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METHOD FOR THE ISOLATION OF HUMAN PANCREATIC ISLETS FOR CLINICAL TRANSPLANTATION IN TYPE 1 DIABETES MELLITUS

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Islet transplantation is a modern method of treating severe type 1 diabetes mellitus (T1D). However, multiple factors can negatively affect both the efficiency of islet isolation and subsequent transplantation outcomes. Consequently, ongoing efforts to optimize isolation techniques have led to the emergence of new unique protocols. The **objective** of this study is to evaluate the effectiveness of a modified method for isolating pancreatic islets (islets of Langerhans) from deceased donors for clinical transplantation in recipients with T1D. **Materials and methods.** Pancreatic islets were isolated using a modified technique that excluded the use of the Ricordi islet isolator during the enzymatic digestion stage, as well as density gradient centrifugation during the purification stage. Islet identification was performed using dithizone staining. Islet viability was assessed by fluorescent staining with acridine orange and propidium iodide. Functional activity was evaluated by determining the stimulation index using enzyme-linked immunosorbent assay (ELISA). Histological examination of the native pancreas (n = 3) included routine staining, as well as immunohistochemical analysis targeting the main types of islet cells. **Results.** Pancreatic islets isolated from the pancreas of deceased donors using the modified technique retained their structural integrity and demonstrated variability in size and shape. The total islet yield was 630,000 ± 30,000, with a viability rate of 90 ± 3% and a stimulation index of 1.41 ± 0.01. These findings indicate the high quality of the isolated biomaterial and confirm the ability of β-cells to respond to changes in glucose content. Morphological assessment of the donor pancreas demonstrated preserved structural integrity of the islet apparatus, supporting the potential for obtaining viable and functionally active islets. The efficiency of islet isolation during enzymatic pancreatic processing was also confirmed. **Conclusion.** Optimization of the methodological approach enabled the development and validation of a modified technique for processing the pancreas of deceased donors, resulting in the isolation of a substantial number of viable and functional islets of Langerhans. The characteristics of the obtained islet graft support its suitability for clinical transplantation in T1D patients.

Keywords: pancreatic islets, pancreas, transplantation, type 1 diabetes mellitus.

INTRODUCTION

Pancreatic islet (PI) transplantation can be considered an alternative to whole-pancreas transplantation, as it is less invasive, sparing patients from major surgery [1, 2]. It represents a modern treatment for type 1 diabetes (T1D), particularly in patients complicated by a high susceptibility to severe hypoglycemia and glycemic instability [3, 4].

Clinical studies conducted over the past three decades suggest that restoration of endogenous insulin production through islet transplantation – which secretes a full spectrum of bioactive peptides – may achieve metabolic outcomes that are not attainable with conventional insulin therapy alone [5–7].

Evidence also indicates the beneficial effects of islet transplantation in slowing the progression of secondary complications of T1D, including improved outcomes in diabetic nephropathy and reduced progression of diabetic retinopathy and neuropathy [8, 9]. It has been found that islet transplantation may support sustained graft function and prolong the survival of concomitant kidney allografts in most recipients [10].

PI transplantation in patients with T1D is primarily based on the Edmonton Protocol introduced by Shapiro et al. in 2000 [11]. The key stages of successful PI isolation include: 1) Enzymatic digestion of the pancreas, which leads to dissociation of pancreatic tissue and release of the islets from the surrounding exocrine tissue; 2) Purification of isolated islets from exocrine tissue using techniques such as incubation, filtration, and density-gradient centrifugation [12].

After isolation and purification, the islet cell suspension is evaluated for identity, purity, viability, functionality,

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and sterility in order to ensure the safe transplantation of an adequate quantity of insulin-producing cells into the recipient [1, 5].

It is important to note that, despite substantial advances in clinical PI transplantation, several challenges continue to limit its widespread implementation. Achieving insulin independence in a single recipient often requires islets obtained from more than one donor pancreas because of the complexity and unpredictability of islet isolation outcomes. At each stage of islet preparation, numerous factors may adversely affect isolation efficiency and, consequently, transplant outcomes [13].

According to data from the Collaborative Islet Transplant Registry, the success rate of islet graft isolation is approximately 52.5% [14]. As a result, ongoing research is focused on optimizing islet isolation techniques, leading to the development of new and improved protocols [15–17].

