DOI: 10.15825/1995-1191-2019-4-45-53

COMPARATIVE ANALYSIS OF THE SECRETORY CAPACITY OF ISLETS OF LANGERHANS CULTURED WITH BIOPOLYMER-BASED COLLAGEN-CONTAINING HYDROGEL AND TISSUE-SPECIFIC MATRIX

N.V. Baranova, L.A. Kirsanova, A.S. Ponomareva, E.A. Nemets, Y.B. Basok, G.N. Bubentsova, V.A. Surguchenko, V.I. Sevastianov

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Introduction. Creation of a biomedical cell product – a bioengineered pancreatic construct – is hampered by problems associated with maintaining the viability of functionally active isolated islets of Langerhans (ILs). Both biopolymer and tissue-specific scaffolds can contribute to maintaining the structure and function of isolated ILs *in vitro* and *in vivo*. The most preferred tissue-specific scaffolds for cells can be obtained via decellularized pancreas matrix scaffold (DP matrix scaffold). **Objective:** to conduct a comparative analysis of the secretory function of isolated ILs of rats cultured in biopolymer-based collagen-containing hydrogel (BCH) and tissue-specific DP matrix scaffold, respectively. **Materials and methods.** ILs from rat pancreas was isolated using classical collagenase technique with some modifications. ILs were cultured in BCH and tissue-specific scaffold under standard conditions. Tissue-specific DP matrix scaffold was obtained through decellularization of rat pancreas. The DP matrix scaffold was examined for cytotoxicity and DNA presence; it was subjected to morphological study. The secretory function of ILs was studied through enzyme-linked immunosorbent assay (ELISA). **Results.** The secretory function of islets cultured in BCH and DP scaffolds is significantly higher than in the monoculture of islets. The advantage of using tissue-specific DP matrix scaffolds when creating bioengineered constructs of the pancreas over BCH matrix scaffolds was identified. **Conclusion.** BCH and tissue-specific DP scaffolds contribute not only to preserving the viability of isolated ILs, but also to prolonging their secretory capacity for 10 days, compared with ILs monoculture.

Keywords: islets of Langerhans, biopolymer-based hydrogel, decellularized pancreas, matrix scaffold, cultivation, insulin secretion.

INTRODUCTION

The creation of a biomedical cell product – a bioengineered construct of the pancreas – is hampered by problems associated with maintaining the viability of isolated Langerhans islets (LI) [1, 2].

In the process of isolation, LIs are known to lose vascularization, innervation, and their connection with the extracellular matrix (ECM) which plays a significant role in the regulation of many aspects of the islets' physiology, including survival, proliferation and insulin secretion [3, 4]. The structure conservation and isolated LIs functioning *in vitro* and *in vivo* can be facilitated by ECM biomimetic matrices with the properties characteristic of the native pancreatic microenvironment [5–7]. Among biomimetics simulating the ECM composition, there is the biopolymer microheterogenous collagen hydrogel (MHCH matrix), a multicomponent product made of natural compounds which includes partially hydrolyzed collagen peptides, glycoproteins, uronic acids, and biologically active ECM substances, including growth factors necessary for the vital functioning of cells [8].

At incubation with collagen-containing matrices, isolated LIs are known to retain integrity, viability, and secretory function for a long time in comparison with LI monocultures [9, 10]. It was shown earlier that the cultivation of isolated rat LI with the MHCH matrix contributes to the preservation of the vitality and characteristic structure of LIs *in vitro* for 14 days [11].

With all their advantages, resorbable matrices of biopolymer materials do not possess tissue specificity. Recently, the development of bioengineered constructs based on tissue-specific matrices of decellularized tissues with the preservation of the structural, biochemical and biomechanical properties of the native ECM with subsequent recellularization by cellular components has been intensively forming [12, 13]. When developing protocols for P decellularization, it is important to take into account the conservation of its architectonics and

Corresponding author: Baranova Natalya Vladimirovna. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Tel.: (499) 190-42-66, (917) 568-98-22. E-mail: barnats@yandex.ru

microvasculature with the most complete removal of cellular material, including DNA, to minimize the immune response during implantation of the bioengineered P structure with minimal damage to ECM components [14, 15]. The presence of such native ECM components in the decellularized P matrix (DCP matrix) as structural proteins (various types of collagen, elastin, fibronectin and laminin), glycoproteins, and cell adhesion factors, allows creating conditions for prolonged vital activity of islet cells and simulate ECM almost completely [16]. The 3D ECM structure determines the topographic location of endocrine P cells, which also affects the survival and secretory activity of LIs [17]. The islets cultured in the presence of the DCP matrix have been shown to increase insulin secretion compared with isolated LIs in monoculture [18].

