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Original Article

Allogeneic islet cells implant on poly-L-lactide matrix to reduce hyperglycaemia in streptozotocin-induced diabetic rat☆

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ABSTRACT

Objective: To demonstrate the effects of allogeneic islet cell matrix implants for glycaemic control in rats with induced diabetes.
Method: Sprague-Dawley rats were used as allogeneic donors of islet cells. Cells were seeded on three-dimensional proprietary poly-(L-lactide) matrices. Animals were rendered diabetic and a week later a matrix seeded with islet cells (IMI group) or a control matrix (placebo group) was implanted in the small bowel mesentery. Blood glucose levels were measured weekly for 12 weeks. After sacrifice, implant sections were Gomori stained for beta-cells and immuno-stained for insulin 3, 4, 5, and 6 months post implantation.
Results: 82% of seeded islet cells attached to the matrices. In the IMI group blood glucose levels were significantly reduced after implantation compared with before implantation across several time points. In the IMI group beta-cells and insulin-positive cells were identified at the implant site.
Conclusion: The islet cell matrix implant reduced the blood glucose levels although complete normoglycaemia was not established. The islet cell matrix implant may serve as an additional option for islet cell transplantation using 3D scaffold platforms for better survival and function of the islet cells.

Introduction

Treatment with exogenous insulin not only prevents diabetic mortality but also improves the quality-of-life (QoL) for patients with chronic pancreatitis and diabetes related diseases. However, it does not resolve the long-term complications of hyperglycaemia. Pancreas transplantation is a successful therapeutic approach to end dependence upon regular insulin injections [1] but can be accompanied by high mortality and morbidity after surgery and suitable donor organs are rare [2].

Allogeneic islet cell transplantation, through e.g. intra-portal infusion, is an alternative treatment capable of ameliorating the burden of diabetes and can eliminate the need for exogenous insulin therapy [3]. Despite its many benefits, a five-year follow-up study after clinical islet transplantation revealed a rate of insulin independence of 10% and a median duration of insulin independence of 15 months. These low percentages may be attributed to an

☆ This work was conducted at Tarumanagara Human Cell Technology Laboratory, Faculty of Medicine, Tarumanagara University, Indonesia and Bimana Indomedical Corporation, Bogor, Indonesia.

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insufficient residual islet mass or impaired function of transplanted islet cells [4,5]. The restricted mass of engrafted islet tissue and the gradual but slow vascularization of the engrafted islets may not be sufficient to handle the metabolic demand and might therefore affect islet function over time [6]. The vascular architecture associated with transplanted islets in the liver after engraftment does not represent that seen to be associated with islets in a native setting [4]. Therefore, it is necessary to develop alternative procedures for islet cell transplantation that better maintain the function of islet cells, especially the functional production of insulin by the beta cells, and allow a better glycaemic control in diabetics.

Developments in tissue engineering have shown that biomaterials facilitate extra hepatic transplants by providing a structural support for islet attachment at the implant site for longer periods of time [7]. Moreover, the use of scaffolds has been shown to promote vascularization and to minimize inflammation [8]. Biodegradable matrices are fabricated from synthetic polymers such as poly lactic acid (PLA), poly glycolic acid (PGA), and their copolymers poly lactic acid-co-glycolic acid (PLGA). PLA, PGA and PLGAs are among the polymers that are approved by the FDA as medical devices for clinical applications [9]. These materials are regularly used in "disappearing sutures" and can also be used to provide structural support for cell transplants in addition to aiding generation of a more optimal transplant site. Poly-L-lactide matrices, for example, have been used with success in humans in liver cells co-transplantation with pancreatic cells [10].

The implantation site is another factor that affects the survival and functionality of an implant. Extra-hepatic sites for implantation have been developed, such as the mesentery of the small intestine. An implantation site in the mesentery provides sufficient volume for the transplantation of a large number of matrices carrying the cells as well as a sufficient density of ingrowing vasculature to secure the blood supply [11]. The kidneys, with sub capsular implants, the peritoneal cavity, and the omentum have also been suggested as promising alternative transplant sites in both rodents and humans [12].

Herein we describe a method to seed cells of islets of Langerhans from rats using a specific three dimensional proprietary poly(L-lactide) matrix. The seeded cells are a mixture of alpha and beta cells (capable of insulin production), and delta endocrine cell subsets. In parallel to this method, we have recently performed a modified clinical phase I study in humans where we used isolated hepatocytes co-transplanted with cells of islets of Langerhans to improve the symptoms of liver insufficiency (Baer *et.al*, submitted). The method used in the present work has been modified to allow implantation of islet cells in rats.

In rats, the anatomy of the pancreas differs markedly from that in humans. Rat pancreatic tissue does not form a delineated organ but rather small, scattered, but nonetheless macroscopically distinct, patches in the omentum. Dissection of the tissue most often results in damage of the main bile duct or other organs. For this reason, it is not possible to perform a total pancreatectomy and remove all pancreatic tissue with certainty. In addition, the incidence of diabetes after pancreatectomy in the rat is variable. Kaufmann et al. have reported inter-strain variations in rats that ranged between 15 and 80% [13]. The complicated removal of pancreatic tissue in the rat and the high variability of induced diabetes after pancreatectomy hinders any autogeneic transplant study, therefore we used pancreatic tissue from donor rats of the same but outbred strain, hence this study can be considered as allogeneic.