Previously, researchers at Shumakov National Medical Research Center of Transplantology and Artificial Organs developed and tested an original method for isolating the islets of Langerhans from rat and rabbit pancreases, as well as from fragments of human pancreatic tissue. This technique reportedly enabled a 2.1-fold increase in islet yield while preserving their morphofunctional properties when compared with previously published protocols [18–22].

This study evaluated a modified protocol for PI isolation from deceased donors. The proposed technique eliminates the need for the costly Ricordi perfusion system during the enzymatic digestion stage, as well as the use of a Ficoll density gradient during the purification process.

The **aim of the study** was to assess the effectiveness of the modified method for isolating PIs from deceased-donor pancreases for subsequent clinical transplantation in recipients with T1D.

MATERIALS AND METHODS

Pancreatic islet isolation

PIs were isolated using a modified technique developed on the basis of traditional isolation protocols [23, 24].

The pancreas specimens were obtained during multiorgan procurement from deceased donors (n = 3) with confirmed brain death who met established criteria for optimal pancreatic donation.

The harvested pancreas was removed from a triple-layer sterile bag containing cold preservation solution (+4 °C) (Custodiol; Dr. Franz Köhler Chemie, Germany) and placed in a sterile tray. Surrounding adipose tissue, blood vessels, and adjacent connective tissue were carefully removed. Under sterile conditions, a sample of the preservation solution was collected for bacteriological assessment of pancreatic explant sterility.

The pancreas was subsequently weighed and rinsed with cold Hanks' Solution (PanEco, Russia) supplemented with a 2% solution containing penicillin, streptomycin, and amphotericin B (Thermo Fisher Scientific, USA). The gland was then cut into 6–8 equal parts. A working enzymatic solution consisting of collagenase NB1 (activity: 20 PZ U/g tissue) and neutral protease NP (activity: 1.5 DMC U/g tissue) (SERVA Electrophoresis GmbH, Germany) was added at a volume of 2 mL per gram of pancreatic tissue.

The stretched pancreatic tissue was mechanically minced into approximately 1×1×1 cm^3 fragments, transferred into a sterile container, and incubated at 37.0 °C for 12–15 minutes until free islets became visible in samples of the digested tissue suspension. Enzymatic activity was terminated by adding three times the volume of cold (+4 °C) Hanks' solution.

The islets were purified from exocrine tissue by filtering the enzymatically digested pancreatic tissue through a 600 μm mesh filter (Sigma-Aldrich, USA). An equal volume of complete culture medium based on DMEM supplemented with 1.0 g/L glucose (PanEco, Russia), 10% fetal bovine serum (HyClone, USA), 1% HEPES buffer, 2 mM alanyl-glutamine (PanEco, Russia), and a 1% antibiotic-antimycotic solution containing penicillin, streptomycin, and amphotericin B (Thermo Fisher Scientific, USA) was added to the filtrate, which was subsequently allowed to sediment for 40 minutes. The filtrate was then centrifuged once for 2.5 minutes at 110 g using a Rotina 38R centrifuge (Hettich Zentrifugen, Germany). Freshly isolated islets obtained from the resulting pellet were resuspended and transferred into culture flasks containing complete growth medium.

The islets were incubated at 37 °C in a CO_2 incubator (Series 8000 WJ, Thermo Fisher Scientific, USA) under humidified conditions (100% relative humidity, 5% CO_2) for 12 hours to enhance purification and facilitate removal of dead or apoptotic cells.

Following incubation, the islets were collected for subsequent infusion by centrifugation for 3.5 minutes at 180 g. The resulting islet pellet was washed twice more in Hanks' solution under identical centrifugation conditions to remove residual culture medium. The final islet suspension was then resuspended in the prepared infusion solution for transplantation.

Identification of pancreatic islets

Immediately after isolation, the islets were identified by staining the suspension with dithizone (Lenreactiv, Russia). This staining method not only confirms the presence of insulin-producing beta-cells among the isolated cells but also enables assessment of the purity of the islet suspension by identifying other cellular components that do not stain with dithizone.

The working dithizone solution was prepared immediately prior to staining by dissolving 10 mg of dithizone in 2 mL of dimethyl sulfoxide (PanEco, Russia) and 8 mL of Hanks' solution, followed by filtration through a sterile syringe filter with a pore size of 0.22 μm (Corning, USA).

