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FUNCTIONAL EFFICIENCY OF PANCREATIC CELL-ENGINEERED CONSTRUCT IN AN ANIMAL EXPERIMENTAL MODEL FOR TYPE I DIABETES

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The creation of a cell-engineered pancreatic construct (CEPC) from islets of Langerhans and biocompatible matrix carrier (framework/scaffold), which imitates the native microenvironment of pancreatic tissue, is an approach to the treatment of type I diabetes mellitus (T1D). **Objective:** to conduct a comparative analysis of the functional efficacy of CEPC and isolated rat islets of Langerhans after intraperitoneal administration into rats with experimental T1D. **Materials and methods.** T1D was induced in rats by injecting low-dose (15 mg/kg) streptozotocin (STZ) for 5 days. CEPC samples were created using viable and functional allogeneic isolated islets of Langerhans and tissue-specific scaffold obtained by decellularization of human pancreatic fragments. The rats received intraperitoneal injection of allogeneic islets of Langerhans (experimental group 1, n = 4) and CEPC (experimental group 2, n = 4). Control group rats received no treatment (n = 4). Blood glucose levels in the rats were measured, and the pancreas and kidneys of the experimental animals were examined histologically. The follow-up period for all animals continued for 10 weeks. Results. In experimental group 1, on day 7 after injection of Langerhans islets, glycemia decreased significantly from 28.2 ± 4.2 mmol/L to 13.4 ± 2.6 mmol/L. This fall persisted for 7 weeks, following which blood sugar increased to nearly their initial levels (prior to islets administration). In experimental group 2, on day 7 after CEPC administration, there was a more noticeable drop in blood sugar levels from 25.8 ± 5.1 mmol/L to 6.3 ± 2.7 mmol/L compared to experimental group 1. By the 10th week of the experiment, the average glucose level was two times lower than it was at the beginning. Blood glucose levels dropped more sharply in the CEPC group than in the islet group (by 75.6% and 52.5%, respectively). **Conclusion.** In T1D rats, CEPC has a more potent antidiabetic effect than islets of Langerhans. Thus, it has been shown that a tissue-specific scaffold may be used to create bioartificial pancreas in order to increase the functional efficiency of islets.

Keywords: type I diabetes mellitus, islets of Langerhans, cell-engineered construct, pancreas, decellularization, tissue-specific scaffold.

INTRODUCTION

Replacement of damaged beta cells in patients with severe type I diabetes mellitus (T1D) through pancreatic islet transplantation is an effective treatment method that allows to establish long-term stable euglycemia [1], improve the quality of life and reduce secondary complications compared to insulin therapy [2]. However, during pancreatic islet isolation, their functional activity decreases due to a number of damaging factors, such as loss of vascularization, innervation and connections with the extracellular matrix (ECM). After transplantation, the islet revascularization process is completed in 10–14 days. During this period, oxygen and nutrients are delivered to islet cells only by diffusion, which may be insufficient to maintain cell viability in the central part of the islet [3, 4]. Thus, the limited graft functioning time is to some extent connected with islet hypoxia in the posttransplant period [5].

It seems essential to provide the islets of Langerhans with the conditions conducive to maintaining their viability and function *in vitro* and *in vivo*. There is a need to develop new strategies for beta-cell replacement, including the creation of a bioequivalent of pancreas based on insulin-producing cells and a carrier matrix (scaffold) mimicking the pancreatic ECM. Thus, decellularized pancreas with preserved biochemical, spatial and vascular structure of the native ECM can be used as a scaffold for subsequent recellularization with insulin-producing cells and further transplantation [6–9].

Decellularized pancreatic tissue most closely mimics the microenvironment of native pancreatic ECM, i.e. they possess tissue specificity with characteristic features of structure and composition. A significant reduction

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in immunogenicity, achieved by removing cellular and nuclear material from decellularized scaffolds, provides an ideal support system for islet cells in transplantation [10, 11]. Successful fabrication and recellularization of scaffold from decellularized pancreatic tissue is an essential component of pancreatic tissue engineering [12].

Previous studies have allowed us to develop protocols for the preparation of tissue-specific scaffolds from decellularized rat [13] and human [14] pancreas. *In vitro* experimental studies have found that culturing islets of Langerhans in the presence of such scaffolds helps preserve their structure, prolongs their viability and insulin-producing function in comparison with islets cultured under standard conditions [6, 13–15]. Some reports [5, 9, 10] have shown that a scaffold from decellularized pancreas also provides islet cells with prolonged survival and functioning *in vivo*.

Decellularized pancreatic tissue scaffolds seeded with islet cells represent a formed cell-engineered pancreatic construct (CEPC), often referred to as bioartificial pancreas. CEPCs appear to be feasible for clinical transplantation to T1D patients to compensate for the endocrine function of the pancreas [8].

The **aim** of the present work was to comparatively analyze the functional efficacy of CEPC and isolated rat islets of Langerhans after intraperitoneal injection in T1D rats.

