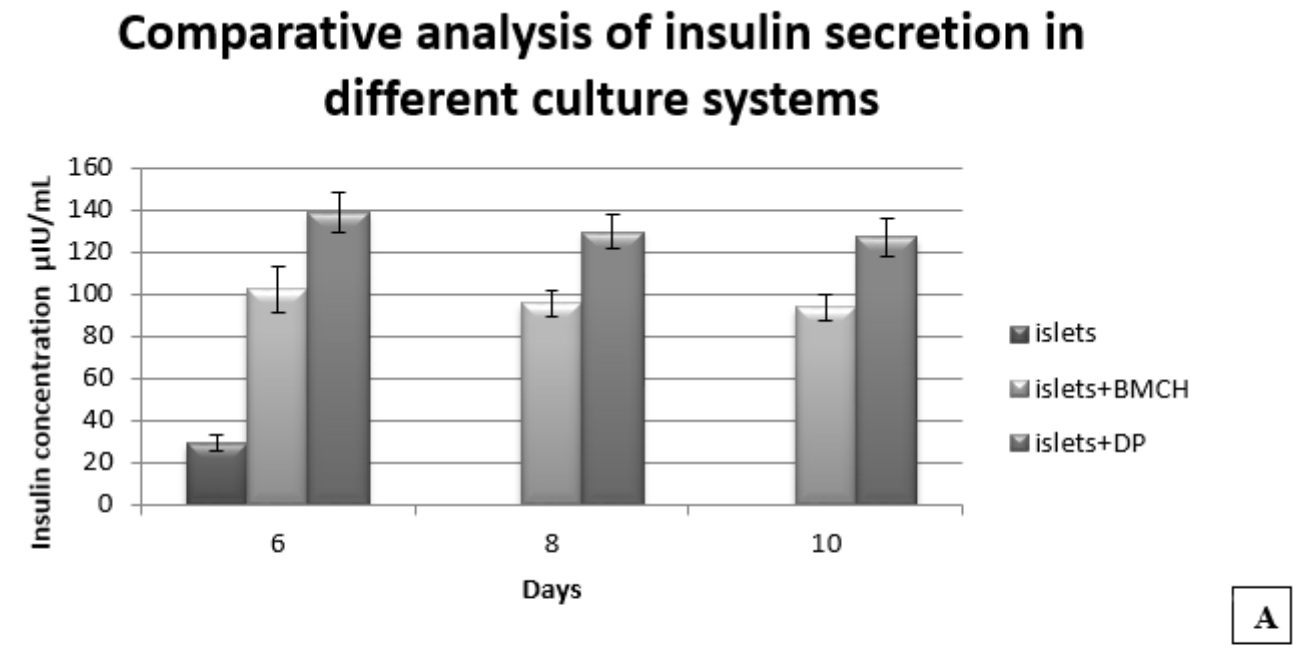


Figure 5

Rat pancreatic islets during culturing with the DP scaffold, Day 14. (Left panel) A – inverted microscope. x 100; (Middle panel) B – fluorescent staining with acridine orange and propidium-iodide. x 100; (Right panel)C – phase contrast microscopy. x100.



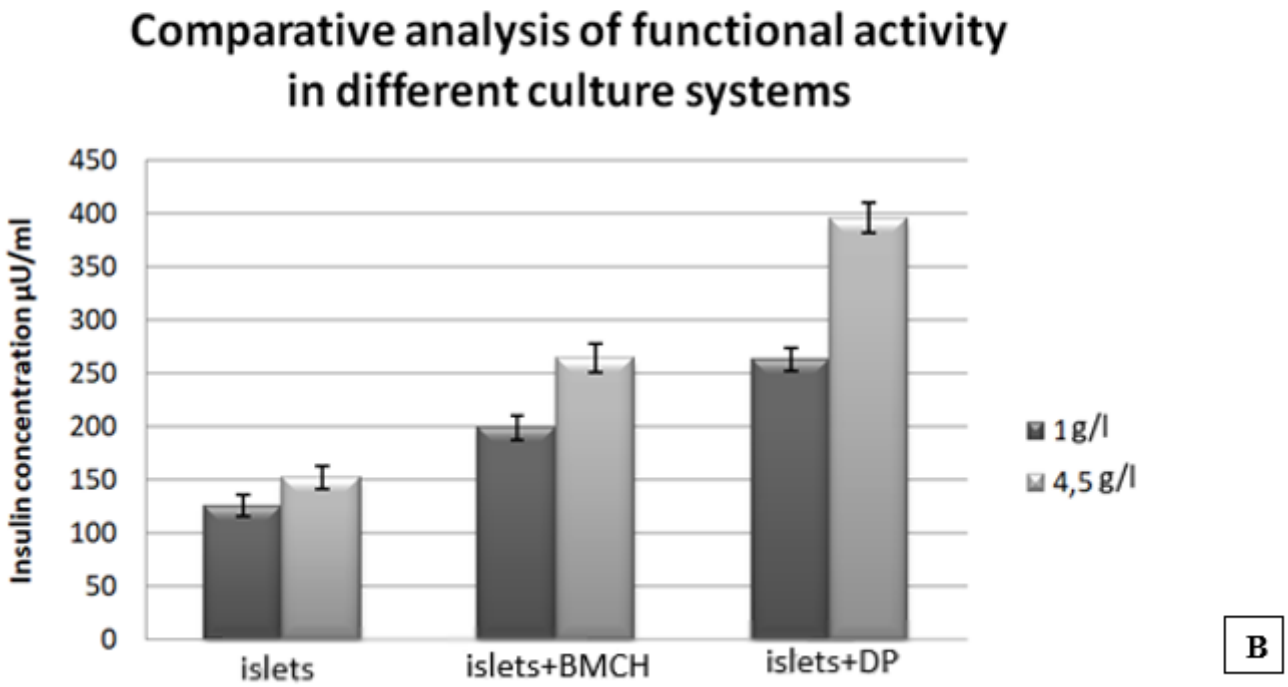


Figure 6

Secretory ability and functional activity of rat pancreatic islets in different culture systems. A – quantification insulin in the control (monoculture islets) and experimental (islets cultured with the BMCH scaffold and the DP scaffold) groups, Day 3 of culturing; B – quantification insulin before and after glucose stimulation in the control and experimental groups, Day 3 of culturing.