A Retrievable, Efficacious Polymeric Scaffold for Subcutaneous Transplantation of Rat Pancreatic Islets

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Objective: We aim on developing a polymeric ectopic scaffold in a readily accessible site under the skin.

Summary Background Data: The liver as transplantation site for pancreatic islets is associated with significant loss of islets. Several extrahepatic sites were tested in experimental animals, but many have practical limitations in the clinical setting and do not have the benefit of easy accessibility.

Methods and Results: Functional survival of rat islets was tested during 7 days of culture in the presence of poly(D,L-lactide-co-ε-caprolactone) (PDLLCL), poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer, and polysulfone. Tissue responses were studied in vivo after subcutaneous implantation in rats. Culture on PEOT/PBT and polysulfone profoundly disturbed function of islets, and induced severe tissue responses in vivo. Modification of their hydrophilicity did not change the suitability of the polymers. PDLLCL was the only polymer that promoted functional survival of rat islets in vitro and was associated with minor tissue reactions after 28 days. Rat islets were transplanted in the PDLLCL scaffold in a diabetic rat model. Before islet seeding, the scaffold was allowed to engraft for 28 days to allow the tissue response to dampen and to allow blood vessel growth into the device. Islet transplantation into the scaffold resulted in normoglycemia within 3 days and for the duration of the study period of 16 weeks.

Conclusions: In conclusion, we found that some polymers such as PEOT/PBT and polysulfone interfere with islet function. PDLLCL is a suitable polymer to create an artificial islet transplantation site under the skin and supports islet survival.

Keywords: pancreatic islet transplantation, polymers, rat, scaffold, type 1 diabetes

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Pancreatic islet transplantation is a promising approach for the treatment of type 1 diabetes mellitus.[1,2] Long-term success rates, however, are still too low to merit large-scale clinical application. Some institutes are able to induce insulin independence in 100% of the diabetic immunosuppressed patients, but after 5 years only 50% is still normoglycemic and insulin-independent, whereas the other 50% have to inject insulin again.[1,2] The cause for the low long-term success rates is multifactorial, but inadequacies of the transplantation site have repeatedly been mentioned as one of the causal factors.[3,4] Currently, islets are infused into the liver via the portal vein.[3] Disadvantages of the liver as site for islet transplantation include insufficient vascularization for transplanted islets,[5] low oxygen tension,[6,7] and instant blood-mediated inflammatory reactions.[8] Unfortunately, the human body does not provide other adequate islet transplantation sites.[3] Therefore, a number of groups, including ours, concentrate on designing a polymeric ectopic scaffold that provides a niche for optimal function of islets.[9–14]

Before human application of such a polymer scaffold can be considered, several requirements have to be met. The polymer should not induce severe inflammation, islet toxicity, or a severe foreign body response, and simultaneously support vascularization. These are very pertinent considerations for islet transplantation as there is a severe scarcity of cadaveric donors. Therefore, loss of functionality or viability after transplantation should be reduced to a minimum.[3] Furthermore, preferably polymers already approved by the U.S. Food and Drug Administration (FDA) should be applied to avoid long-term testing procedures and regulatory issues.

In this study, 3 FDA-approved polymer candidates—poly(D,L-lactide-co-ε-caprolactone) (PDLLCL), poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer, and polysulfone—were tested for their compatibility with pancreatic rat islets in vitro and their tissue response in vivo in rats. The hydrophilicity of a material can influence cell morphology and behavior.[15] Therefore, to distinguish the effect caused by the chemistry of the polymer from the hydrophilicity differences, we used a hydrophobic and a hydrophilic counterpart of each polymer. We first determined the effect of the polymers on islet functionality and survival in vitro in the absence of possible interfering tissue responses. Subsequently, the tissue responses and vascularization of the 6 polymers in scaffolds were compared at 2 time points in vivo in rats. This was done not only to select a scaffold with minimal tissue responses, but also to select a time point at which the inflammatory responses associated with surgery and inflammation are dampened.[16] Islets have been shown to be very sensitive for these types of inflammatory responses,[17,18] as illustrated in previous studies demonstrating that up to 60% of the graft can be destroyed during tissue responses against polymers in the immediate period after implantation.[17,19] Finally, to prove that our selected polymer supports islet function, rat islets were transplanted in a subcutaneous-placed polymer scaffold in a diabetic rat model.

METHODS

Polymeric Materials

Polyganics (Groningen, The Netherlands) kindly provided PDLLCL (poly($^{68}/_{32}$[$^{15}/_{85}$ $^{D}/_{L}$-lactide]-co-ε-caprolacton) and PDLLCL-PEG (polyethylene glycol). PEOT/PBT 150 (150PEOT50PBT50) and PEOT/PBT 4000 (4000PEOT30PBT70) were obtained from

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PolyVation (Groningen, The Netherlands). Polysulfone was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) and PEG 20000 from Merck Milipore (Amsterdam, The Netherlands).