MATERIALS AND METHODS

Experimental animals

For modeling streptozotocin-induced T1D followed by CEPC implantation (recipient animals) and isolation of islets of Langerhans (donor animals), we used male Wistar rats weighing 300–380 g (n = 30) from a laboratory animal nursery belonging to KrolInfo LLC. Acclimatization and maintenance of the animals was carried out in accordance with interstate standard GOST ISO 10993-2-2009 "Medical Devices. Assessment of the Biological Effect of Medical Devices". Part 2. "Requirements for the Treatment of Animals".

Manipulations with animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (2005) and the Rules of Laboratory Practice approved by Order No. 708 of the Russian Ministry of Health dated August 23, 2010. Approval (Protocol No. 280121-1/1e, dated January 28, 2021) for the experimental studies was obtained from the Local Ethics Committee of Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Modeling of type I diabetes

To induce T1D, the rats were intermittently injected with streptozotocin (STZ) (Biorbyt, India): 15 mg/kg/day intraperitoneally for 5 consecutive days (total dose 75 mg/kg) [16]. The injection agent was prepared ex tempore by diluting STZ in 0.9% sodium chloride solution and injected into the peritoneal cavity. The dynamics of blood sugar levels was determined daily using an Accu-Chek Active glucometer (Roche, Switzerland).

Testing the stability of an experimental model of type I diabetes

To rule out spontaneous reversion of diabetic status, the animals were monitored for the next 14 days after the last STZ injection. Fasting blood glucose and body weight were monitored weekly, appearance and amount of water consumed by the animals were assessed daily.

T1D was considered stable if blood glucose level in the rats exceeded 20.0 mmol/L two weeks after the last dose of STZ injection.

In rats with glucose concentration of less than 20.0 mmol/L, clinical signs of diabetes were mild. Such animals were not used in the experiment. Animals with glucose levels exceeding the glucose meter limit (33.0 mmol/L) were considered unsuitable for further CEPC implantation.

CEPC preparation technology

To create CEPC, tissue-specific finely dispersed scaffold derived from decellularized human pancreatic fragments (DHP scaffold) was chosen as an ECM biomimetic [13, 14]. Studies have shown that DHP scaffold retains the morphofunctional properties of native ECM of pancreatic tissue, contains basic fibrillar proteins (type I collagen, elastin), has low immunogenicity (≤0.1% DNA) and is not cytotoxic with respect to adhesion and proliferation of cell cultures. Allogeneic isolated rat islets of Langerhans were used as an insulin-producing component of CEPC. Freshly isolated islets were identified by dithizone staining (Sigma-Aldrich, USA) and cultured in complete growth medium containing DMEM (1.0 g/L glucose) (PanEco, Russia), 10% fetal calf serum (FBS) (HyClone, USA), Hepes (Thermo Fisher Scientific, USA), 2 mM alanyl-glutamine (PanEco, Russia), 1% antibiotic/antimycotic (Thermo Fisher Scientific, USA) for 24 hours, under standard conditions at 37 °C, in a CO_2 incubator, in a humidified atmosphere containing 5% CO_2.

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

The functional activity of islets was assessed after 24 hours of cultivation using the Rat Insulin ELISA Kit (Thermo Fisher Scientific, USA) to measure insulin levels under the load of hormone secretion stimulant (glucose).

A concentrated suspension of islets was obtained by centrifugation in complete growth medium for 2 minutes at 1200 rpm, then washed from the growth medium in

Hanks' Balanced Salt Solution (HBSS) under the same regime.

For each CEPC sample, 2000 islets obtained from 1–2 rat pancreas were selected, resuspended in 1.0–1.2 mL of Hanks' solution, and mixed with finely dispersed sterile DHP scaffold (10.0 ± 0.1 mg in 100 μL of HBSS) from human pancreas. The resulting CEPC samples were placed into 2-mL syringes with a 23 G needle size.

Intraperitoneal injection of CEPC to rats with experimental type I diabetes

The functional efficacy of CEPC was studied in rats with severe and stable T1D induced by daily intermittent injection of low-dose STZ.

The animals selected for the experiment were categorized into groups:

1) Control group (4 rats, untreated, no administration of islets of Langerhans or CEPC);
2) Experimental group 1 (4 rats, intraperitoneal injection of 2000 allogeneic islets of Langerhans in the form of a suspension);
3) Experimental group 2 (4 rats, intraperitoneal injection of CEPC created from 2000 allogeneic islets of Langerhans and tissue-specific scaffold (DHP scaffold) from decellularized human pancreas).

Follow-up period continued for 10 weeks. Blood glucose levels in the animals were measured on an empty stomach weekly, 12 hours after the last meal.

Histological examination

A morphological study identified some structural disorders in the parenchyma of the pancreas and kidneys. Extracted organs of the experimental animals were fixed in 10% buffered formalin for 24 hours, then dehydrated in alcohols of ascending concentration, kept in a mixture of ethanol and chloroform, pure chloroform, and then embedded in paraffin.

Using a microtome RM2245 (Leica, Germany), 5 μm thick slices were obtained and stained with hematoxylin and eosin and for total collagen using Masson's method. The cellular composition of pancreatic islets in the pancreas of rats from the control and experimental groups was assessed by immunohistochemical staining of the main types of islet cells using antibodies to insulin and glucagon (Abcam, UK) and Rabbit Specific HRP/DAB (ABC) Detection IHC kit visualization system (Abcam, UK).