Materials and methods

Preliminary study

To establish the highest yielding technique for cell harvesting we assessed 3 different islet cell isolation techniques. Harvested cells consist of alpha cells, insulin producing beta cells, and delta endocrine cell subsets. In animals from group A (n = 9), we isolated islet cells without perfusion [10]. Surgical procedures were performed after intraperitoneal anesthesia (ketamine 10%, 40–80 mg/kg and xilazil 2%, 5–10 mg/kg). Under aseptic conditions, the abdominal cavity was opened; the pancreatic tissue was dissected and removed, minced with two scalpels and digested enzymatically with 10 U/mL collagenase type I (Gibco, Grand Island, NY) and 0.01 mg/mL hyaluronidase solution (Sigma Aldrich Co., St. Louis, MO), then sieved (1 mm mesh size) and washed twice with William's E medium (Sigma) completed with 2 mM L-Glutamine (Sigma), 1 mM Na-pyruvate (Sigma), fetal bovine serum 10% (Gibco) and antibiotic-antimicotic solution 1% (Sigma).

In group B (n = 9), we performed *in situ* perfusion with EGTA solution (Sigma) before islet cell isolation as per the method of Gotoh et al. [14] The abdominal cavity was opened and the inferior vena cava was injected with 0.3 mL heparin solution. An intravenous winged needle (27G) was inserted into the portal vein and perfused with 130 mL of pre-warmed (37 °C) and oxygenated 0.5 mM EGTA solution (Sigma), with peristaltic pump (flow rate adjusted to 15 mL/min). Once successful cannulation was confirmed, the inferior vena cava was cut to permit free blood outflow. After the perfusion was completed, pancreas tissue was dissected and the islet cells were isolated as per the method of Gotoh et al. [14] In group C (n = 9), we performed *in situ* perfusion with EGTA and treatment with enzyme solution before islet cell isolation. The perfusion technique was performed as described above. The portal vein was perfused with 60 mL of 0.5 mM EGTA solution, 37 °C, 15 mL/min, followed by 70 mL of 10 U/mL collagenase type IV solution (Gibco Grand Island, NY), 37 °C, 10 mL/min, before the pancreas tissue was dissected.

Main study

Experimental setting

Thirteen outbred male Sprague-Dawley (SD) rats (National Agency of Drug and Food Control, Jakarta) weighing 180–200 g were used as donors and recipients of islet cells. Outbred SD rats maintain diversity at the level of the individual; thus, this study is allogeneic. Recipients included animals that received islet cell matrix implants (IMI group) (n = 16), and matrix implants without cells (placebo group) (n = 16). Recipients were rendered diabetic one week before implantation by single dose intraperitoneal injection of Streptozotocin (STZ) (50 mg/kg bw; Selleck Chemicals). Blood samples were obtained from the tail vein of the animals for glucose assay. Recipients were considered diabetic if after 3 days after the STZ injection the non-fasting blood glucose was >200 mg/dL, and presented with at least 3 days of persistent hyperglycaemia. STZ injection may induce secondary hypoglycaemia due to insulin release associated with acute beta cell destruction to below the expected level, or cause death of the animal [15]. Rats were maintained by administration of glucose solution (5%) when their blood glucose was below 100 mg/dL, or by insulin administration (1–2 units) (Lantus Solostar) when the blood glucose was above 400 mg/dL, until the implantation day, 4 days later.

Two recipient animals were simultaneously injected with STZ. After confirmation of diabetes, they were allocated either to the IMI group or the placebo group. They were allowed free access to standard laboratory food and water and housed individually in cages in a controlled environment (23 °C ± 3 °C, 30–70% humidity, and a 12:12 h light:dark cycle). All surgical procedures were performed by veterinary surgeons of Bimana Indomedical Corporation, (Bogor, Indonesia) according to the guidelines of the Animal Care and Use Review Committee of Bimana Corporation.

Matrix fabrication and preparation

The matrix production process has been described previously [16]. The matrices are made from synthetic polymer poly(L-lactide) (PLA) (Resomer® L 207 S), produced by Boehringer Ingelheim (now Evonik) of Germany. The matrices are three-dimensional circular shaped sponges with a diameter of 20 mm and a thickness of 2 mm. The porosity of the sponge is more than 90% with a pore size of approx. 400 μm (Fig. 1). The matrices were jointly developed by the senior author, the head of the Tarumanagara Human Cell Technology laboratory and the group of J.C. Briceño (Department of Biomedical Engineering, Universidad de Los Andes, Bogotá, Colombia). Matrices were sterilized in the surgical theatre department of the Hirslanden Clinic in Zurich, Switzerland, by plasma sterilization, vacuum packed and delivered to Jakarta, Indonesia. Before use, the hydrophobic matrices were coated with type I collagen (calf skin) to render them hydrophilic and to allow cell adhesion [17].

