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# INTRAPERITONEAL INJECTION OF CELL-ENGINEERED PANCREAS IN RATS WITH EXPERIMENTAL TYPE I DIABETES (PRELIMINARY RESULTS)

*A.S. Ponomareva, N.V. Baranova, A.O. Nikolskaya, L.A. Kirsanova, N.A. Onishchenko, Z.Z. Gonikova, G.N. Bubentsova, E.A. Volkova, Yu.B. Basok, V.I. Sevastianov*

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

Creation of a bioartificial pancreas, including a cell-engineered construct (CEC) formed from pancreatic islets (islets of Langerhans) and a biocompatible matrix mimicking the native microenvironment of pancreatic tissue, is one of the approaches to the treatment of type 1 diabetes mellitus (T1D). **Objective:** to conduct preliminary *in vivo* studies of the functional efficacy of intraperitoneal injection of a cell-engineered pancreatic endocrine construct and a suspension of rat pancreatic islets in an experimental T1D model. **Materials and methods.** Tissue-specific scaffold was obtained by decellularization of human pancreatic fragments. The viability and functional activity of rat islets isolated with collagenase were determined. Experimental T1D was modeled by intraperitoneal injection of low-dose streptozotocin and incomplete Freund's adjuvant into rats. The rats were intraperitoneally injected twice with pancreatic CEC (n = 2) or islet suspension (n = 1). Glucose levels in the blood and urine of the rats were assessed. Histological examination of organs (pancreas and kidneys) of the experimental animals was carried out. **Results.** After the first injection, blood glucose levels gradually decreased in all animals by more than 47% of the initial values; by follow-up day 24, the glucose level rose to the initial hyperglycemic values. After repeated administration, a 63.4% decrease in glycemic level was observed in the rats with pancreatic CEC and a 47.5% decrease in the one with islet suspension. At week 5 of the experiment, blood glucose levels gradually increased in all animals. At the same time, the glycemic index of the rat with injected pancreatic CEC was 62% lower than the glycemic index of the rat with injected islets. **Conclusion.** Allogeneic pancreatic islets in pancreatic CEC increase the duration of stable glycemic level in T1D rats.

*Keywords: pancreas, islets of Langerhans, cell-engineered construct, tissue-specific scaffold, diabetes model.*

## 1. INTRODUCTION

T1D is an autoimmune disease characterized by critical loss of insulin-producing beta cells. Treatment of T1D with cell therapy seems promising. The current method of treatment of severe T1D islets is pancreatic islet transplantation according to the Edmonton Protocol [1–4], which requires a significant mass of islets from several donors [5]. After transplantation, the risk of diabetes complications is reduced, the number of hyperglycemic episodes is reduced, and in some cases complete insulin-independence is achieved [6, 7].

However, the problem of organ shortage and the limited functioning time of islets *in vivo* stimulates the search for tissue engineering and regenerative medicine technologies aimed at long-term preservation of their viability and functional activity. In the process of isolation, cultivation and transplantation, islets lose vascularization, innervation, and connection to extracellular matrix (ECM), which makes them even more susceptible to oxidative stress [8]. In addition, post-transplant islet damage is associated with immediate blood-mediated inflammatory responses, immune response, hypoxia and toxic effects of immunosuppressants [9–11].

At present, improvement of biotechnological methods gives hope for promising application of technologies based on creation of a tissue equivalent of the pancreatic endocrine, formed on the basis of insulin-producing cell components preserving long-term viability and functional activity and biocompatible matrix that provides them with the best conditions. The advantage of using islets in the creation of a tissue equivalent of pancreas over insulin-producing cells of other origin is that beta cells retain paracrine connections with all types of islet cells [12].

ECM components are important components of the pancreatic tissue equivalent, preventing cellular stress and contributing to preservation of islet viability and function. Biomatrices with ECM components are universal platforms for creating a tissue equivalent, as they provide structural and mechanical support to islets, serve

**Corresponding author:** Anna Ponomareva. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (499) 196-26-61; (926) 585-23-73. E-mail: a.s.ponomareva@gmail.com

as a reservoir of growth factors, cytokines, antioxidants, and transmit signals to islet cells via integrins [13–15].

Previously, studies of biomatrices for pancreatic tissue equivalents have focused on artificial construction of scaffolds similar to the native pancreatic ECM, but none of them could accurately mimic the complexity of the actual ECM composition and structure [16–18].

Buitinga et al [19] reported that allogeneic islets transplanted on a porous scaffold platform to mice with experimental T1D were able to restore stable normoglycemia compared to islets transplanted without a scaffold. It was shown that when cultured on collagen-containing matrices for a long time after isolation, the islets remained viable and exhibited secretory activity [20, 21]. Transplantation of islets cultured on such matrices became more successful [22].