Detection and assessment of the degree of structural disorders in the kidneys of experimental animals was performed by staining the sections with hematoxylin and eosin.

Statistical analysis

Statistical data processing was done using Microsoft Excel (2016) software. Differences were considered statistically significant if the significance level of p did not exceed the threshold value of 0.05.

RESULTS

Experimental type I diabetes

Rats (n = 13) with fasting blood glucose levels ranging from 20.4 mmol/L to 32.6 mmol/L two weeks after the end of STZ administration were used for experiments to investigate the functional efficacy of CEPC (Table 1). Clinically, these animals showed hypodynamia, a tendency to form wounds and pustules, polyuria, significant

Table 1

Changes in glucose levels and body weight in T1D rats

	Glucose level (mmol/L)			Body weight (g)		
Days after the last STZ dose	1	7	14	1	7	14
Norm (n = 4)	5.5 ± 0.7	5.9 ± 0.3	5.8 ± 0.9	345 ± 15	350 ± 18	365 ± 24
T1D (n = 13)	17.8 ± 3.3	19.1 ± 3.5	26.5 ± 6.1	310 ± 25	295 ± 27	280 ± 23

Note. $p < 0.05$ compared with the values of similar indicators of the norm (intact rats).

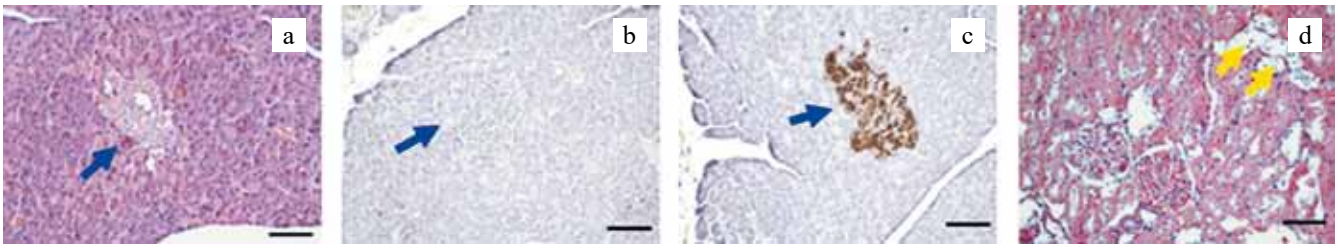

Fig. 1. Pancreas (a–c) and kidney (d) of a rat with experimental T1D; H&E stain (a, d); immunohistochemical staining for insulin (b); immunohistochemical staining for glucagon (c). Blue arrows indicate islets of Langerhans. Yellow arrows indicate tubular epithelial Armanni–Ebstein cells. Scale bar: 100 μm

polydipsia and decreased body weight compared to the intact animals.

To confirm the development of T1D, histologic examination of the pancreas of rats with a blood glucose level of 25.3 mmol/L was performed two weeks after the last STC injection. It was revealed that the exocrine parenchyma was generally preserved; moderate focal lymphoid-cell infiltration was noted only in some lobules. Vacuolated cells (probably beta cells) were detected in few irregularly shaped islets of Langerhans (Fig. 1, a). When stained with antibodies against insulin, a negative reaction was observed, which indicated the death of beta cells (Fig. 1, b). At the same time, glucagon-positive alpha-cells were found not only in the periphery, but also in the central part of the islets and in a much larger number than in healthy rats (Fig. 1, c). Histological examination of rat kidneys with clinical signs of T1D revealed pronounced changes, including the presence of multiple vacuolated tubular epithelial cells (Armanni–Ebstein cells, which are pathognomonic for diabetes) (Fig. 1, d). Foci of inflammatory infiltration (lymphoid cellular with admixture of plasmacytes) were observed. Protein cylinders were occasionally detected in the tubule lumen.

In order to create an experimental model of type 1 diabetes, intermittent injections of STZ were used since this method prevented animal death and spontaneous remission of diabetes while maintaining a stable diabetic state in the animals [16]. The obtained T1D was characterized by stable hyperglycemic indicators (from 20 mmol/L to 33 mmol/L) in the blood of experimental animals and other signs of diabetes: polydipsia, polyuria.

Morphofunctional analysis of isolated rat islets of Langerhans before CEPC creation

Freshly isolated islets of Langerhans of predominantly rounded shape mostly retained the integrity of the macrostructure intact during islet isolation (Fig. 2, a). Pancreatic islets selectively acquired red-orange color when stained with dithizone, while acinar cells remained unstained (Fig. 2, b).

To determine the viability of rat pancreatic islets cultured for 24 hours, staining with vital acridine orange/propidium iodide stain (AO/PI) was performed. Islet viability was found to be higher than 96% (Fig. 2, c).