Individual islet cell isolation, matrix seeding and assessment of viability

In our study we applied the most efficient technique to obtain individual islet cells from healthy donor animals as described in the preliminary study (group B) using a modified *in-situ* perfusion and isolation method [10,14]. The surgical procedures were performed after intraperitoneal anesthesia (ketamine 10%, 40–80 mg/kg and xilazil 2%, 5–10 mg/kg). Under aseptic conditions the abdominal cavity was opened, the portal vein was exposed, penetrated with an IV winged needle, and perfused with 130 mL of 0.5 mM EGTA solution, 37 °C, at 15 mL/min. The pancreatic tissue was dissected and kept in 20 mL sterile PBS solution with 1% antibiotic-antimicotic solution (Sigma) at 2–4 °C. Animals were euthanized by exsanguination after the perfusion by opening the caval vein.

The pancreatic tissue was prepared as described in the preliminary study section. The islet cells were counted and viability was determined with the Trypan blue exclusion assay method [18].

Cell suspensions of 400 μL with a density of ±350,000 cells per 100 μL were pipetted dropwise onto the top surface of each matrix and placed in a well plate. Complete William's E medium was added to each well (50 μL at once, 50 μL the next morning). The cell-containing matrices were incubated at 37 °C, with 5% CO_2 and 95% relative humidity for 3 d. Following incubation, remaining cells not attached to the matrices were counted. The 2 cm diameter matrix was cut into smaller size pieces to fit the dimensions of the small intestine mesenteries of the rat. The small matrices were transferred to a new sterile well plate previously filled with fresh medium.

Matrix implantation

One matrix previously seeded with islet cells was implanted in each animal of the IMI group. Animals of the placebo group received a non-seeded matrix. One hour before surgery, the recipient rats were administered with subcutaneous antibiotics (amoxicillin 150 mg/kg bw) and oral administration of paracetamol 100 mg/kg bw. Anesthesia was induced by intraperitoneal administration of ketamine 10%, 40–80 mg/kg bw and xilazil 2%, 5–10 mg/kg bw.

Under aseptic conditions, a 2-cm midline incision was made. The matrices were placed between the serosal surface of two adjacent mesenteric loops of the small intestine covering both the upper and lower surface of the implant with serosa (Fig. 1c). They were fixed using 6-0 prolene running sutures. Absorbable 4-0 running sutures were used to close the abdominal wall. After surgery, the rats received subcutaneous administration of amoxicillin, 150 mg/kg bw, and oral paracetamol, 100 mg/kg bw, twice a day for five consecutive days.

Blood glucose levels were checked daily (in the morning, before the meal) for one week post implantation and insulin was administered if blood glucose was >400 mg/dL. Two weeks after the implantation, blood glucose levels and body weights were measured weekly thereafter until 12 weeks post implantation.

Histological analysis

Animals were euthanized at 3, 4, 5, and 6-months post implantation (four animals per group and per time point). Euthanasia was performed on deeply anesthetized animals by injection of sodium pentobarbital (200 mg/kg bw, intracardiac). Histological specimens from pancreatic tissue and site of implantation were harvested. Specimens were fixed in 10% formalin, stained with hematoxylin and eosin (HE), Gomori stained for beta cells, and immuno-stained for insulin.

Data analysis and statistical methods

Results are expressed as means ± SD. The normal distribution of the data was assessed using the Shapiro-Wilk test. For multiple comparisons One-Way Analysis of Variance (ANOVA) was used. The significance between two independent groups was determined by independent Student's t-test and the Mann-Whitney test. An unpaired *t*-test was performed if the data were normally distributed. Otherwise, a Mann-Whitney test was used. Statistical analysis was 2-tailed and values were considered statistically significant at $p < 0.05$.

Results

Individual islet cell isolation and implantation

We performed 3 different islet cell isolation techniques to

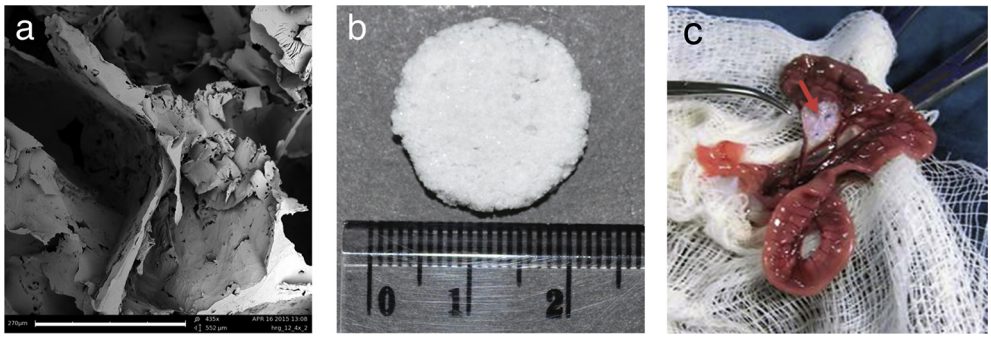

Fig. 1. (a) Scanning electron micrograph of a matrix composed of PLA polymer (production THCT lab according to the Liao et al. method [16]. Scale bar 270 μm. (b) PLA matrix (2 mm thick, 2 cm diameter). (c) Mesenteric loops of the small intestine as matrix implantation site. The matrix is indicated by the red arrow.