Recently, pancreatic decellularization technologies have been used as an alternative to obtaining a matrix with preserved features of pancreatic tissue structure and composition. When developing the protocols for obtaining tissue-specific decellularized scaffold, it is important to consider the preservation of native ECM components: structural proteins, glycoproteins and cell adhesion factors for its functional activity while removing DNA to minimize immune response during implantation of the pancreatic tissue equivalent [23–27].

It was found that islets cultured in the presence of tissue-specific scaffold from decellularized pancreas enhanced insulin secretion compared to isolated islets in monoculture [28, 29]. Wu et al [30] showed on an experimental T1D model that the pancreas tissue, recellularized by a population of insulin-producing cells, is able to control blood glucose levels in mice compared to the same cells cultured on Petri dishes.

We have previously described approaches to obtaining tissue-specific matrices from decellularized rat [31] and human [28] pancreas, showing preservation of structure, prolongation of viability and function of islets cultured with tissue-specific scaffold compared to islets cultured without biomatrix.

Thus, the *in vitro* functionality of the pancreatic tissue equivalent *in vitro* is directly related to preservation of the cellular component comprising it, which may ultimately be of crucial importance *in vivo* [32].

The **objective** of this work was to carry out preliminary *in vivo* studies of the functional efficacy of pancreatic CEC and isolated rat pancreatic islets when administered intraperitoneally to diabetic rats.

## 2. MATERIALS AND METHODS

### 2.1. Experimental animals

Experiments were carried out on male Wistar rats obtained from the laboratory animal nursery belonging to KrolInfo Ltd. A veterinary certificate confirming the absence of infectious diseases in the farm was presented. Acclimatization and maintenance of the laboratory animals were done in accordance with the interstate standard GOST ISO 10993-2-2009 "Medical products. Evaluation of biological effect of medical products." Part 2. "Requirements for the treatment of animals."

All manipulations with animals were performed in compliance with the bioethical principles approved by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 2005, and in accordance with the Rules of Laboratory Practice, approved by the Ministry of Health of Russia, No. 708 on August 23, 2010. A report on approval of experimental studies was received from the local ethics committee of Shumakov National Medical Research Center of Transplantology and Artificial Organs, dated January 28, 2021, Protocol No. 280121-1/1e.

### 2.2. Experimental type I diabetes model

T1D was modeled according to a known proprietary method on male Wistar rats for 16 days (Table 1) by intraperitoneal injection of low-dose streptozotocin every 7 days [33]. Streptozotocin was administered after 12 hours of fasting; the dose of the drug was determined at the rate of 25 mg/kg of animal body weight in the first injection, 20 mg/kg in the second injection, and 25 mg/kg in the third injection. To induce autoimmune inflammation, the animals were intraperitoneally injected 1 mL of incomplete Freund's adjuvant three times a day before each streptozotocin injection.

The animals were observed daily, their physical appearance was assessed, and the amount of water consumed by them was measured. Every week, we measured blood and urine glucose levels on an empty stomach, and monitored the dynamics of weight changes. Rats whose high glycemic level remained stable for two weeks were selected for further study.

### 2.3. Technology for obtaining tissue-specific scaffold from decellularized pancreatic tissue

To form a pancreatic tissue equivalent, a tissue-specific finely dispersed matrix was chosen as an ECM biomi-

Table 1

**Scheme for TID modeling**

| Day | 1 | 2 | … | 8 | 9 | … | 15 | 16 |
|---|---|---|---|---|---|---|---|---|
| Injection of streptozotocin | | 25 mg/kg | | | 20 mg/kg | | | 25 mg/kg |
| Injection of incomplete Freund's adjuvant | 1 mL | | | 1 mL | | | 1 mL | |

metic, obtained by decellularization of human pancreas fragments (DHP scaffold) according to a protocol developed earlier [28]. The studies showed that DHP scaffold retains morphofunctional properties of the native ECM of pancreatic tissue, contains basic fibrillar proteins (type I collagen, elastin), has low immunogenicity (≤0.1% DNA), is not cytotoxic with respect to adhesion and proliferation of cell cultures [28].

The experimental studies were approved by the local ethics committee, Shumakov National Medical Research Center of Transplantology and Artificial Organs, dated March 16, 2018, Protocol No. 160318-1/1e. It was for the development of the technology for obtaining and studying tissue-specific matrices.

## 2.4. Isolation, identification and cultivation of rat pancreatic islets

Islets were isolated from the pancreas of male Wistar rats (Pr). They were subjected to inhalation euthanasia using Isoflurane (Kariozo Laboratories, Spain), and then the islets were excised under sterile conditions and immediately placed in a Petri dish with cold (+4 °C) Hanks' Balanced Salt Solution (HBSS) without $Ca^{2+}$ and $Mg^{2+}$ ions (Thermo Fisher Scientific, USA) containing amphotericin B. All further manipulations requiring sterility were performed in a laminar flow hood providing sterile air flow.