In the culture medium samples taken after 24 hours of islets culturing, basal insulin level was 178.6 ± 13.3 μIU/mL; after stimulation with "hyperglycemic" glucose

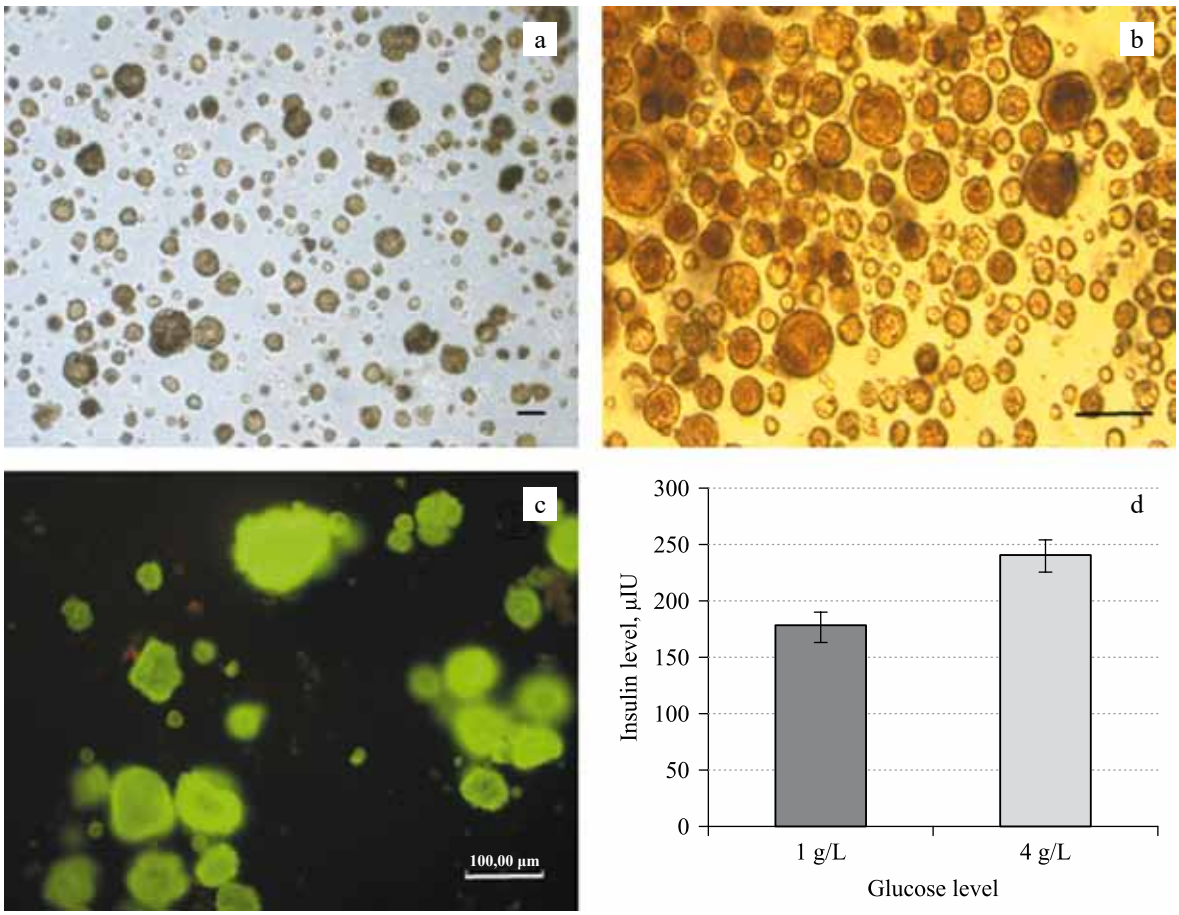

Fig. 2. Isolated islets of Langerhans of a healthy rat. a, phase-contrast microscopy; b, dithizone staining; c, islets cultured for 24 hours, acridine orange and propidium iodide (AO/PI) fluorescent staining; d, functional activity of isolated islets of Langerhans of a healthy rat cultured for 24 hours. Scale bar: 100 μm

level of 4.5 g/L (25 mmol/L), it increased by 35.4% (241.8 ± 14.2 μIU/mL), which confirmed the functional activity of isolated islets (Fig. 2, d).

Thus, it was found that isolated rat islets of Langerhans by their morphofunctional state (level of viability and functional activity) can be used as a cellular insulin-producing component for the creation of CEPC.

Comparative analysis of the functional efficiency of CEPC and islets of Langerhans in rats with experimental type I diabetes

The functional efficacy of CEPC, formed from allogeneic islets of Langerhans and tissue-specific scaffold from decellularized human pancreas, was assessed for 10 weeks in relation to its ability to restore physiological blood glucose levels in rats with STZ-induced T1D.

Control group

All animals in the control group maintained pronounced clinical signs of T1D throughout the follow-up period, and the general condition of the rats worsened. Polydipsia, polyuria, diminished strength, non-healing purulent wounds on tails, further loss of body weight (from 280 ± 23 g to 170 ± 35 g) were noted in the animals. The level of hyperglycemia in the blood in the control group (n = 4) increased during the follow-up period (Table 2). Of the four control rats, two died at 3 and 8 weeks.