The **purpose** of the present study was to make the comparative analysis of secretory activity of isolated rat LIs cultured with biopolymer microheterogenous collagen-containing hydrogel and tissue-specific matrix.

MATERIALS AND METHODS

Research animals

The studies were performed on mature male rats of the Wistar breed (180–220 g) from the laboratory bank of the FGUP OPKH Manikhino. The laboratory animals were acclimatized and kept in accordance with the interstate standard GOST ISO 10993-2-2009, "Medical devices. Assessment of the biological effects of medical devices" Part 2. "Animal Handling Requirements".

All manipulations with animals were carried out in accordance with the "Rules for Working with Research Animals" of 1973 and the Rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123, Strasbourg, 1986).

Hydrogel mimetic ECM

As an ECM hydrogel mimetic, the injection form of the MHCH matrix was selected from the *Sphero*®GEL composition line (BIOMIR Service JSC, Russia), intended for use in cell technologies with the following characteristics: average microparticle size 145.79 ± 0.09 microns; modulus of elasticity 1170 ± 12 Pa; viscosity modulus 62.9 ± 7.9 Pa; swelling – not lower than 86.6 ± 3.0 mass. %; resorption time up to 9 months. Previous studies have shown that this MHCH matrix is optimal for creating cell and tissue engineered constructs [19].

Tissue-specific ECM

As a tissue-specific ECM, a matrix derived from decellularized rat P tissue (DCP matrix) was selected.

For decellularization, a subtotally removed rat P crushed manually using eye scissors to a fragment size of not over 1×1×2 mm was used. P fragments were processed at room temperature under continuous stirring (MultiBio RS-24 rotational system, 5 rpm) and sequentially in 0.1% solution of sodium dodecyl sulfate (SDS) in distilled water for 3 hours, in a 0.1% SDS solution on 1N NaCl for 3 hours and in a 0.1% SDS solution in phosphate-buffered saline (PBS, pH = 7.35) for 18 hours. At the final stage of the preparation of the DCP matrix, the decellularized fragments of pancreatic tissue were washed from surfactant residues for 72 hours in three PBS shifts with an antibiotic/antimycotic. Once a day the solution was changed. Samples of the DCP matrix (DCP fragments in PBS) were put in cryovials, frozen and subjected to γ-sterilization (1.5 Mrad). The sterile DCP matrix was stored at 4–6 °C and immediately before the experiment was additionally crushed to an average microfragment size of 500 ± 45 μm to reduce the degree of microheterogeneity.

Morphological study of DCP matrix

For morphological studies, DCP matrix samples were fixed in 10% buffered formalin, dehydrated in ascending concentration spirits, kept in chloroform/ethanol mixture, in chloroform, and embedded in paraffin. 4–5 μm sections from the RM2245 microtome (Leica, Germany) were dewaxed, rehydrated and stained with hematoxylin and eosin for total collagen (Masson), elastic fibers (Unna–Taenzer), and DAPI stained for the qualitative determination of nuclear material in a DCP matrix.

DNA quantification in the DCP matrix

To determine the degree of immunogenicity of the decellularized material by the residual amount of nuclear material in a DCP matrix, DNA was isolated and fluorescent stained [20].

DNA was isolated from DCP matrix samples with the DNeasyBlood & TissueKit (QIAGEN, Germany) according to the manufacturer manual. For DNA quantification according to the protocol, PicogreenQuant-iT fluorescent dye™ was used (Invitrogen, USA), with its action activated by 480 nm wavelength radiation. The obtained thermoionic emission was analyzed on a Spark 10M microplate reader (TecanTrading AG, Switzerland) at 520 nm wavelength. To determine the absolute amount of DNA, a bacteriophage λ DNA calibration curve (Invitrogen, USA) in the range of 0.0–1000 ng/ml was used.

Study of DCP matrix for cytotoxicity

The cytotoxicity of *in vitro* DCP matrix samples was evaluated by direct contact in accordance with the interstate standard GOST ISO 10993-5-2011 "Medical devices. Assessment of the biological effects of medical

devices. Part 5. Study of cytotoxicity: *in vitro* methods" on the culture of mouse fibroblasts, line L929. A sample culture medium with 10% fetal calf serum (HyClone, USA) was taken for negative control. A single element aqueous standard of 10,000 μg/ml (Sigma-Aldrich, USA) was used as a positive control sample. All procedures were performed in aseptic conditions. The culture was visually evaluated with the Eclipse TS100 (Nikon, Japan) inverted microscope.

The metabolic activity of fibroblasts after contacting matrix samples was evaluated after 24 h with prestoBlue™ CellViabilityReagent vital dye (Invitrogen™, USA) according to the manufacturer protocol. The change in optical density was recorded with Spark 10M microplate reader (TecanTrading AG, Switzerland) with SparkControl™ Magellan V1.2.20 software at 570 and 600 nm wavelengths.