We injected 2 mL of collagenase NB 1 solution (activity 20 PZ U/g tissue) with neutral protease NP (activity 1.5 DMC U/g tissue) (Serva, Germany) intraparenchymatously into the pancreatic tissue by successive injections. Pr stretched tissue, without cutting, was carefully separated into 10–12 approximately equal parts with microtweezer, transferred into a vial, and incubated for 7–10 minutes in an orbital shaker incubator (Biosan, Latvia) at 37.0 °C with a rotation speed of 150 rpm. The action of collagenase was stopped by adding cold (+4 °C) HBSS. The formed small fragments were filtered through a 100 μm cell strainer (Corning-Costar, USA); the filtrate was collected into conical tubes, and centrifuged for 1 minute at 800 rpm. The supernatant was washed twice with fresh HBSS for 1–1.5 minutes at 1200–1300 rpm to obtain an islet suspension.

Islets were identified by dithizone staining (Sigma-Aldrich, USA) immediately after isolation. For this purpose, part of the suspension was mixed with dithizone solution (1 mg/mL) in a 2 : 1 volume ratio and incubated for 20–30 minutes at 37 °C.

Freshly isolated islets were resuspended in complete growth medium containing DMEM (glucose 1.0 g/L) (PanEco, Russia), 10% fetal calf serum (HyClone, USA), Hepes (Thermo Fisher Scientific, USA), 2 mM alanyl glutamine (PanEco, Russia), 1% antibiotic/antimycotic (Thermo Fisher Scientific, USA), were added to culture vials and cultured for 24 hours under standard conditions at 37 °C in a $CO_2$ incubator in a humidified atmosphere containing 5% $CO_2$.

## 2.5. Assessment of viability and functional activity of pancreatic islets

The viability of islets cultured for 24 hours was determined by fluorescent acridine orange/propidium iodide (AO/PI) staining (PanEco, Russia).

For staining, a portion of the islet suspension was placed in a Petri dish, mixed with the prepared working dye solution in a volume ratio of 2 : 1, and incubated in the dark for 15–30 minutes. Viable islets were counted using a Nikon Eclipse 50i fluorescent microscope (Nikon, Japan) at 10× magnification.

To determine the functional activity of islets after 24 hours of cultivation, insulin content was measured under the influence of a traditional hormone secretion stimulant. For this purpose, the growth medium was replaced with a fresh medium with a low glucose content of 1.0 g/L (2.8 mmol/L). After a 60-minute incubation under standard conditions, culture medium was sampled. The growth medium was then removed and replaced with fresh medium with a high glucose concentration of 4.5 g/L (25 mmol/L). After 60 minutes of incubation under standard conditions, the culture medium was also sampled (2 samples for each culture period) for enzyme-linked immunosorbent assay (ELISA) using Rat Insulin ELISA Kit (Thermo Fisher Scientific, USA).

## 2.6. Obtaining a cell-engineered pancreatic construct

A purified suspension of cultured islets was used as the cellular insulin-producing component of the pancreatic CEC. The islet suspension was obtained by centrifugation in a growth medium for 2 minutes at 1200 rpm, then purified in HBSS under the same regime.

For each pancreatic CEC sample, 2000 islets were selected, obtained from an average of 1.5 rat donor pancreas, resuspended in 1.0–1.2 mL of HBSS, and mixed with finely dispersed sterile DHP scaffold (10.0 ± 0.1 mg) from human pancreas.

The resulting pancreatic CEC sample was placed in a syringe with a 23 G needle size just before administration to recipient rats.

## 2.7. Intraperitoneal injection of cell-engineered pancreatic construct and islets of Langerhans

Three rats with severe and stable autoimmune T1D were selected for corrective therapy: a pancreatic CEC sample (2000 allogeneic islets of Langerhans with DHP scaffold) was injected intraperitoneally into the lower third of the abdomen of rats 1 and 3; rat 2 received 2000 allogeneic pancreatic islets in the form of a suspension.

All animals were observed for more than 12 weeks. The amount of water consumed by them was monitored daily. Body weight was monitored weekly, blood and urine glucose levels were determined. Capillary glycemic level was measured on an empty stomach weekly using Accu-Check Active glucose meter (Roche, Switzerland). All manipulations were performed in the morning hours (between 9 a.m. and 12 p.m.). The levels of glucose and ketone bodies in the urine were evaluated using Ketogluc-1 indicator strips (Biosensor AH, Russia).

### 2.8. Histological examination

The extracted pancreas and kidneys of all experimental animals were fixed in 10% neutral buffered formalin (NBF), dehydrated in alcohols of ascending concentration, then incubated in a chloroform/ethanol mixture, then transferred to chloroform and embedded in paraffin blocks.