In histological samples of the pancreas of rats in control group after withdrawal from the experiment (10 weeks), few irregularly shaped islets of Langerhans were found, with almost complete absence of insulin-positive beta cells and numerous glucagon-positive alpha-cells (Fig. 3, a–c). Rats in the control group had their kidneys examined histologically, and the results showed changes in the organ's morphology, as well as the presence of many Armanni–Ebstein cells and foci of inflammatory infiltration. These findings are indicative of diabetic nephropathy developing on the backdrop of T1D without treatment (Fig. 3, d). In addition, deposits of dense material – protein cylinders – were detected in the lumen of individual tubules.

Experimental group 1 (injection of islets of Langerhans)

In rats from experimental group 1 (n = 4), after intraperitoneal injection of islets of Langerhans, characteristic clinical signs of T1D slowed down within a week, weight gain was noted (from 280 ± 23 g to 305 ± 15 g). Moreover, three days following islets administration, hyperglycemia levels in all recipient rats decreased significantly by 12.5 ± 3.2 mmol/L (Table 3). Thereafter, blood glucose levels in three rats stabilized at a level that was on average 2.5 times lower compared to the level before islet administration. However, after 7 weeks of follow-up, glucose levels in experimental group 1 began to rise. By week 10, it had reached the level above the initial level (before islets administration) in two rats, and had dropped by 7.3 mmol/l below the initial level in one rat (Fig. 4, a). At week 6, the rat with blood glucose level of 27.3 mmol/L died, even though after islet administration, there was a stable decrease in hyperglycemia.

The histological picture of the pancreas of rats from experimental group 1 revealed good preservation of endocrine parenchyma without signs of inflammation and irregularly shaped islets, as in the pancreas of rats from control group, with rare vacuolated cells, probably beta

Table 2

Dynamics of blood glucose level in control group rats without treatment

Day	Blood glucose (mmol/L)			
	Rat 1	Rat 2	Rat 3	Rat 4
0	22.1	20.7	29.5	32.2
4	20.2	22.0	≥33.0	31.5
7	22.7	19.3	30.7	≥33.0
14	27.8	18.8	28.8	24.3
21	25.3	25.5	dead	28.0
28	28.0	26.3	–	≥33.0
35	30.4	28.0	–	≥33.0
42	≥33.0	26.8	–	≥33.0
49	≥33.0	28.4	–	≥33.0
56	dead	26.5	–	≥33.0
63	–	31.2	–	≥33.0
70	–	32.5	–	≥33.0

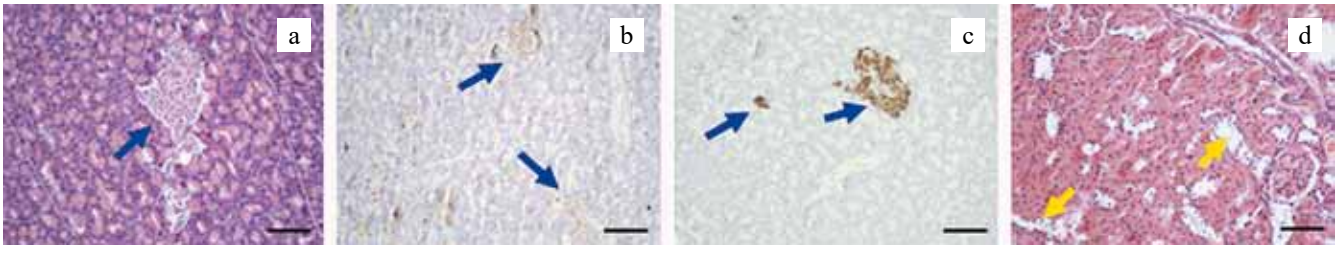

Fig. 3. Pancreas (a–c) and kidney (d) of control group rats with experimental T1D; H&E stain (a, d); immunohistochemical staining for insulin (b); immunohistochemical staining for glucagon (c). Blue arrows indicate islets of Langerhans. Yellow arrows indicate tubular epithelial Armanni–Ebstein cells. Scale bar: 100 μm

cells (Fig. 4, a–c). In two rats (rat 2 and rat 4), immunohistochemical staining revealed no insulin-positive beta cells, while numerous glucagon-positive alpha-cells were present in the islets. In one rat (rat 3), there were single beta cells in the islets (Fig. 4, d). Morphological study of the kidneys of rats from experimental group 1 did not reveal severe degenerative changes in the vascular and tubular apparatus; however, numerous vacuolated cells – Armanni–Ebstein cells – were detected in the tubular epithelium (Fig. 4, e). Flaky material was visualized in the lumen of some tubules.

Table 3

Changes in blood glucose levels in T1D rats of experimental group 1 after injection of islets of Langerhans

Day	Blood glucose (mmol/L)			
	Rat 1	Rat 2	Rat 3	Rat 4
0	26.7	30.7	23.0	32.5
4	11.6	15.3	12.9	23.1
7	13.0	13.5	9.5	18.8
14	11.5	16.0	15.3	10.9
21	15.8	22.3	14.8	12.3
28	23.0	20.9	15.7	14.9
35	27.3	22.6	12.4	14.1
42	dead	18.9	12.1	16.3
49	–	19.1	14.8	15.9
56	–	26.1	23.0	18.7
63	–	25.4	22.3	21.7
70	–	33.0	27.6	25.2

Experimental group 2 (CEPC injection)