A sample of the islet suspension was mixed with the working dithizone solution in a 2:1 volume ratio and incubated at 37 °C for 20 minutes. Evaluation of staining results and counting of the stained islets were performed using a Nikon Eclipse TS100 inverted fluorescence microscope (Nikon, Japan).

Assessment of islet viability

Viability of the isolated islets was assessed after 12 hours of incubation by fluorescent staining with acridine orange and propidium iodide (PanEco, Russia). For staining, a portion of the islet suspension was transferred into a Petri dish and mixed with the working dye solution at a 2:1 volume ratio, followed by incubation in the dark for 20 minutes. Viable islets were subsequently identified and counted using an inverted fluorescence microscope.

Assessment of the functional activity of pancreatic islets

The functional activity of the isolated islets incubated for 12 hours was evaluated using the stimulation index, defined as the ratio of insulin concentration secreted under glucose stimulation to the basal insulin concentration.

To determine basal insulin secretion, the conditioned culture medium was replaced with a fresh medium containing a low glucose concentration (2.8 mmol/L). After a 60-minute incubation under standard conditions, medium samples ($n = 5$) were collected for measurement of basal insulin levels.

Subsequently, the medium was replaced with a fresh medium containing a high glucose concentration (25 mmol/L) to stimulate insulin secretion. After an additional 60-minute incubation under standard conditions, medium samples ($n = 5$) were again collected. Insulin levels in all samples were determined using an enzyme-linked immunosorbent assay (ELISA) reagent kit manufactured by Vector-Best.

Histological examination

Samples of the pancreas ($n = 3$) and pancreatic tissue obtained after enzymatic digestion ($n = 3$), were subjected to morphological examination using standard histological staining methods. The samples were fixed in 10% buffered formalin, dehydrated through a graded alcohol series, cleared with chloroform, and embedded in paraffin. Sections 4–5 μm thick were prepared using an RM2245 microtome (Leica Microsystems, Germany), followed by deparaffinization and rehydration.

The sections were stained with Mayer's hematoxylin and eosin (BioVitrum, Russia), as well as by the Masson staining method using a ready-to-use reagent kit (BioVitrum, Russia). Histological evaluation and photomicrography were performed using a Nikon Eclipse 50i microscope equipped with a digital camera (Nikon, Japan).

To identify the main types of islet endocrine cells, immunohistochemical staining was performed using antibodies against insulin (Abcam, UK) and glucagon (Merck, Germany). Detection was carried out using a standard horseradish peroxidase method with the Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (Abcam, UK).

Statistical analysis

Statistical analysis was performed using SPSS software (version 26.0). The Student's t-test was applied to assess the statistical significance of differences in mean values between samples when evaluating the functional activity of the isolated PIs. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characteristics of the islet graft

A schematic representation of the isolation of PIs from a donor pancreas using the modified method is presented in Fig. 1.

The modified technique enabled the isolation of a substantial number of islets of varying sizes from donor pancreases ($n = 3$) while preserving their structural integrity. The isolated islets were predominantly round with smooth surfaces. In some preparations, minor surface irregularities were observed, corresponding to residual fragments of surrounding exocrine tissue (Fig. 2a).

Dithizone staining imparted a characteristic red-orange coloration to the islets (Fig. 2b), facilitating their identification and counting. From a single human pancreas, we were able to isolate 630,000 ± 30,000 islets.

Observation showed that the isolated islets retained their original morphological characteristics after 12 hours of incubation. Fluorescent staining with acridine orange and propidium iodide demonstrated predominantly green fluorescence within the islets, confirming their viability (Fig. 2c). Isolated propidium iodide–positive non-viable acinar cells were detected only in the surrounding culture medium. Overall, viability assessment using vital staining indicated that 90 ± 3% of the isolated islets remained viable.

After 12 hours of incubation, the insulin level in culture medium samples was 4.4 ± 0.8 mU/L, demonstrating the functional ability of beta-cells within the isolated islets to secrete insulin. After glucose stimulation, insulin level increased to 6.2 ± 0.7 mU/L, representing a 41% increase (Fig. 2d). Accordingly, the calculated stimulation index was 1.41 ± 0.01, indicating the ability of beta-cells to respond to changes in glucose concentration.