Slices, 5 μm-tick, were obtained using an RM2245 microtome (Leica, Germany) and further stained with hematoxylin and eosin (H&E) and with Masson's trichrome for total collagen content. Immunohistochemical staining of main islet cell types was performed using antibodies to insulin and glucagon (Abcam, UK) and the Rabbit Specific HRP|DAB (ABC) Detection IHC kit imaging system (Abcam, UK).

### 2.9. Statistical analysis

Microsoft Excel software (2016) was used to perform statistical data processing. Student's t test was used to determine the statistical significance of differences in the mean between the samples when assessing the functional activity of the islets of Langerhans of healthy rats (insulin content in culture medium during glucose stimulation). The differences were considered statistically significant at $p < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Experimental type I diabetes model

Four diabetic rats with fasting blood glucose – 15.8; 26.7; 28.1; 18.6 mmol/L – were selected for the experiment. Significant loss of body weight (from 350 g to 260 g), hypodynamia, non-healing wounds on the tail, yellowing of hair, thinning of hair, polyuria, and marked polydipsia were noted in the animals. Each animal consumed an average of 209 ± 6 mL of water per day – a healthy rat drinks 14 ± 3 mL of water/day.

Histological examination of rat pancreas samples with glycemic level of 18.6 mmol/L revealed changes in both exocrine parenchyma and pancreatic islets compared with the morphological picture of a healthy animal (Fig. 1, a–c). In the parenchyma, some lobules underwent dystrophic changes, and up to necrosis in some cases. Infiltration by inflammatory cells was observed in the islets: polymorphonuclear leukocytes and solitary macrophages were detected in the islet thickness and along its perimeter. Vacuolated and necrotized insulocytes were detected in the center of the islet, the shape of the islet also changed (Fig. 1, d). Immunohistochemical staining with antibodies to insulin was negative, which confirmed the death of beta cells (Fig. 1, e), while glucagon-positive cells in the islets were preserved (Fig. 1, f). The results

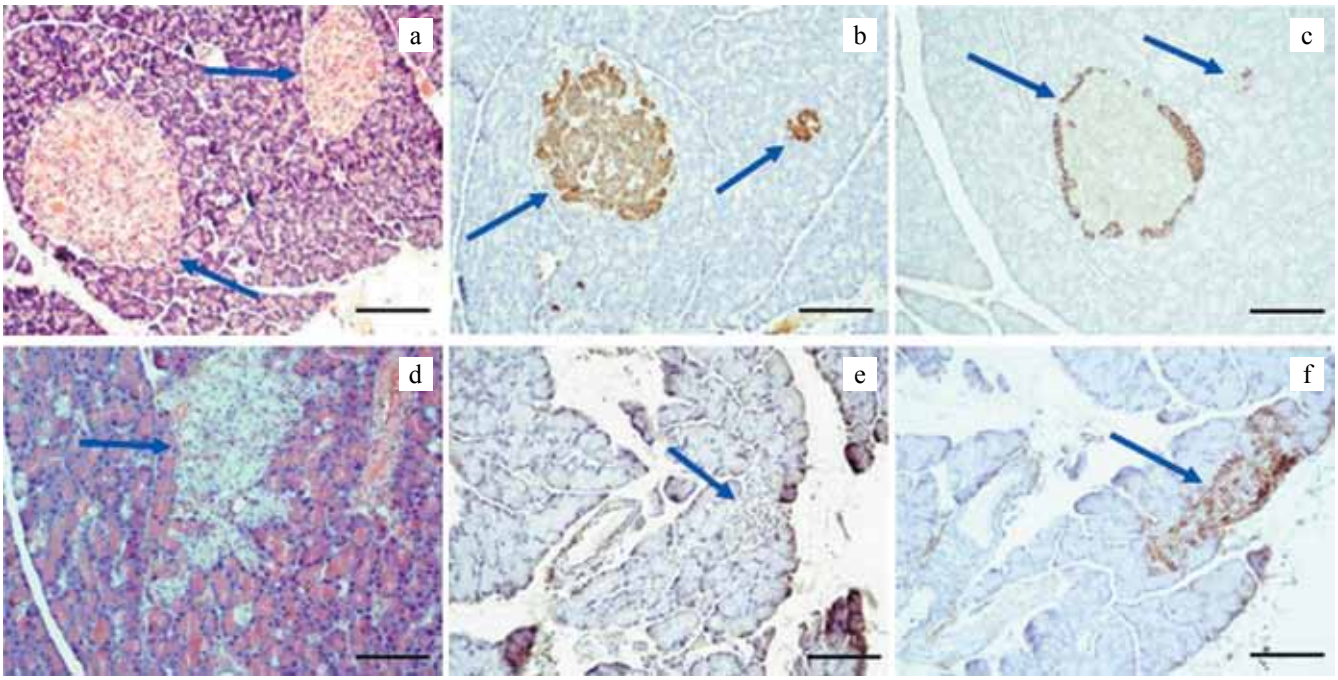


Fig. 1. Rat pancreas. a–c, healthy rat; d–f, rat with experimental T1D; a and d, H&E stain; b and e, insulin immunohistochemical staining; c and f, glucagon immunohistochemical staining. Arrows indicate pancreatic islets. Scale bar = 100 μm

obtained may indicate the effectiveness of the T1D induction model.