The obtained data were statistically processed with Microsoft Excel 2007. All results are presented in the form of "mean value ± standard deviation". Differences were considered significant at $p < 0.05$.

Langerhans islets isolation and identification

The procedure for isolating LIs from P of mature rats (n = 6) was carried out on the basis of classical protocols using collagenase [21, 22] with some modifications [11].

Islets were identified by dithizone staining. The dye selectively stained LIs, while the acinar cells remained unstained [22]. Resuspended isolated LIs were subsequently used in the experiment.

LIs cultivation in the presence of MHCH and DCP matrices

Freshly isolated LIs were resuspended in DMEM/F12 (1/1) medium with 10% ETS, 2 mM L-glutamine, 1 M Hepes and 50 mg/ml gentamicin and approximately equal number of islets (n = 300 ± 25) were introduced into three 25 cm^2 culture flasks. The islets cultured without matrix addition (culture flask 1) served as control. 0.2–0.3 ml of carefully resuspended MHCH matrix (experimental group I) and 0.2–0.3 ml of a suspension of DCP matrix with an average microfragment size of 500 ± 45 μm (experimental group II) were added to culture flasks 2 and 3, respectively.

All culture systems were incubated under standard conditions at 37 °C in a CO_2 incubator in humidified atmosphere with 5% CO_2. The cultivated islets were monitored and photographed with Nikon Eclipse TS100 digital camera inverted microscope (Nikon, Japan). The culture medium was changed on the 1^{st}, 2^{nd}, 3^{rd}, 6^{th}, 8^{th} and 10^{th} days to take samples for subsequent tests on insulin content.

Enzyme-linked immunosorbent assay for the determination of insulin content in the culture medium

Before determining insulin content in the samples from the culture flasks at the indicated incubation times, the growth medium was removed and replaced with fresh nutrient medium. After 1 hour of incubation under previous conditions (37 °C, 5% CO_2), growth medium samples were taken out and frozen (–23 °C) for the subsequent study [23].

To determine the basal concentration of insulin in the culture medium, the solid-phase sandwich method Rat Insulin ELISA Kit (Thermo scientific, USA) was used according to the manufacturer's manual. In this option, ELISA uses a pair of antibodies specific for spatially remote epitopes of the studied antigen, thus allowing high sensitivity and specificity in the antigen (insulin) determination.

The optical density was measured with Spark 10M microplate reader (TecanTrading AG, Switzerland) with Spark Control™ Magellan V1.2.20 software at 450 and 550 nm wavelengths to account for the microplate optical artifacts.

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

RESULTS AND DISCUSSION

Morphological study of DCP matrix

A preliminary morphological study confirmed the classical pattern of the rat P structure without signs of ischemic damage (Fig. 1). Decellularized P samples used in the experiment showed the integrity of the stroma architectonics as a whole and were represented by a laced fiber mesh net – like structure. The surviving cells and individual cell nuclei in the samples were not detected. Specific DAPI staining confirmed the absence of cell nuclei and fragments of nuclear material in the matrix, thereby indicating the effectiveness of the procedure for the decellularization of pancreatic tissue (Fig. 2, a). Samples Masson staining made it possible to visualize collagen fibers in the obtained matrix composition (Fig. 2, b), and orsein staining also revealed the presence of elastic fibers, which indicated the preservation of the main fibrillar proteins of the matrix (Fig. 2, c).

DNA quantification in the DCP matrix

Quantitative analysis of the native and decellularized rat P tissue showed that the matrix of decellularized P compared to the original tissue was significantly ($p < 0.05$) purified from DNA (Table 1).