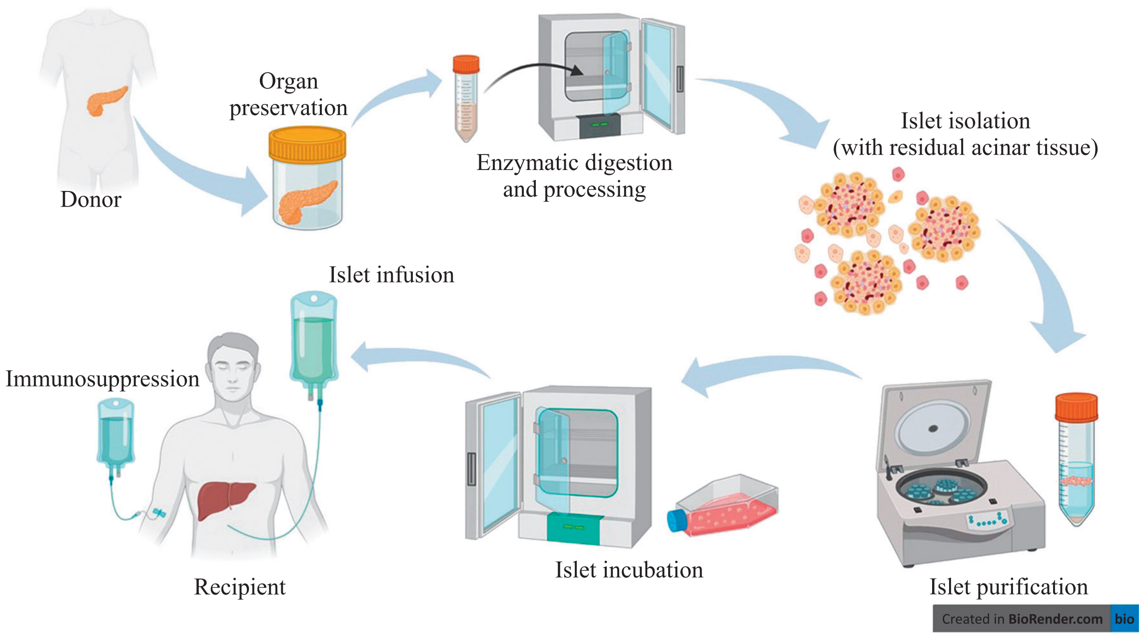

Fig. 1. Schematic representation of the isolation of pancreatic islets from the pancreas of a deceased donor