### 3.2. Viability and functional activity of isolated pancreatic islets

A large proportion of freshly isolated islets were round or oval in shape and mostly retained their integrity, indicating that the macrostructure of the islets was not damaged during isolation (Fig. 2, a). Dithizone selectively stained the pancreatic islets red-orange, while the acinar cells remained unstained (Fig. 2, b).

A day after isolation, the rat pancreatic islets were stained with vitamin dye to determine their viability. It was found that more than 95% of the islets remained viable (Fig. 2, c).

The functional activity (presence of hormonally active beta cells) of cultured islets was confirmed by the results of culture fluid samples taken at day 1 after incubation before and after stimulation with "hyperglycemic" glucose level of 4.5 g/L (25 mmol/L) (Fig. 3). The pre- and post-stimulation insulin levels in the samples were 185.4 ± 16.4 μIU/mL and 251.7 ± 16.6 μIU/mL, respectively, a 35.8% increase.

### 3.3. Results of intraperitoneal injection of pancreatic cell-engineered construct and islets of Langerhans

Animal follow-up continued for 87 days after the first injection of pancreatic CEC in rats 1 and 3 and islet suspension in rat 2 (Fig. 4, Table 2). Pancreatic CEC and islet suspension were reintroduced on day 24 of the experiment. Rat 1 was removed from the experiment on day 65 for histological analysis of the pancreas condition.

On day 5 after intraperitoneal injection of pancreatic CEC, glycemic level in rats 1 and 3 decreased by 28.5% and 23.5% from the initial values, respectively, while that in rat 2 (administration of islet suspension) decreased by 13.9%. On day 10, the glycemic level in rat 1 reached a minimum value of 9.2 mmol/L (65.5% decrease). In rat 3, the minimum glucose level was 14.7 mmol/L (47.7% decrease) by day 17 of follow-up. In rat 2, in the first 3 weeks after pancreatic CEC injection, glycemic levels reached its lowest level (a 48.1% decrease) on day 17.

On day 24, all the rats witnessed a sharp rise in glucose levels. So, it was decided to re-inject pancreatic CEC and islet suspension in the same animals. One week later, all rats had a sharp decrease in glycemic levels,

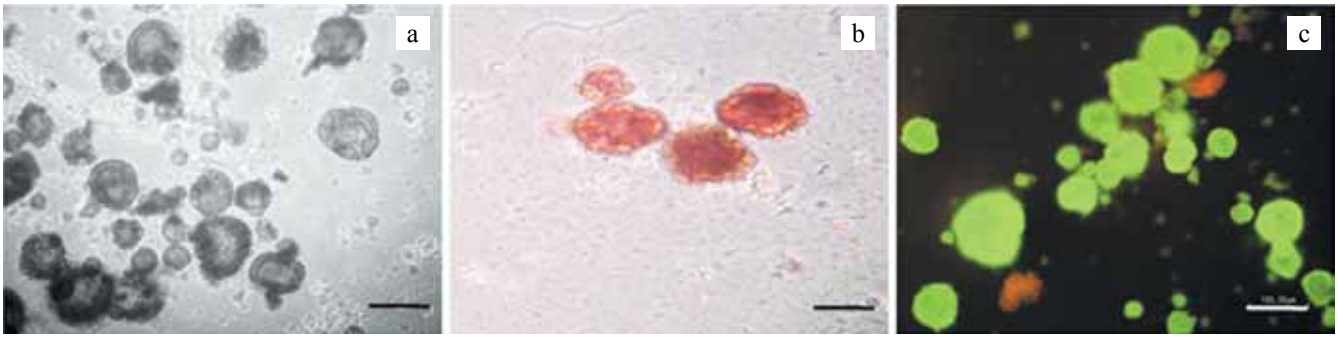


Fig. 2. Isolated rat pancreatic islets. a, light microscopy without staining; b, dithizone staining; c, islets cultured for 24 hours, acridine orange/propidium iodide (AO/PI) staining. Scale bar = 100 μm