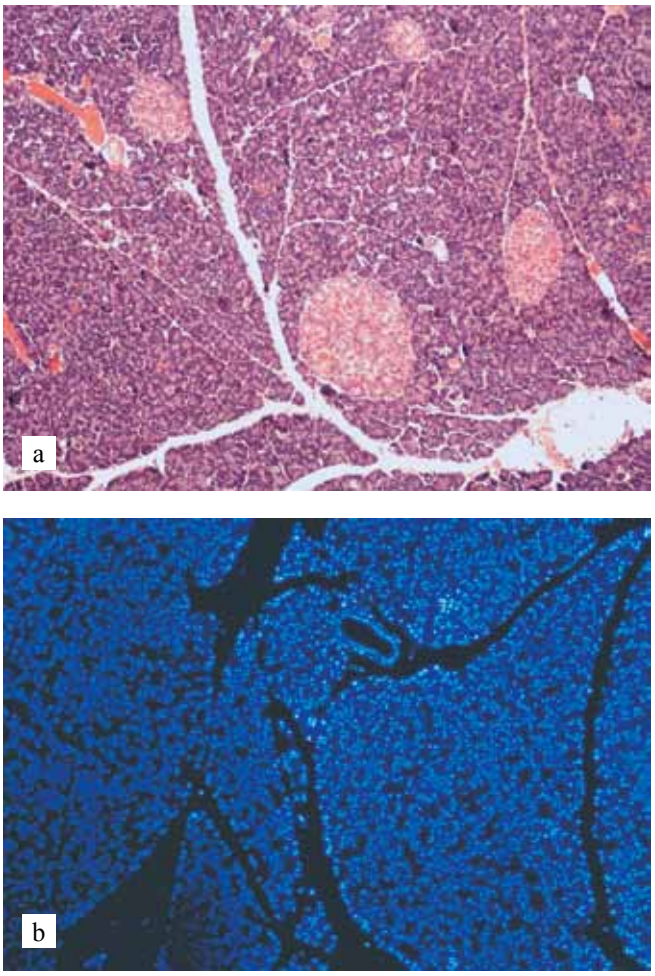

Fig. 1. Histological structure of rat pancreas: a – H&E staining; b – nuclear DAPI staining. ×100

Table 1

Quantitative content of DNA in native and decellularized rat pancreatic tissue

Rat P tissue sample	DNA, ng Mean ± SD
Native rat P	1,354.8 ± 168.7
Decellularized rat P	1.3 ± 0.3

Thus, decellularization resulted in not over 0.1% of DNA retained in the tissue, which indicated a high decellularization efficiency, and, accordingly, low immunogenicity of the obtained matrix (Fig. 3).

Cytotoxicity of DCP matrix

The results were analyzed by the evaluation scale of the degree of cell response after incubation with DCP matrix samples (n = 3).

Table 2 shows the values featuring the proliferative activity of L929 fibroblasts relative to the negative control (response degree 0). After contacting DCP-matrix samples, the proliferative activity of fibroblasts relative to the negative control remained above 90% (response degree 0), which showed the absence of cytotoxic effect of the samples of this matrix. The positive control in this experiment showed sharp cytotoxicity (response degree 4).