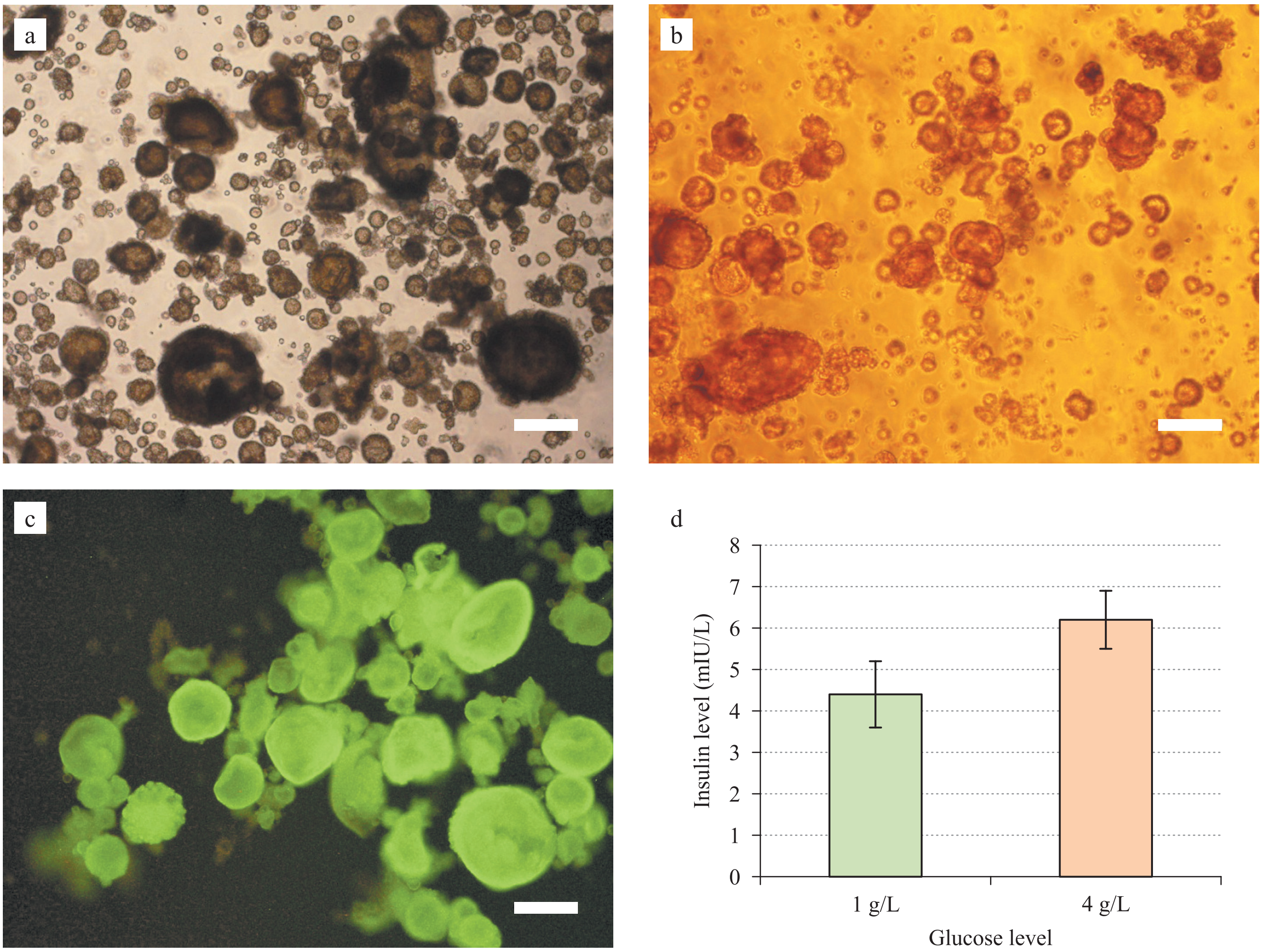

Fig. 2. Freshly isolated human pancreatic islets. (a) phase-contrast microscopy without staining; (b) dithizone staining; (c) islets incubated for 12 hours, assessed by fluorescent staining with acridine orange and propidium iodide; (d) comparative analysis of insulin secretion by islets before and after glucose stimulation. Incubation time: 12 hours. Scale bar: 100 μm

Morphological study

The initial condition of the pancreatic tissue was assessed by examining donor pancreases with comparable baseline characteristics (donor age: 49–54 years; gland weight: 97–100 g). Histological evaluation revealed no significant pathological alterations in the pancreatic parenchyma, indicating adequate preservation of the organ in the preservation solution.

The glandular parenchyma retained an intact lobular architecture in all examined samples. A mild degree of lipomatosis was observed in some exocrine lobules (Fig. 3a, 3c). No signs of fibrosis was detected; collagen