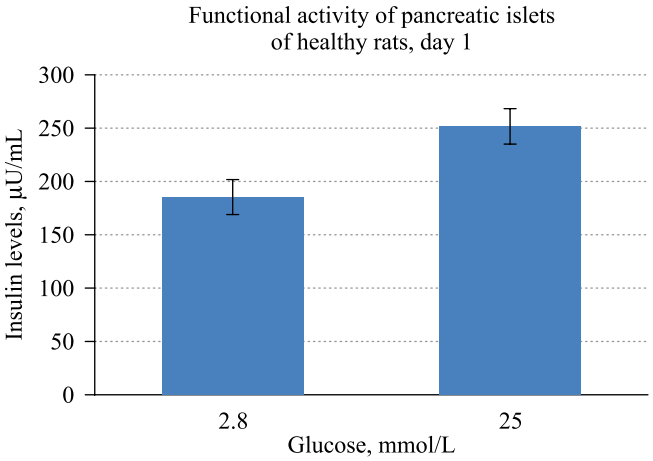


Fig. 3. Comparative analysis of insulin levels before and after glucose stimulation of cultured 1-day isolated rat pancreatic islets

decreasing by 47.5% in rat 2 and by 65.5% and 61.2% in rats 1 and 3, respectively.

Over the next 3 weeks, glycemic levels in rat 1 continued to fall, reaching 4.2 mmol/L by day 52, then gradually increasing. At the time rat 1 was withdrawn from the experiment (day 65), its blood sugar level was 11.7 mmol/L.

At these time periods up to day 87, rat 3 showed slight fluctuations in blood sugar levels with a tendency to increase (from 10.9 to 16.9 mmol/L).

In rat #2, we observed an unstable glycemic level up to day 73, then there was a return to the initial blood glucose concentrations (above 20 mmol/L), and an excess by 34.8% over the initial values. By day 87, the glucose level in rat 3 was 62% lower than that in rat 2.

After the first intraperitoneal injection, there was a decrease in urine glucose levels, which correlated with blood glucose levels in all animals. However, urine glucose concentration by day 24 increased sharply to the maximum (Table 3). On repeated administration, a significant decrease in urine glucose was observed in all rats up to day 65, with rat 1 having no glucose in its urine for 3 weeks, while blood normoglycemia was stable (4.2–6.7 mmol/L). After 65 days, rat 1 had a sharp jump in urine sugar levels with some increase in blood sugar (11.7 mmol/L). This was the reason for removing the animal from the experiment to evaluate the condition of the pancreas and kidneys. In rat 3, urine glucose levels fell to 2.8 mmol/L, and then it gradually increased. Rat 2 also had a short-term decrease in urine glucose levels, followed by an increase to 112 mmol/L by day 87, which in turn correlated with blood sugar levels.