Similar to the animals in the experimental group, a slowdown in the clinical manifestations of T1D was seen in rats of experimental group 2 (n = 4) one week following intraperitoneal injection of CEPC based on allogeneic islets of Langerhans with DHP scaffold. T1D signs, including polydipsia and polyuria, virtually vanished in rat 3, and by week 10, the rats gained body weight dramatically from 303 g at the time of CEPC injection to 555 g. In recipient rats 3 and 4, there was a prolonged decrease in blood glucose levels (BGLs) by more than 3-fold compared to baseline, along with individual measurements reaching normoglycemic values of up to 7.5 mmol/L (Table 4). By the end of the experiment (10 weeks of follow-up), BGLs of 9.5 and 7.9 mmol/L, respectively, were recorded in the above rats, which was much lower than the initial average level (25.8 ± 5.1 mmol/L). Rat 1 had no persistent reduction in hyperglycemia: after CEPC injection, blood glucose level ranged from 4.9 to 17.3 mmol/L. At the time of withdrawal from the experiment (70 days), blood glucose in rat 1 was 17.0 mmol/L, not exceeding the baseline value.

In week 4 of follow-up, rat 2 with a glucose level of 5.9 mmol/L died.

Histological examination of the pancreas of rats from experimental group 2 revealed that the exocrine parenchyma was preserved without inflammation signs and with no infiltrates (Fig. 5, a, d). Few vacuolated cells were observed in both round and irregularly shaped is-

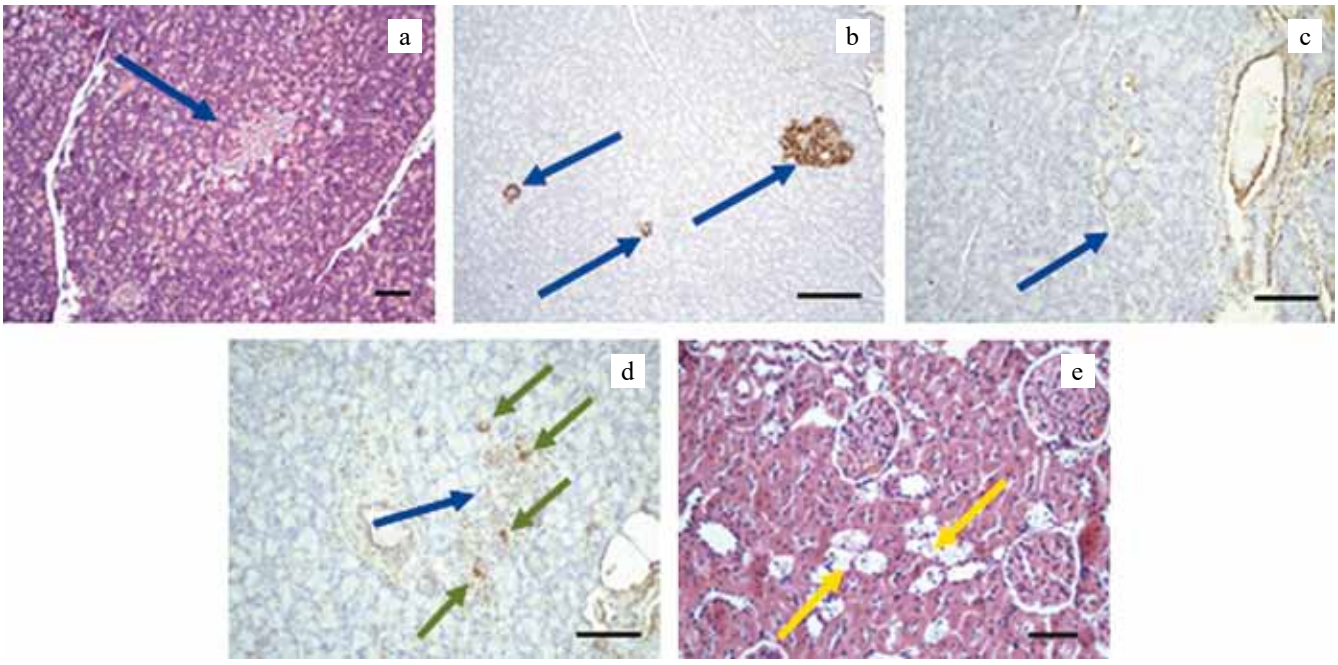

Fig. 4. Pancreas (a–d) and kidney (e) of experimental group 1 rats with T1D after intraperitoneal injection of islets of Langerhans; H&E stain (a, e); immunohistochemical staining for glucagon (b); immunohistochemical staining for insulin (c, d). Blue arrows indicate islets of Langerhans. Yellow arrows indicate tubular epithelial Armanni–Ebstein cells. Scale bar: 100 μm

lets. Immunohistochemical staining for insulin revealed the presence of few beta cells in the pancreatic islets of rats 1 and 4 (Fig. 5, b); in rat 3, the increase in the count of insulin-positive cells turned out to be more significant (Fig. 5, e), while there was lower count of glucagon-positive cells in the islets (Fig. 5, e, f). Experimental data [16] suggest that in addition to its direct antidiabetic effect, CEPC implantation can have a positive effect on the processes of restoring the pool of actively functioning beta cells of the recipientю This has been confirmed by our histological study. Rat kidneys from experimental group 2 showed a different morphological picture in contrast to kidneys from the control group and experimental group 1, showing fewer Armanni–Ebstein cells and no protein cylinders in the tubular lumen (Fig. 5, g).