determine which method resulted in the highest number of harvested cells. Analysis of the data revealed that the *in situ* perfusion through portal vein with EGTA solution islet cell isolation technique implemented in group B animals recovered the highest number of harvested cells (Fig. 2). Accordingly, this methodology was selected for the main study. Routinely, at least 2.8×10^6 islet cells per pancreas with a viability of 98% were isolated from the donor animals (n = 13), and an average of $1.37 \pm 0.15 \times 10^6$ cells with a viability of 98% were seeded onto the matrices. After 3 days of incubation, 82% of the islet cells seeded onto the matrices remained attached to the matrices. These seeded matrices were implanted in the mesenteric small bowel leaves of each animal in the IMI group.

Blood glucose monitoring

As reference for normo-glycaemia we measured the blood glucose levels of recipient rats before STZ injection and determined 100–140 mg/dL to be normal. The mean blood glucose levels pre-STZ injection were similar in both groups (IMI group: 120 ± 26 mg/dL, placebo group: 117 ± 28 mg/dL, $p = 0.678$). We compared the effect of the STZ injection on blood glucose levels between the IMI and the placebo group (Table 1). The mean blood glucose levels at 3 days post STZ injection was similar in both groups (IMI group: 333 ± 55 mg/dL, placebo group: 340 ± 78 mg/dL, $p = 0.769$). The mean blood glucose level at implantation day was not significantly different between groups (IMI group: 266 ± 73 mg/dL, placebo group: 291 ± 67 mg/dL, $p = 0.335$). Table 1 and Fig. 3 show the compared results of the weekly blood-glucose measurements.

Fig. 3 shows a graphical display of the mean blood glucose levels results over time. Overall, the blood glucose levels of the IMI group were lower than those of the placebo group. We detected statistically significant differences in the 2, 3, 4, 5, and 7 weeks post implantation samples when comparing the two groups. In the IMI group, the blood glucose levels were significantly reduced after implantation at all time points when compared with those measured before implantation ($p < 0.001$ as determined by repeated test ANOVA).

Body weight monitoring

On the day of implantation, the mean body weight of the IMI group was significantly lower compared to the placebo group, (179.6 ± 25.8 g vs. 202.3 ± 34.1 g; $p = 0.04$). Chance and the small sample size may be responsible for the lower body weight in IMI rats. Animals in the IMI group gained body weight throughout the study period significantly when compared to the measured weights at the date of implantation W1 ($p = 0.001$), while in the placebo group there was no significant increment ($p = 0.918$). At 12 weeks post implantation the mean body weight of the two groups did not differ significantly (IMI group: 228 ± 59 g vs. placebo group: 210.9 ± 58.5 g, respectively, with $p = 0.291$).

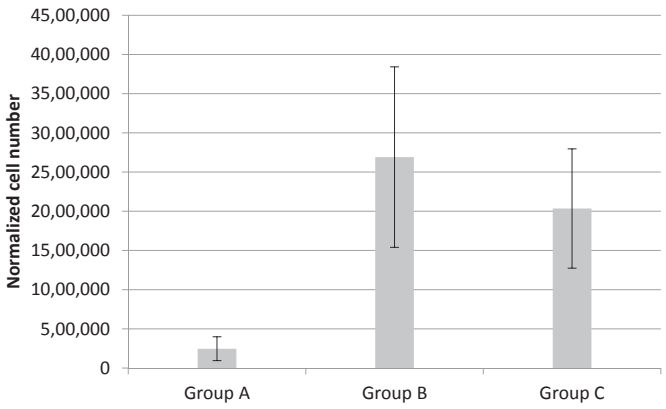

Fig. 2. Comparison of cell yield between the 3 groups (mean ± SD). Cell number represents number of cells per pancreas.

Histopathology analysis

Microscopic evaluation (HE staining) of the pancreatic cells revealed a reduction in the size of the islet of Langerhans in rats treated with Streptozotocin (Fig. 4b) compared with nontreated rats (Fig. 4a), indicating the effectiveness of the Streptozotocin treatment. No other major abnormalities were observed besides the infiltration of a small number of lymphocytes. The mean number of beta cells per islet of Langerhans in non-treated rats was 176 ± 43. The mean number of beta cells per islet of Langerhans was not statistically different in both recipient groups after STZ injection (IMI group: 40 ± 18 vs. placebo group: 47 ± 16, respectively, $p = 0.266$).