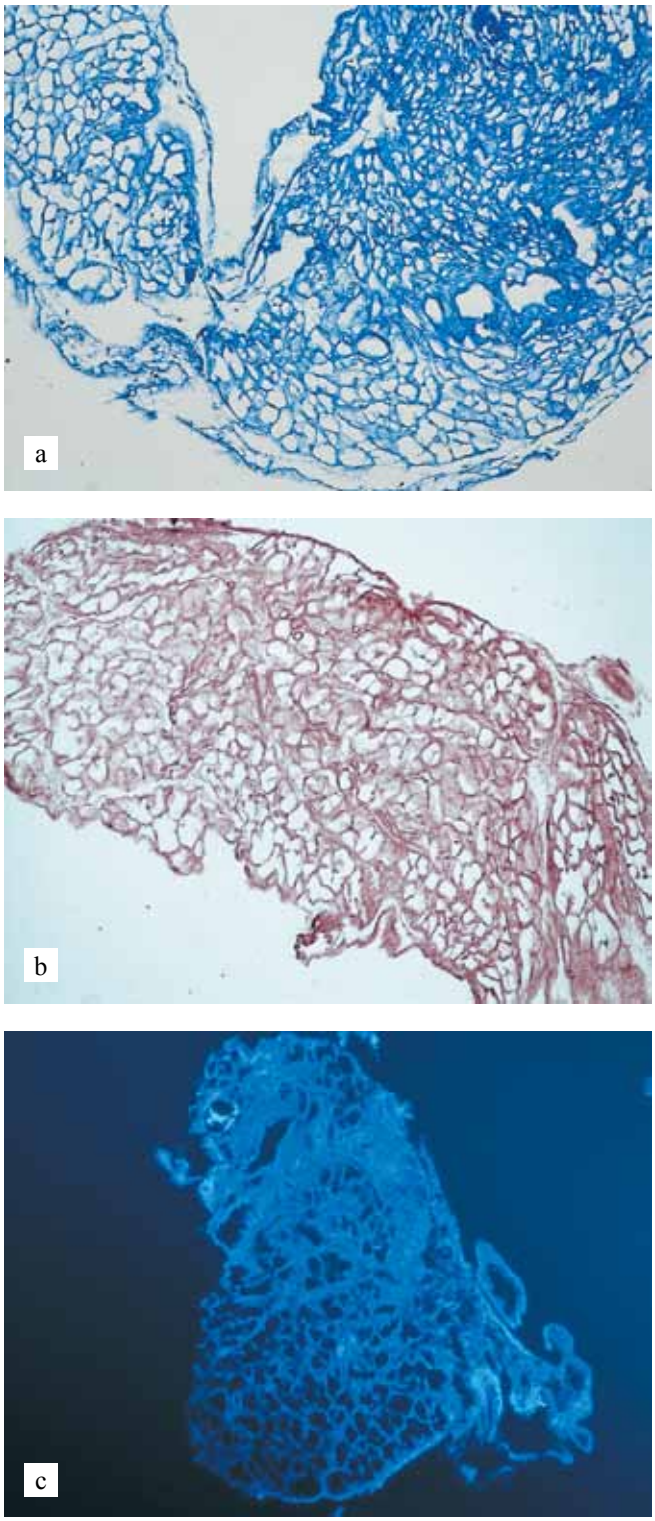

Fig. 2. Histological structure of decellular rat pancreas (DP matrix): a – Masson's trichrome staining demonstrated complete absence of cells and preservation of collagen fibres; b – Unna–Tentser's staining revealed preservation of elastic fibres; c – nucliar DAPI staining confirmed absence of nuclear material in DP matrix. ×200

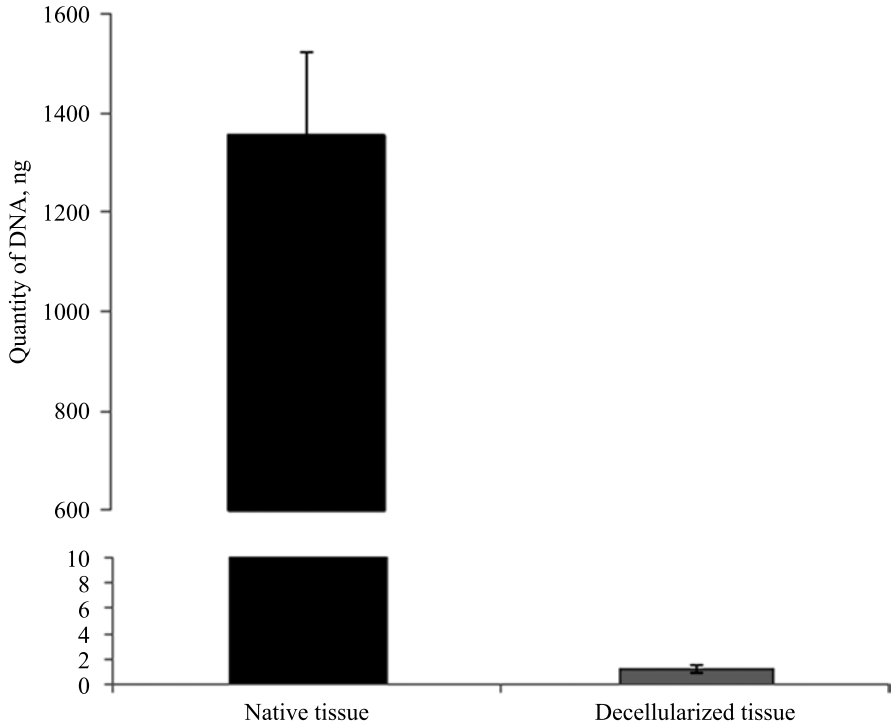

Fig. 3. Quantification DNA in native and decellularized rat pancreatic tissue

Table 2

Results of DP matrix cytotoxicity study

Sample No.	Sample name	Proliferating cells relative to the negative control (%)	Cell response degree
1	DCP matrix	96.25 ± 1.69	0
2	Positive control	7.84 ± 2.34	4

Morphofunctional properties of LIs upon incubation with MHCH and DCP matrices

The resulting LIs were round or oval in shape and maintained integrity, which indicated that their macrostructure was not affected during the isolation (Fig. 4).

After 24h of cultivation, significant morphological changes in the LI monoculture (control group) were not detected. However, after three days, the first signs of destruction were revealed: cavities appeared in some islets, and their surface became tuberous. At the turn of six days of cultivation, most of the control islets underwent fragmentation.

In the experimental group I, after one day of cultivation, a significant part of the islets showed adhesive properties and attached to the surface of the MHCH matrix, while the remaining islets continued to float (Fig. 5). This pattern practically did not change during the entire observation period (10 days). At this, LIs were visually preserved.

In contrast to the experimental group I, the islets cultured with the DCP matrix (experimental group II) did not exhibit adhesive properties and, being in close proximity to the matrix, remained intact up to 5 days of incubation. Subsequently, at least half of the cultured LIs were deposited on the surface of the DCP matrix (Fig. 6). During the entire observation period, as in the experimental group I, no pronounced signs of destruction of the islets were detected.