Fig. 6 shows a comparative diagram of the dynamics of changes in BGLs in animals of the control and experimental groups. The graph of glycemic indicators of the control group is given by the BGLs of one rat, not exceeding the limit value of the glucometer – 33.0 mmol/L, throughout the entire follow-up period. In the control group, hyperglycemia indicators steadily increased throughout the experiment.

After injection of islets of Langerhans in experimental group 1, there was a marked decrease in BGLs from 28.2 ± 4.2 mmol/L to 13.4 ± 2.6 mmol/L, which persisted for 7 weeks, after which it increased to levels close to the initial values (before islets administration).

Table 4

Changes in blood glucose levels in T1D rats of group 2 after CEPC injection

Day	Blood glucose (mmol/L)			
	Rat 1	Rat 2	Rat 3	Rat 4
0	20.4	25.6	24.7	32.6
4	17.9	23.0	5.7	19.5
7	4.9	3.9	10.0	6.5
14	9.0	7.4	3.6	7.9
21	10.8	5.9	6.9	9.2
28	13.2	dead	10.7	10.7
35	17.3	–	13.9	7.2
42	15.6	–	9.6	8.5
49	11.8	–	12.4	10.3
56	12	–	10.6	6.8
63	11.1	–	6.5	8.9
70	17.0	–	9.5	7.9

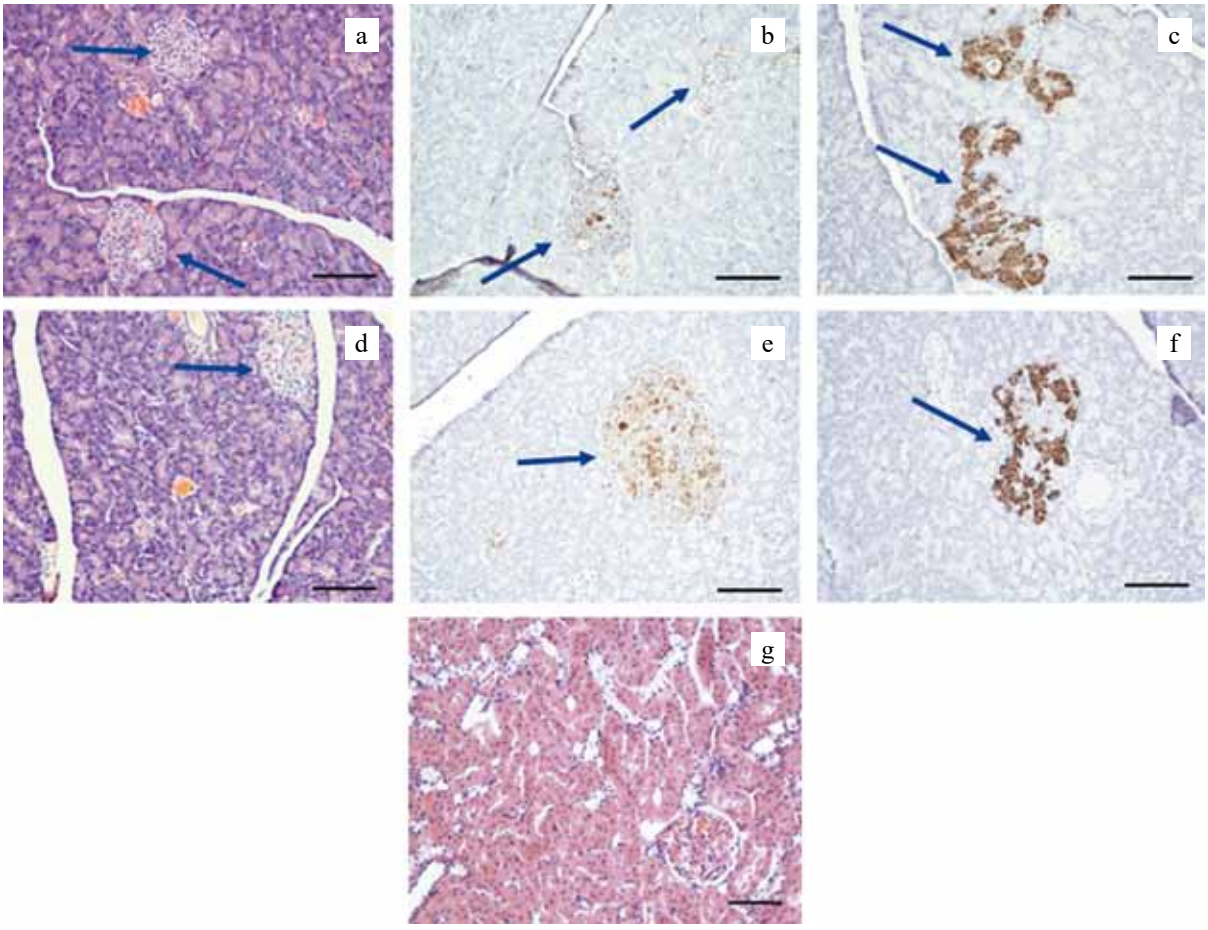

Fig. 5. Pancreas (a–f) and kidney (g) of rats of experimental group 2 with T1D after intraperitoneal injection of CEPC; (a, d, g), H&E; (b, e), immunohistochemical staining for insulin; (c, f), immunohistochemical staining for glucagon. Arrows indicate islets of Langerhans. Scale bar: 100 μm