Microscopic evaluation of the mesenteric site of the implants in animals of the placebo group revealed regions of the tissue that were predominantly populated by adipocytes. In animals of the IMI group, regions of the tissue were dominated by a population of cuboidal to polygonal shaped cells with relatively clear cell boundaries, a moderate cytoplasm, and a round to oval nucleus. Occasionally we observed 4–10 multinucleated giant cells. No infiltration of mononuclear cells was observed and vascularization was observed around regions of implantation site of the IMI group.

Six-months post implantation, we performed Gomori and anti-insulin staining on dissected tissue from the islet grafts and identified several insulin-stained (brown) beta-cells demonstrating the insulin production capability of implanted beta cells (Fig. 5). To determine the proliferative capability of the implanted cells, we sacrificed animals at 3, 4, 5, and 6 months after implantation and counted the number of beta cells and insulin-positive cells on the site of implantation based on Gomori and immuno-staining. Statistical analysis of the data detected significant differences in neither the mean number of beta cells per field of view at 3, 4, 5, and 6-months post implant ($p = 0.208$) nor in the mean number of insulin-positive cells per field of view ($p = 0.728$) (Table 2).

Discussion

Clinical studies in humans have shown encouraging results following the transplantation of islet cells into the portal vein for the control of glycaemia [19,20]. The median duration reported for insulin independence was of 15 months but longer effects were limited, probably due to inefficient islet cell engraftment [4], inadequate or impaired beta cells, and/or impaired vascularization [6].

To address these limitations, our study focused on the development of 3D poly(L-lactide) matrices to provide a more appropriate attachment site, a methodology to obtain a high number of viable and functional cells for the transplant, and an alternative site for implantation in rats.

A critical success factor in islet cell transplantation is an appropriate isolation method to obtain a high yield of viable and functional islet cells. We isolated individual cells of the islets by *in situ* perfusion through the portal vein with EGTA solution. This method resulted in the highest yield compared with the standard method without perfusion or compared with the *in situ* perfusion through the portal vein followed by collagenase type IV treatment.

In this work, the implanted seeded scaffolds successfully maintained the rat glucose levels below 250 mg/dL for up to 3-

Table 1
Mean differences between groups of blood glucose levels pre and post implantation. Values are represented as means ± standard error of difference of n recipients. IMI group (n = 16). Placebo group (n = 16).

Time point	Mean group difference (Placebo minus IMI group) blood glucose level (mg/dL)
Pre-implant	7 ± 24
Weeks post implant	
1	54 ± 26
2	104 ± 32[a,b]
3	87 ± 43[a,b]
4	100 ± 41[a,b]
5	79 ± 43[b]
6	74 ± 40
7	76 ± 41[b]
8	56 ± 42
9	71 ± 43
10	46 ± 40
11	59 ± 45
12	64 ± 42

[a] Statistical significance calculated using the unpaired *t*-test.
[b] Statistical significance calculated using the Mann Whitney *U* test.

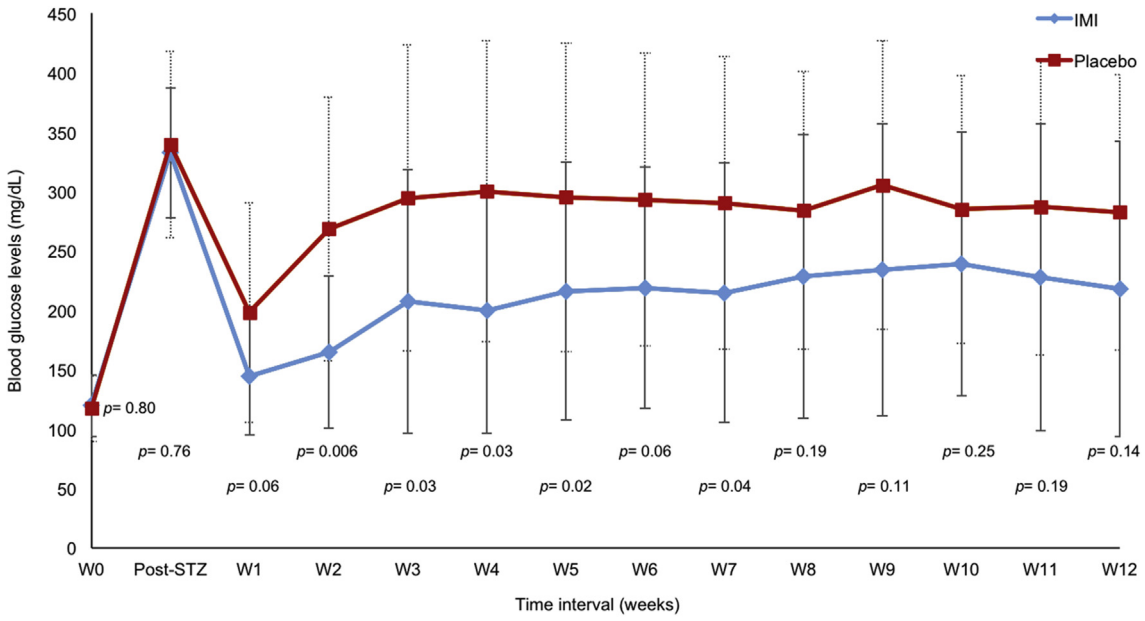

Fig. 3. Comparison of mean blood glucose levels between groups (mean ± SD. Standard deviation for the placebo group is a dotted line). W0 represents the blood glucose measures recorded in the morning before the STZ injection, therefore W0 is defined as normo-glycaemia. Post-STZ represents the glucose level measures three days after STZ injection. Animals received the matrix-implants at week one (W1).