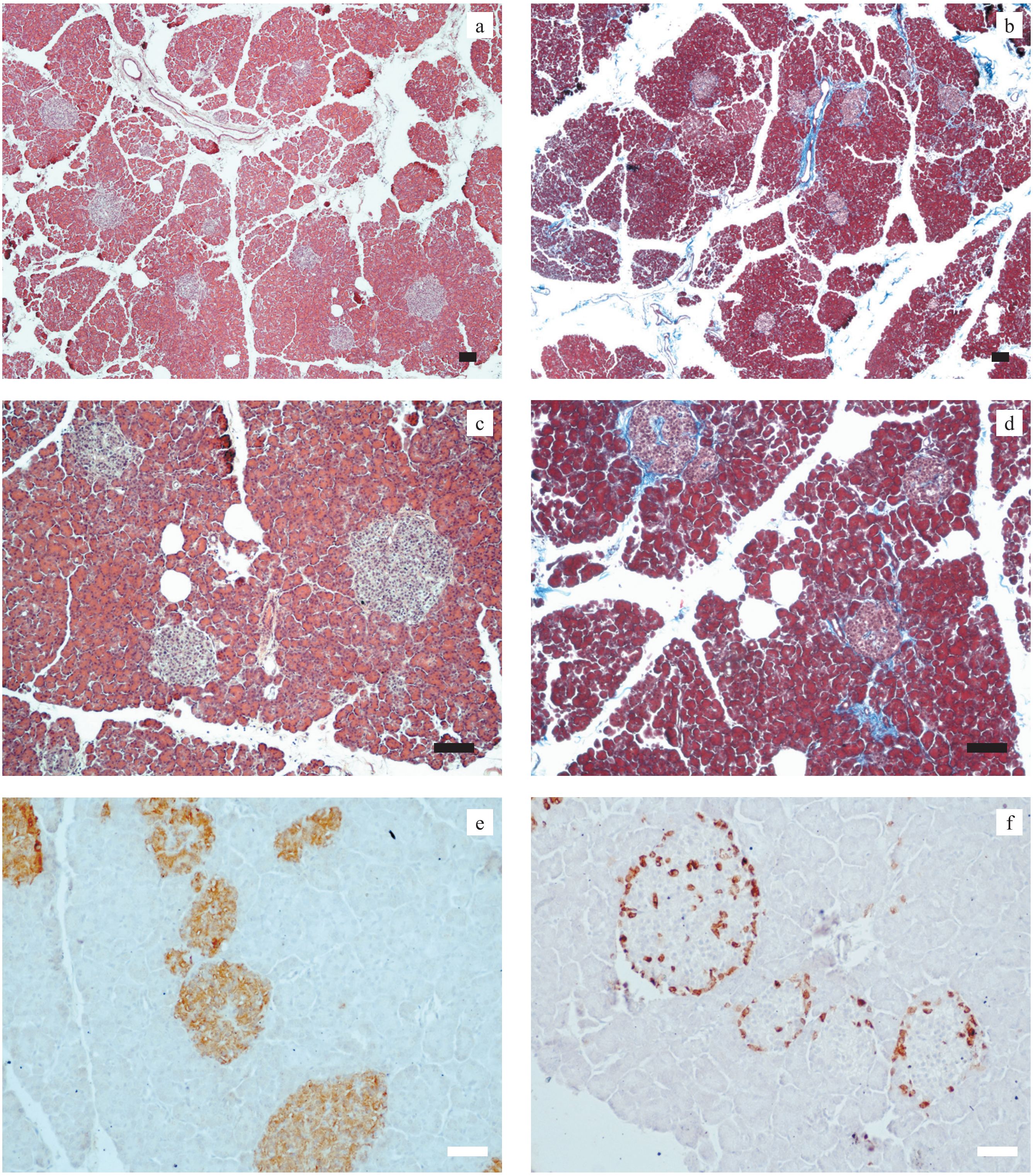

Fig. 3. Donor pancreas. (a, c) H&E staining; (b, d) Masson's trichrome staining; (e) Immunohistochemical staining for insulin; (f) Immunohistochemical staining for glucagon. Scale bar: 100 µm