LI secretory function at incubation with MHCH and DCP matrices

After 24 hours of cultivation, the insulin concentration was higher by 26.2% (258.4 ± 9.7 μIU/mL) and 48.7% (304.9 ± 12.2 μIU/mL) in the experimental groups I and II compared to the control group (205.1 ± 11.5 μIU/mL), on the third day of incubation it was higher by 62.1% (149.0 ± 12.3 μIU/mL) and 102.9% (186, 5 ± 10.9 μIU/mL), respectively, compared to the control group (91.9 ± 7.8 μIU/mL) (Table 3).

The revealed differences in hormone concentration in the control and experimental groups at these time points can be explained by the positive effect of matrices on the functional ability of LIs. On the sixth day of cultivation, an even more significant difference was observed between the insulin concentrations in the experimental groups I and II (102.1 ± 10.6 and 138.3 ± 9.6 μIU/mL, respectively) and the control (29.2 ± 4.1 μIU/mL). This correlates with the morphological data on destructive

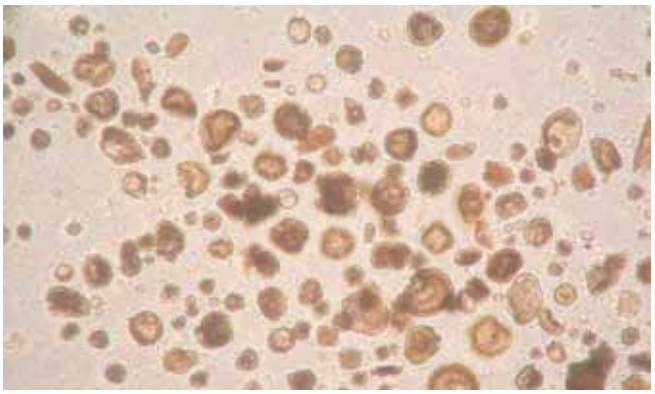

Fig. 4. Isolated Langerhans islets, inverted microscope. ×200

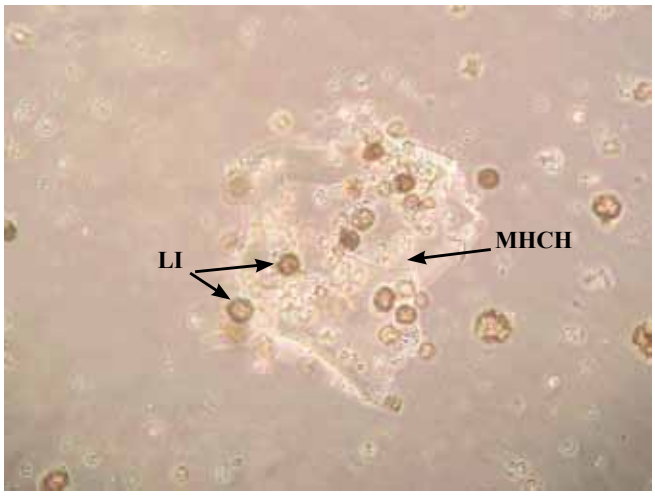

Fig. 5. Langerhans islets cultivated with MHCH, 7 days, inverted microscope. ×100

Table 3

Comparative analysis of insulin in experimental groups relative to the control group (LI monoculture), %

Days	LI + MHCH (experimental group I)	LI + DCP (experimental group II)
1	26.2 ± 3.8	48.7 ± 4.0
2	31.6 ± 6.2	71.6 ± 5.7
3	62.1 ± 8.3	102.9 ± 5.8
6	249.6 ± 10.4	373.6 ± 6.9

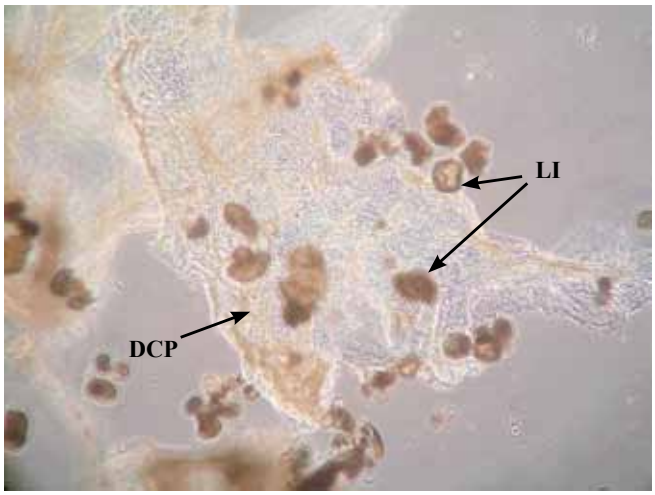

Fig. 6. Langerhans islets cultivated with decellular rat pancreas (DP matrix), 7 days, inverted microscope. ×100