months post implantation. Other studies, using intra portal allogeneic islet transplants, reduced blood levels in diabetic SD rats to 140 mg/dL until 7-days post-transplant, but in later stages blood glucose levels increased to over 250 mg/dL [6]. The cause of this difference may lie on the chosen implant site for the seeded matrix. The microenvironment provided by the surface of the serosa of the small bowel mesentery may be more suitable for immediate oxygen supply and nutrient delivery than the intra portal implant microenvironment. As reported by Kneser et al., cell matrix implants with hepatocytes that were co-seeded with islet cells successfully attached to capillaries within one month, securing vascularization for supply of oxygen and nutrients to the implanted islet cells [21]. In our study, histological examination of the tissue provided evidence for insulin production by the beta cells at the site of the implant and blood glucose levels were reduced. However, the levels we obtained are higher than those of normo-glycaemia. In our experiments we seeded ~1.5 million islet cells per matrix and implanted one matrix per rat. The three dimensional poly(L-lactide) matrices used in our study provided a structural scaffold where implanted cells attached, survived, and maintained their functionality, probably by promoting vascularization towards the implanted cells and offering a favorable microenvironment for the cells. And although we achieved an adhesion rate of 82%, this may still be an insufficient islet cell mass. The adhesion rate depends primarily on the scaffold characteristics, in particular on the density of available sites at which cell binding occurs, which is influenced by the pore size [22]. Therefore, identifying a suitable pore size of the scaffolds may lead to better adhesion rates.

Our specifically designed scaffolds may emulate the extracellular matrix providing an attachment site to the seeded cells and cells of the surrounding tissues. Better adhesion rates result in physical contact between the seeded cells and may encourage intercellular communication and encourage viability. SEM images performed in other investigations and in our own work have confirmed this close contact between implanted cells.