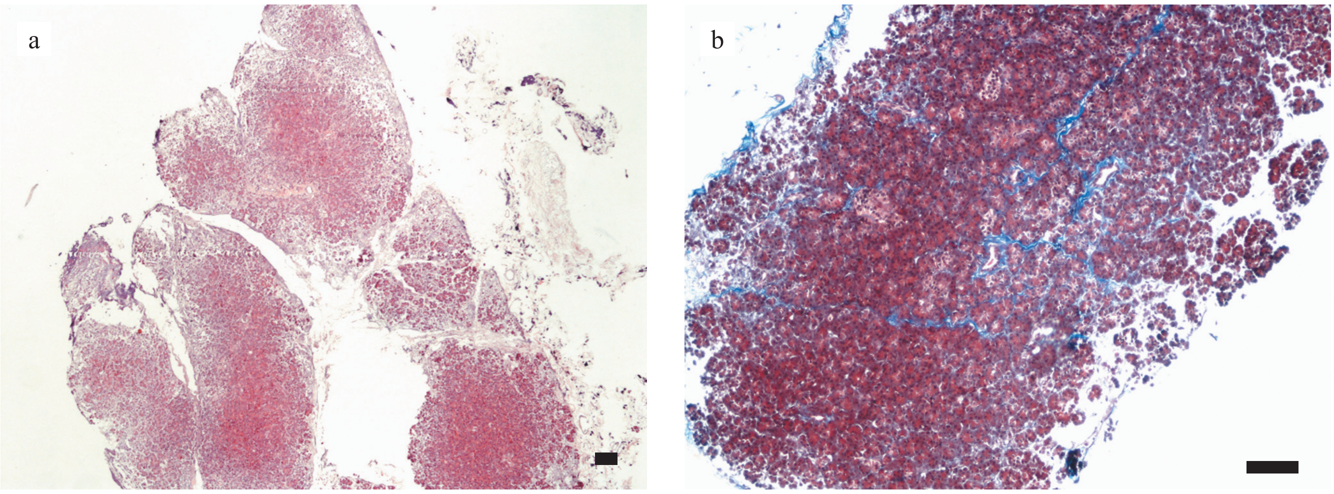

Fig. 4. Pancreatic tissue after enzymatic digestion. (a) H&E staining; (b) Masson's trichrome staining. Scale bar: 100 μm

fibers (stained blue) were identified predominantly in the periductal regions (Fig. 3b, 3d).

Occasionally, isolated lobules contained small focal areas of pancreatocytes with mild dystrophic changes. Numerous well-defined islets of Langerhans were distributed throughout the parenchyma. These islets exhibited a typical rounded morphology and compact structure without signs of pathological alteration (Fig. 3a–d).

Immunohistochemical staining of the main types of islet cells (beta- and alpha-cells) demonstrated positive immunoreactivity for insulin and glucagon in all examined samples (Fig. 3d, 3e). Brown granules of the precipitate abundantly filled the insulin-positive beta-cells, which constituted the major cellular component of the islets (Fig. 3d). The less abundant glucagon-positive alpha-cells were scattered in a mosaic pattern throughout the islet structure (Fig. 3e).

These findings indicate preservation of the structural integrity of the islet apparatus in the examined pancreases and confirm their potential for yielding viable and functionally competent islets.

An assessment of the efficiency of islet yield from the pancreas during enzymatic processing was further supported by morphological analysis of the digested pancreatic tissue. The results demonstrated tissue dissociation within pancreatic lobules, with either complete absence or only minimal residual islets remaining in the processed lobular structures (Fig. 4a, 4b).

A modification of the traditional protocol was introduced to optimize the methodological steps for isolating PIs, with the aim of increasing yield while preserving their morphofunctional integrity. Thus, particular attention was paid to minimizing the adverse effects associated with individual stages of pancreatic processing [25], thereby reducing structural damage and loss of islet viability during isolation.

In the modified approach, the enzyme solution was administered intraparenchymally rather than intraductally. This adjustment reduced ischemia duration and significantly accelerated and facilitated pancreatic tissue distension. Specific skills for intraparenchymal injection ensured uniform distribution of the enzymatic solution throughout the tissue.

Given that certain properties of density gradient media – such as hypertonicity, high viscosity, and possible endotoxin contamination – may adversely affect the morphofunctional state of isolated islets [26], the density gradient purification step was abandoned. Instead, purification was performed using complete growth medium and Hanks' saline solution under controlled centrifugation conditions. The selected centrifugation regime proved to be equivalent and productive due to efficient separation of cellular and tissue components.

We believe that eliminating the potentially undesirable effects associated with density gradient media and avoiding additional islet washing may have a positive impact on islet viability.

Our results were comparable with those reported in similar studies on PI isolation using the Ricordi perfusion system and density gradient during the purification process [5, 14].

In global clinical practice, the established quality criteria for an islet product include a yield exceeding 5,000 islet equivalents per kilogram of recipient body weight, viability of at least 70%, and a stimulation index greater than 1 [14]. The islet grafts obtained using the modified method met these internationally accepted requirements, demonstrating an adequate yield, confirmed cellular identity, high viability, and satisfactory functional activity.

CONCLUSION

As a result of optimizing methodological techniques, a modified protocol for processing the pancreas from deceased donors was validated, enabling the isolation of a substantial number of viable and functionally active PIs.

The mean yield of isolated islets was 630,000 ± 30,000, with a viability of 90 ± 3% and a stimulation index of 1.41 ± 0.01, indicating high-quality preservation of the isolated biomaterial and the ability of beta-cells to respond to changes in glucose levels.

The characteristics of the obtained islet graft show that the resulting cellular material is suitable for transplantation in patients with T1D.

The authors declare no conflict of interest.

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