changes occurring in the islets after three days of suspension cultivation. On the 8th to 10th day of incubation, no surviving islets were found in the control group; for this reason, it seemed irrelevant to study the culture medium. At the same time, at the same time points in the experimental groups, the insulin concentration remained practically unchanged: group I – 93.7 ± 6.2 μIU/mL, group II – 126.9 ± 8.9 μIU/mL, while the level of insulin secretion in group II (LI in the presence of a tissue-specific DCP matrix) was 35.5% higher than in group I (LI in the presence of MHCH matrix) (Fig. 7). Despite the fact that the insulin concentration, expressed in absolute terms, decreased with an increase in the cultivation period, the positive tendency of the effect of the matrix (MHCH and DCP) on the secretory function of the islets in percentage persisted throughout the entire observation period.

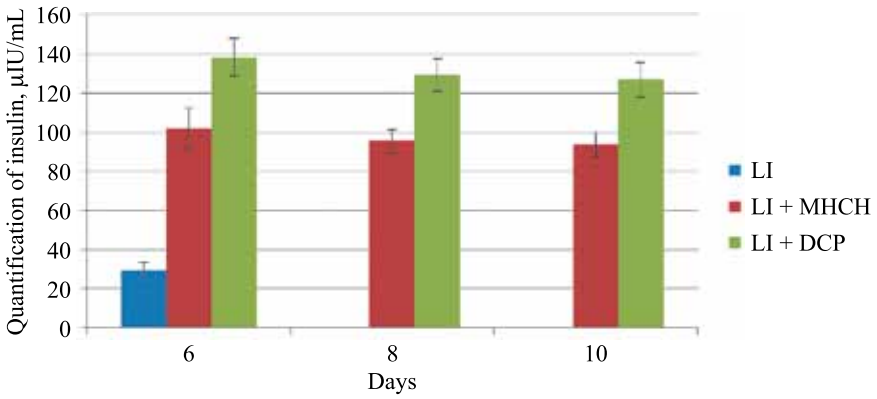

Fig. 7. Quantification of insulin in the control (monoculture LI) and experimental (LI, cultured with BMCH and DP matrix, respectively) groups

CONCLUSION

MHCH and tissue-specific DCP matrices contribute not only to the preservation of the viability of isolated LIs, but also to the prolongation of their secretory ability for 10 days compared to LI monoculture. In the experimental condition, the advantage of implying the tissue-specific DCP matrix to create a bioengineered P structure compared with the MHCH matrix is shown.

The authors declare no conflict of interest.