During the first weeks of the study we detected significantly

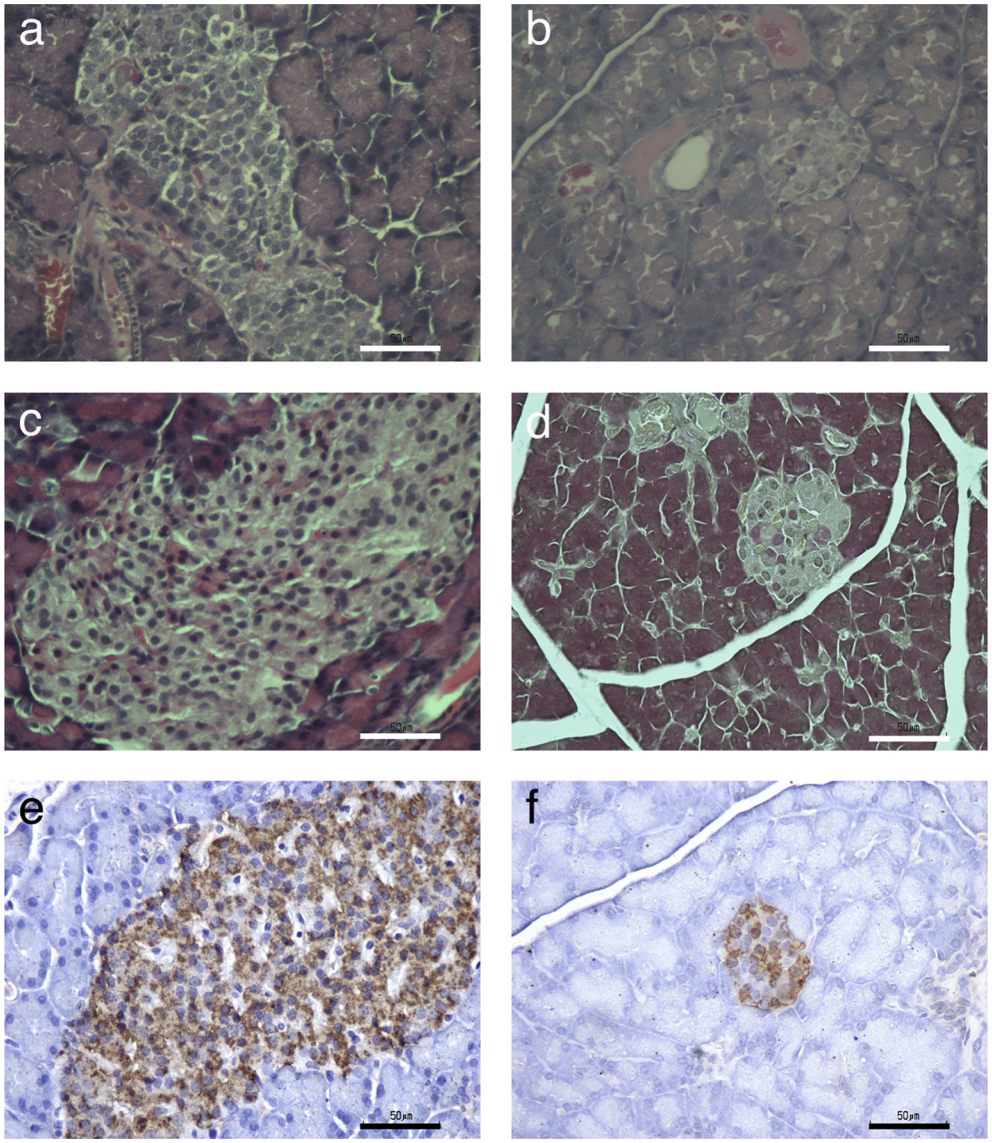

Fig. 4. Histological analysis of islet of Langerhans on non-treated rats (a, c, e) and on STZ-induced diabetic rats (b, d, f); HE staining in panels a and b. Gomori staining in panels c and d. Insulin immunohistochemistry staining in panels e and f. Magnification 40×. Scale bar is 50 μm in all panels.

lower values of glucose in the IMI group when compared to the placebo group. In the second half of the study we found glucose values lower than in the placebo operated rats but not statistically significant. Nevertheless, the lower values of the IMI group and the results from the histological examination demonstrate the presence of functional beta cells.

We cannot determine if cells observed at 3, 4, and 5-month were seeded cells that survived throughout the study period or if these were daughter cells derived from the original seeded cells. The later may be possible if sufficient numbers of these cells attach to the specially prepared matrices and survive the first weeks by diffusive nutrition and proliferate [23]. Beta cells are considered to be highly differentiated cells or end-differentiated cells that are not expected to de-differentiate into a precursor beta cell capable of proliferation. However, latest reports of progenitor cells of adult hepatocytes suggest that they are their own progenitor cells and are not dependent on bone marrow cells [24,25]. The authors suspect that this is also the case for beta cells. This would support our proposed method since seeded beta cells that survive for a longer period of time may start to proliferate. The seeding on the extracellular matrix, or extracellular matrix-like substances, should be further adapted to offer a physical and chemical environment that is similar to the natural environment of cells of islets. These types of cells are not expected to grow easily in the blood stream because they are not hematological cells. They are not adapted to the blood and have a relatively slim chance to survive in the blood stream in an environment deprived of an extracellular matrix.

New developments in the field of tissue engineering and material sciences may lead to better matrix materials with specific cavity dimensions and porosity qualities that are designed to tailor the specific needs of islet cells. Further developments in the science of surface treatment, surface adaptation of scaffold materials within the cavities and pores, may also play an important role in the improvement of the function of the implantable cell matrix products.

The islet cell matrix implant method presented in this study may have clinical implications. It offers the possibility of an autologous islet implant with functional beta cells isolated from a