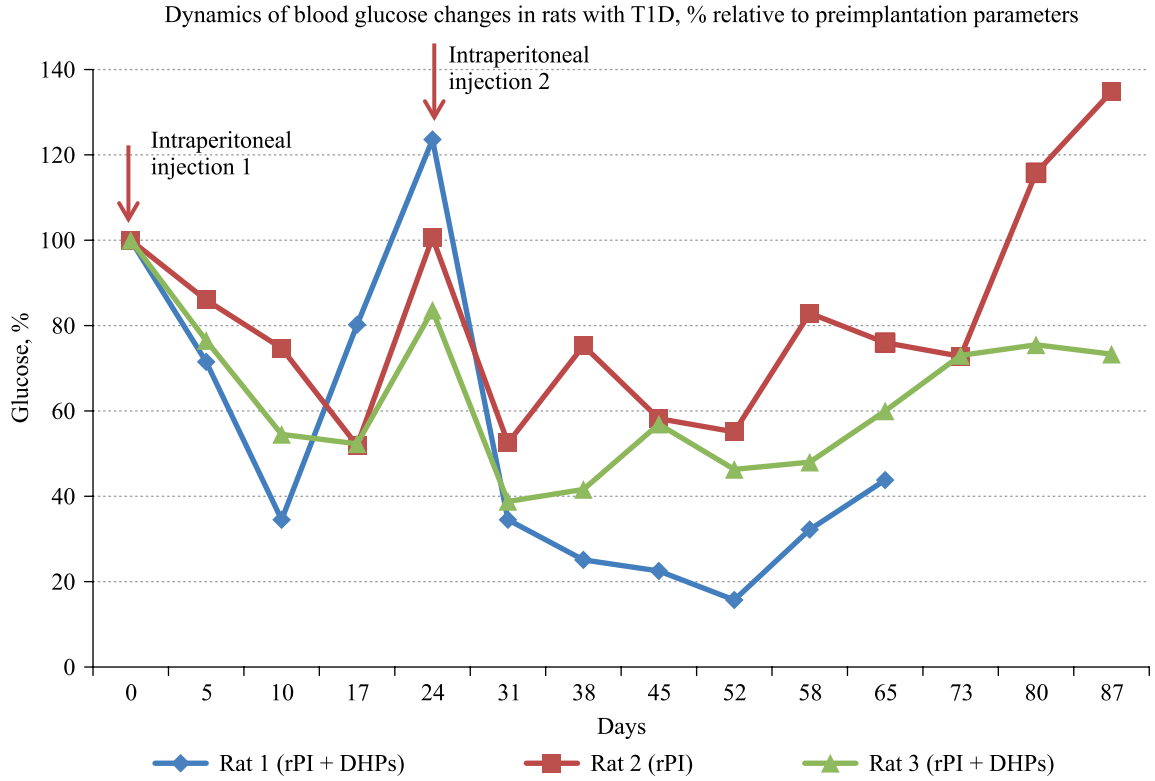


Fig. 4. Changes in blood glucose levels in rats with T1D model after intraperitoneal injection of cell-engineered pancreatic construct or islet suspension. rPI, rat pancreatic islets; DHPs, decellularized human pancreas scaffold

Table 2

**Changes in blood glucose levels in the experimental animals**

| Day | Rat 2 (rPI), blood glucose, mmol/L | Rat 1 (rPI + DHPs), blood glucose, mmol/L | Rat 3 (rPI + DHPs), blood glucose, mmol/L |
|---|---|---|---|
| 0 (injection 1) | 15.8 | 26.7 | 28.1 |
| 5 | 13.6 | 19.1 | 21.5 |
| 10 | 11.8 | 9.2 | 15.3 |
| 17 | 8.2 | 21.4 | 14.7 |
| 24 (injection 2) | 15.9 | 33.0 | 23.5 |
| 31 | 8.3 | 13.3 | 10.9 |
| 38 | 11.9 | 9.2 | 11.7 |
| 45 | 9.2 | 6.7 | 16.0 |
| 52 | 8.7 | 6.0 | 13.0 |
| 58 | 13.1 | 4.2 | 13.5 |
| 65 | 12.0 | 8.6 | 16.9 |
| 73 | 11.5 | 11.7 | 25 |
| 80 | 18.3 | – | 21.2 |
| 87 | 21.3 | – | 20.6 |

Evaluation of the content of ketone bodies in the urine of the animals revealed a decrease in ketone from 1.5 mmol/L to 0.5 mmol/L in all the three experimental rats.

After intraperitoneal injection of pancreatic CEC or islet suspension, all animals reduced their drinking water consumption from 209 ± 16 mL/day to 151 ± 11 mL/day. There was also a 30–40 g increase in body weight, healing of wounds on the tail, and restoration of hair structure and color.

Morphological analysis of pancreas and kidney samples revealed no differences in the organs of the three rats. No immunopositive beta cells were detected in the pancreas, both directly in the islets and in the pancreatic parenchyma as a whole (Fig. 5, a). This indicated the absence of regenerative processes and of the induction

Table 3

**Changes in urine glucose levels in the experimental animals**

| Day | Rat 2 (rPI), urine glucose, mmol/L | Rat 1 (rPI + DHPs), urine glucose, mmol/L | Rat 3 (rPI + DHPs), urine glucose, mmol/L |
|---|---|---|---|
| 0 (injection 1) | 14–28 | 14–28 | 28–56 |
| 5 | 14 | 14 | 28 |
| 10 | 14–28 | 5.6 | 14 |
| 17 | 14–28 | 2.8–5.6 | 2.8 |
| 24 (injection 2) | 56–112 | 112 | 56–112 |
| 31 | 2.8–5.6 | 5.6–14 | 2.8–5.6 |
| 38 | 2.8–5.6 | 5.6–14 | 2.8 |
| 45 | 14 | 0 | 14–28 |
| 52 | 5.6–14 | 0–2.8 | 14–28 |
| 58 | 14–28 | 0–2.8 | 14 |
| 65 | 14–28 | 112 | 5.6–14 |
| 73 | 28 | – | 14–28 |
| 80 | 14–56 | – | 28 |
| 87 | 56–112 | – | 28–56 |