REFERENCES

1. *Kumar N, Joisher H, Ganguly A.* Polymeric scaffolds for pancreatic tissue engineering: a review. *Rev Diabet Stud*. 2018 Winter; 14 (4): 334–353. doi: 10.1900/RDS.2017.14.334.
2. *Lemos NE, de Almeida Brondani L, Dieter C, Rheinheimer J, Boucas AP, BauermannLeitao C et al.* Use of additives, scaffolds and extracellular matrix components for improvement of human pancreatic islet outcomes *in vitro*: a systematic review. *Islets*. 2017 Sep 3; 9 (5): 73–86. doi: 10.1080/19382014.2017.1335842.
3. *Llacua LA, Faas MM, de Vos P.* Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. *Diabetologia*. 2018 Jun; 61 (6): 1261–1272. doi: 10.1007/s00125-017-4524-8.
4. *Stendahl JC, Kaufman DB, Stupp SI.* Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. *Cell Transplantation*. 2009; 18 (1): 1–12. doi: 10.3727/096368909788237195.
5. *Fisher SA, Tam RY, Shoichet MS.* Tissue mimetics: engineered hydrogel matrices provide biomimetic environmentsfor cell growth. *Tissue Engineering*. 2014; Part A, 20 (5, 6): 895–898. doi: 10.1089/ten.tea.2013.0765.
6. *Coronel M, Stabler C.* Engineering a local microenvironment for pancreatic islet replacement. *Curr Opin Biotechnol*. 2013; 24: 900–908. doi: 10.1016/j.copbio.2013.05.004.
7. *Jiang K, Chaimov D, Patel SN, Liang JP, Wiggins SC, Samojlik MM et al.* 3-D physiomimetic extracellular matrix hydrogels provide a supportive microenvironment for rodent and human islet culture. *Biomaterial*. 2019 Apr; 198: 37–48. doi: 10.1016/j.biomaterials.2018.08.057.
8. *Perova NV, Sevastianov VI.* Sfero®GEL – inyektsionniy biodegradiruyemiy implantat. *Prakticheskaya meditsina*. 2014; 8 (84): 111–116.
9. *Abualhassan N, Sapozhnikov L, Pawlick RL, Kahana M, Pepper AR, Bruni A et al.* Lung-derived microscaffolds facilitate diabetes reversal after mouse and human intraperitoneal islet transplantation. *PLoS One*. 2016 May 26; 11 (5): e0156053. doi: 10.1371/journal.pone.0156053.
10. *Szebeni GJ, Tancos Z, Feher LZ, Alfoldi R, Kobolak J, Dinnyes A, Puskas LG.* Real architecture for 3D Tissue (RAFT) culture system improves viability and maintains insulin and glucagon production of mouse pancreatic islet cells. *Cytotechnology*. 2017; 69 (2): 359–369. doi: 10.1007/s10616-017-0067-6.
11. *Baranova NV, Kirsanova LA, Bubentsova GN, SevastianovVI.* Microstructuredcollagen-containing hydrogel as matrix for isolated islets of rat pancreas. *Genes and Cells. Materials of the 3rd National congress on regenerative medicine*. 2017, XII (3): 38–39.
12. *Rana D, Zreigat H, Benkirane-Jessel N, Ramakrishna S, Ramalingam M.* Development of decellularized scaffolds for stem cell-driven tissue engineering. *J Tissue Eng Regen Med*. 2017 Apr; 11 (4): 942–965. doi: 10.1002/term.2061.
13. *Napierala H, Hillebrandt K-H, Haep N, Tang P, Tintemann M, Gassner J et al.* Engineering an endocrine neopancreas by repopulation of a decellularized rat pancreas with islets of Langerhans. *Sci Rep*. 2017 Feb 2; 7: 41777. doi: 10.1038/srep41777.
14. *Wu D, Wan J, Huang Y, Guo Y, Xu T, Zhu M et al.* 3 D culture of MIN-6 cells on decellularized pancreatic scaffold: *in vitro* and *in vivo* study. *Biomed Res Int*. 2015; 2015: 432645. doi: 10.1155/2015/432645.
15. *Keane TJ, Londono R, Turner NJ, Badylak SF.* Consequences of ineffective decellularization of biologic scaffolds on the host response. *Biomaterials*. 2012 Feb; 33 (6): 1771–1781. doi: 10.1016/j.biomaterials.2011/10/054.
16. *Smink AM, de Vos P.* Therapeutic strategies for modulating the extracellular matrix to improve pancreatic islet function and survival after transplantation. *Curr Diab Rep*. 2018 May 19; 18 (7): 39. doi: 10.1007/s11892-018-1014-4.
17. *Salvatori M, Katari R, Patel T, Peloso A, Mugweru J, Owusu K, Orlando G.* Extracellular matrix scaffold technology for bioartificial pancreas engineering: state of the art and future challenges. *J Diab Sci Technol*. 2014; 8 (1): 159–169. doi: 10.1177/1932296813519558.
18. *Mirmalek-Sani S-H, Orlando G, McQuilling J, Pareta R, Mack D, Salvatori M et al.* Porcine pancreas extracellular matrix as a platform endocrine pancreas bioengineering. *Biomaterials*. 2013 July; 34 (22): 5488–5495. doi: 10.1016/j.biomaterials.2013.03.054.
19. *Sevastianov VI, Shagidulin MYu, Skaletskiy NN, Dovzhik IA, Gautier SV.* Preclinical studies of safety and efficacy of biomedical cell products for regeneration of articular cartilage, liver and pancreas. Guidelines for preclinical studies of biomedical cell products. Edited by ac. V.A. Tkachuk. M., 2017: 187–255.
20. *Crapo PM, Gilbert TW, Badylak SF.* An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2011 Apr; 32 (12): 3233–3243. doi: 10.1016/j.biomaterials.2011.01.057.
21. *London NJ, Swift SM, Clayton HA.* Isolation, culture and functional evaluation of islets of Langerhans. *Diabetes Metab*. 1998 Jun; 24 (3): 200–207. PMID: 9690051.
22. *Pang X, Xue W, Feng X, Tian X, Teng Y, Ding X et al.* Experimental studies onislets isolation, purification and function in rats. *Int J Clin Exp Med*. 2015 Nov 15; 8 (11): 20932–20938. PMID: 26885021.
23. *Sigmundsson K, Ojala JRM, Ohmam MK, Osterholm AM, Moreno-Moral A, Domogatskaya A et al.* Culturing functional pancreatic islets on 65-laminins and curative transplantation to diabetic mice. *Matrix Biol*. 2018 Sep; 70: 5–19. doi: 10.1016/j.matbio.2018.03.018.

The article was submitted to the journal on 21.10.2019