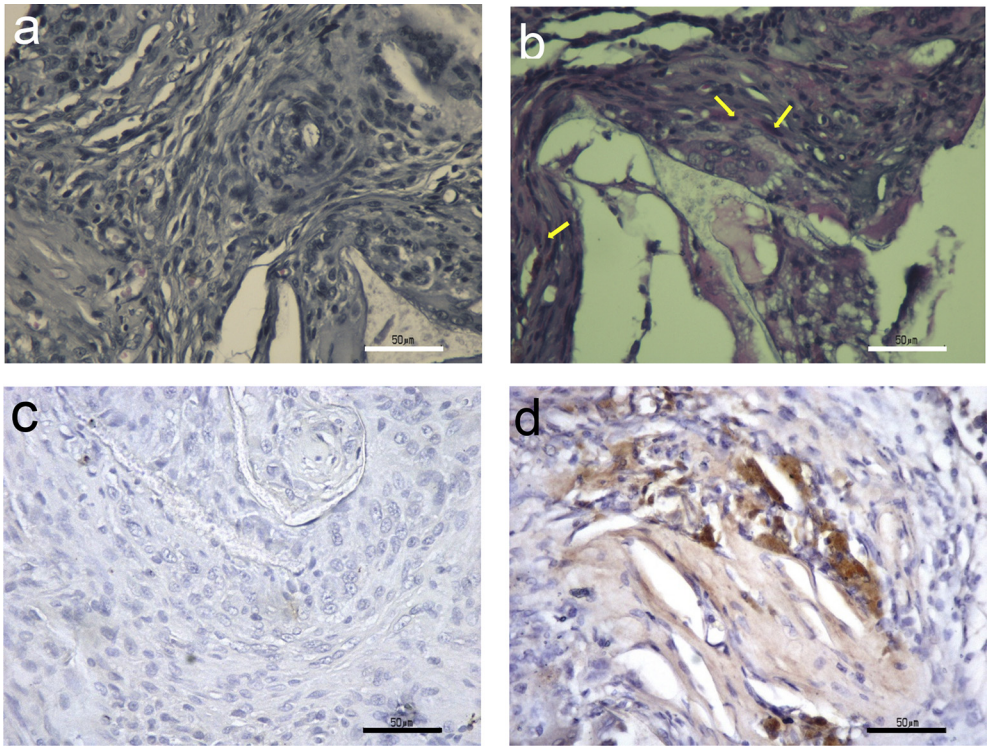

Fig. 5. Histological analysis of the graft site on the small bowel mesentery of the placebo group (a, c) and of the IMI group (b, d). Gomori staining (a, b) and insulin immunohistochemistry staining (c, d). Beta cells are indicated by yellow arrows (b) and insulin-positive cells stained in brown (d) 4-months post implantation, magnification 40×. Scale bar is 50 μm in all panels.

Table 2
Mean number of beta cells per field of view and mean number of insulin-positive cells per field of view on the site of implantation. Four animals from the IMI group were sacrificed at 3, 4, 5, and 6 months after implantation, tissue was Gomori stained for beta cells and immuno-stained for insulin.

Recipient group	Months post implant	Islet graft at implantation site	
		Gomori stained	Immunostained
		Number of beta cells per field of view	Number of insulin-positive cell per field of view
IMI	3	90 ± 30	55 ± 42
IMI	3	135 ± 48	278 ± 65
IMI	3	186 ± 54	23 ± 10
IMI	3	108 ± 29	73 ± 37
IMI	4	181 ± 51	326 ± 81
IMI	4	204 ± 133	6 ± 2
IMI	4	72 ± 34	21 ± 14
IMI	4	107 ± 37	302 ± 69
IMI	5	97 ± 34	220 ± 86
IMI	5	149 ± 20	19 ± 11
IMI	5	114 ± 58	16 ± 11
IMI	5	107 ± 36	268 ± 73
IMI	6	235 ± 132	164 ± 49
IMI	6	140 ± 44	56 ± 23
IMI	6	290 ± 69	11 ± 7
IMI	6	131 ± 45	18 ± 11

patient suffering e.g. from chronic pancreatitis. In these patients partial or total pancreatectomy can be indicated to eliminate the excruciating pain caused by inflammatory cells infiltrating the sheaths of nerves in the inflammatory mass [26]. A study by Chinnakotla et al. showed that a total pancreatectomy combined with islet auto transplantation was efficient in relieving pain and improving quality of life [27]. Moreover, auto transplantations have a better long-term islet graft compared with an allogeneic islet transplant [28].

Our investigation of matrix-supported allogeneic islet cell implantation to control diabetes in rats cannot be directly compared with the clinical results of autologous islet cell implants in humans. However, the IMI method in rats has proven feasible, safe and has resulted in the maintenance of lower glucose levels over 6 months. Moreover, our group has performed a modified clinical phase 1 study using hepatocyte matrix implants with co-implantation of islet cells into the small bowel mesentery in patients with long-standing liver disease. The trial has proven that the matrix implant procedure is safe and feasible (Baer et al. submitted). These results suggest that the use of biodegradable IMI cell implants for the control of hyperglycemia in patients suffering from a loss of pancreatic tissue by chronic pancreatitis may become a viable

treatment in the future.

Diabetes type II, the most common type of diabetes involving 90% of patients, is caused by a reduction of insulin due to a dysfunction of the beta cells combined with insulin resistance. Unfortunately, this IMI method cannot be applied as the success of the treatment relies on the isolation of healthy insulin-producing beta cells. In diabetes Type I, an immunological disorder results in the destruction of the beta cells in a young age. As yet it is not known if allogenic cells could be used but this approach might offer an opportunity.

A 5-year follow-up study using allogeneic islet cell transplantation through intra-portal infusion achieved a rate of insulin independence of 10% and a median duration of insulin independence of only 15 months [4]. We speculated that these results may be attributed to an insufficient residual islet cell mass or an impaired function of transplanted islet cells [5]. In comparison to the study with 5-year follow-up, the duration of our study is short and therefore precludes definitive conclusions on the activity of the islet cells, number, or function during a prolonged period of time. In addition, the number of allogenic islet cells harvested from only one donor rat may be insufficient. The significant increase in body weight in the IMI group may indicate a positive effect of the implants. Yet the weight of IMI rats at the start of the study was lower than the weight of placebo animals, thus preventing conclusions from being drawn regarding the effect of the implants on weight increase. The method of islet cell matrix implantation will benefit from a longer study duration, from a larger number of transplanted islet cells, and from a larger number of study animals. However, the significant reduction of blood glucose levels in the IMI group after implantation, and the survival and insulin-production capability of the implanted beta cells suggests that our cell matrix implants can be used to control glucose levels in diabetic induced rats even in the absence of beta-cell proliferation at the site of the implant. 3D matrices with pores size of ±400 μm are good carriers for islet cells implants. Although complete normo-glycaemia was not established, we observed a reduction of blood glucose levels. Thus, the islet cell matrix implant may serve as an additional option for islet cell transplantation.

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