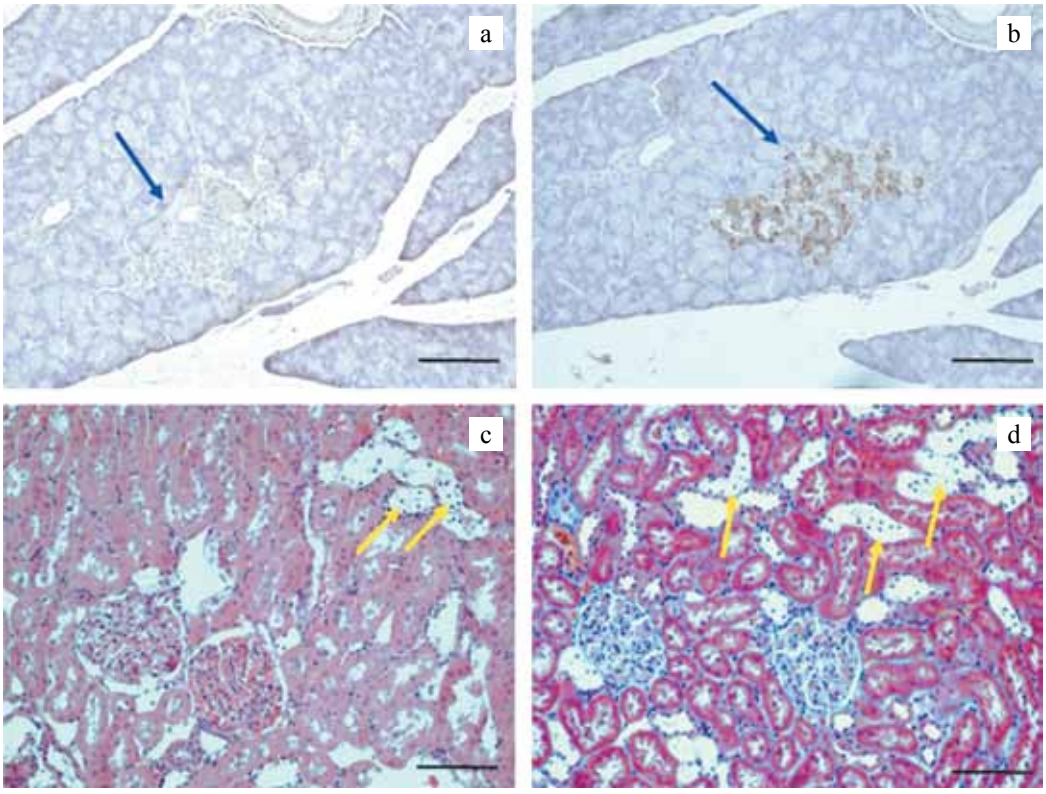


Fig. 5. Pancreas (a, b) and kidney (c, d) of experimental rats. a, no insulin positive beta cells in the islet; b, glucagon positive alpha cells in the islet; c, H&E stain; d, Masson's trichrome stain. Blue arrows indicate islets of Langerhans, yellow arrows indicate Armanni–Ebstein cells in tubular epithelium. Scale bar = 100 μm

effect of the transplanted islets on progenitor cells. At the same time, glucagon-positive alpha cells were clearly detected in the islets (Fig. 5, b). In histological preparations of the kidney, there were no marked degenerative changes in the vascular and tubular apparatus. At the same time, numerous vacuolated cells were detected in the tubule epithelium. We believe that these are the so-called Armanni–Ebstein cells, which are considered to be pathognomonic in diabetes mellitus (Fig. 5, c, d).

In tissue engineering and regenerative medicine, one can distinguish two mechanisms of CEC action *in vivo*, which underlie two approaches to the treatment of pathological organ conditions [34]:

– Stimulation of internal (physiological) regeneration of damaged tissue structures;
– Partial or complete temporary replacement of the functions of the damaged tissue structures.

This also applies to pancreatic CEC, in which tissue-specific scaffolds provide pancreatic islets with a longer survival time and efficient functioning *in vivo.*

Analysis of the results suggests that decreased glycemic level is not due to stimulation of the regeneration (restoration) of beta cells in the native islets, but due to the functional efficiency of allogeneic islets. At the same time, intraperitoneal injection of pancreatic CEC achieved a more pronounced antidiabetic effect in T1D rats compared to intraperitoneal injection of islet suspension.

## CONCLUSION

From preliminary results obtained in the experimental T1D model, intraperitoneal injection of pancreatic CEC (xenogeneic tissue-specific scaffold in combination with allogenic islets of Langerhans) can be assumed to result in a significantly greater decrease in blood glucose concentration in rats compared to the islet suspension. Repeated intraperitoneal injection of pancreatic CEC and islet suspension increases the duration of stable glucose levels, but probably does not induce regenerative regeneration of pancreatic islet tissue.

It should be noted that, firstly, to confirm or refute the results obtained, experiments on a sufficient sample of animals are required in order to get statistically significant results. Secondly, the mechanism of hypoglycemic effect of allogeneic islets may depend on the localization site of pancreatic CEC and islet suspension. Apart from intraperitoneal administration, it is necessary to investigate in the T1D experimental model the functional effect of islets when they are transplanted as a suspension or implanted as part of CEC, for example, into the spleen, mesentery, omentum, or under the kidney capsule.

*The authors declare no conflict of interest.*

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