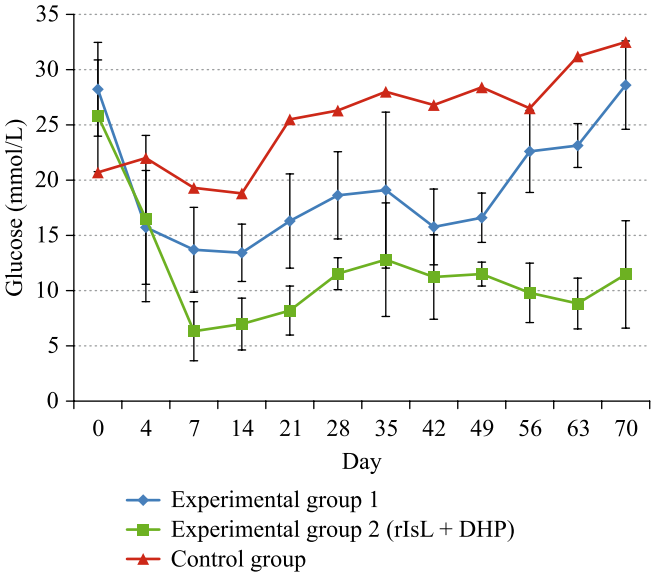

Fig. 6. Changes in blood glucose levels in T1D rats of the control group (without treatment) and experimental groups after intraperitoneal injection of CEPC (islets of Langerhans (rIsL) + decellularized human pancreas (DHP)) or suspension of islets of Langerhans (rIsL). Glycemic indicators of the control group are presented according to blood glucose levels of one rat. Glycemic indicators of experimental group 1 are presented by blood glucose levels of four rats up to 42 days, and then due to the death of one animal, the indicators of three rats were considered. Glycemic indicators of experimental group 2 are presented by blood glucose levels of four rats up to 28 days, and then due to the death of one animal, the indicators of three rats were considered

Experimental group 2 showed a more pronounced decrease in glucose levels from 25.8 ± 5.1 mmol/L to 6.3 ± 2.7 mmol/L compared to experimental group 1. Such concentrations were maintained throughout the follow-up period. The glucose level by week 10 of the experiment was on average 2 times lower than the initial one.

In rats of experimental group 2, after CEPC injection, the maximum decrease in BGLs relative to initial hyperglycemic parameters was by 75.6%; in experimental group 1, it was by 52.5% after administration of islet suspension.

Thus, the studies have shown the *in vivo* functional efficiency of allogeneic islets of Langerhans injected without scaffold and as a part of the human cell-engineered pancreatic construct. There was a more pronounced decrease in blood glucose concentration in recipient rats after administration of experimental CEPC samples compared to the level in recipient rats after administration of islets without scaffold (by 75.6% and 52.5%, respectively).

The obtained results correlate with reports from a preliminary study [17], where, with intraperitoneal CEPC injection, tissue-specific scaffolds provided pancreatic islets with longer survival and effective functioning *in vivo*.

Thus, the important role of tissue-specific scaffold in the creation of a bioartificial pancreas has been demonstrated. The optimal scaffold obtained from decellularized pancreatic tissue for CEPC formation should 1) meet the criteria for effective decellularization, 2) preserve the native structure as much as possible, 3) provide sites necessary for cell adhesion and proliferation, and 4) be evenly populated by insulin-producing cells [6, 14]. Synthetic artificial scaffolds may not meet some of these requirements and, hence, may not positively influence the survival and functioning of islets *in vivo*. The presence of native proteins (various types of collagen, elastin, fibronectin and laminin) in the decellularized pancreatic scaffold, as well as cell adhesion factors, allows to create conditions for prolonged viability of islet cells, thus maintaining the critical mass of islets necessary for transplantation to T1D patients [18].

CONCLUSION

The study found that intraperitoneal administration of CEPC samples in experimental T1D rats resulted in a significant, sustained reduction in fasting blood glucose levels that persisted for 10 weeks. Thus, administration of CEPC revealed a more pronounced antidiabetic effect in T1D rats compared to administration of a suspension of islets of Langerhans. This suggests that in order to improve the functional efficiency of islets, a tissue-specific scaffold could be used to create a bioartificial pancreas. This work lays the groundwork for research on the development of human endocrine cell-engineered pancreatic constructs based on tissue-specific scaffolds made from decellularized human pancreatic tissue and islets of Langerhans of deceased donors in order to partially or completely replace the lost endocrine function of the pancreas in patients with severe T1D.

The authors declare no conflict of interest.

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