Polymer Casting

A 2% (w/w) polymer solution in chloroform of PDLLCL and PDLLCL-PEG (50/50 w/w) were casted in glass petri dishes (40 mm) at Polyganics. Residual solvents evaporated under reduced pressure overnight. PEOT/PBT 150 (13% w/w) and PEOT/PBT 4000 (10% w/w) were dissolved in hexafluoroisopropanol (Sigma-Aldrich, 23% w/w) and chloroform (Merck, 77% w/w). Polysulfone (3% w/w) and polysulfone (2% w/w) with PEG (0.3% w/w) were dissolved in chloroform (100% w/w). These polymer solutions were transferred to glass petri dishes for spin-coating and rotated at high speed using a Convac 1001S spin coater (ST 143 control unit; Convac, Wiernsheim, Germany). Spin-coating resulted in a thin layer (0.5–1.5 mm) of polymer on the bottom of the petri dishes. Until usage, spin-coated dishes were stored at 4°C. All polymer dishes were washed before use by an overnight incubation with Connaught Medical Research Laboratories (CMRL) medium (Gibco; Life Technologies, Bleiswijk, The Netherlands).

Hydrophilicity was quantified by static sessile drop method as described by Rinastiti et al.[20] A custom-made camera-goniometer system was used to photograph and quantify the contact angle. The mean contact angle of 3 samples of each polymer type was determined.

Animals

The Institutional Animal Care and Use Committee of the University of Groningen approved all animal procedures. For our in vitro experiments, male Wistar rats (HsdCpb:WU; Harlan, Horst, The Netherlands) weighing 300 to 380 g served as islet donor. For studying the in vivo tissue responses, 10-week-old male Albino Oxford (AO) rats were used from our own breeding colony. Male AO rats (250–350 g) were also used as islet donors in our transplantation experiment. Male Rowett nude rats (Hsd:RH-Foxn1$^{rnu/rnu}$) of 7 to 10 weeks of age, obtained from Harlan, were used as islet recipients.

Islet Isolation, Culture, Glucose-stimulated Insulin Secretion Test and Viability

Islets were isolated as described by the Serva (Heidelberg, Germany) protocol.[21,22] Islets from Wistar rats were cultured for 7 days on the polymer-coated petri dishes in CMRL containing 8.3 mM glucose (Sigma-Aldrich), 20 mM Hepes (Gibco), 2 mM Glutamax (Gibco), Penicillin Streptomycin (50 U/mL–50 μg/mL, Gibco), and 10% fetal calf serum (Thermo Scientific, Breda, The Netherlands) at 37°C and 5% CO_2. Islets in conventional suspension culture served as controls. At day 1, 3, and 7 of culture, the functional survival of the islets was measured.

A glucose-stimulated insulin secretion (GSIS) test was applied to test functionality as previously described.[18,23] To determine islet viability, 25 islets were incubated with 1 mM calcein AM and 2 mM ethidium bromide (live/dead staining kit Molecular Probes; Life Technologies) as previously described[24] and studied. Stained islets were scanned by confocal microscopy (Leica SP2; Leica Microsystems B.V., Rijswijk, The Netherlands). Image J (1.47) was used to quantify the percentage of viable cells and dead cells.

PDLLCL Scaffold

A 4% (w/v) PDLLCL solution was prepared in chloroform (Sigma-Aldrich). To create a porous structure natriumchloride particles (Sigma-Aldrich) of 250 to 425 μm were added to the PDLLCL solution (10:1 w/w). After evaporation of the solvent, the salt particles were removed by washing with sterile water. The polymer sheet was resized into 10 mm by 15 mm rectangle scaffolds.

Surgery

To study the in vivo tissue responses, polymer sheets with a thickness of 1 mm, a width of 10 mm, and a length of 10 mm were implanted in subcutaneous pockets on the back of AO rats (6 polymers per rat, 2 time points, 6 rats per time point). The polymer sheets were implanted to the left and right about 1 cm from the incision site. The polymer sheets plus surrounding tissue were dissected from the subcutaneous pocket at day 14 and 28 after implantation.

For islet transplantation, Rowett nude rats were used. These rats have a normal innate immune system and foreign body response against the scaffold, but have no adaptive immunity preventing an immune response against the allogenic islet graft. This allows for sound interpretation of the survival of islets in the scaffold in the absence of a rejection process. The nude rats were made diabetic with 60 mg/kg streptozotocin IV (Sigma-Aldrich) injected via the tail vein. After 2 consecutive blood glucose measurements of 20 mmol/L or higher during a period of 14 days, the rats received a Linplant insulin tablet (LinShin, Scarborough, Canada). Four weeks before islet transplantation, PDLLCL scaffolds were subcutaneously implanted on the back of the diabetic rats to allow vascularization of the scaffold ($n = 4$). At the day of transplantation, a small incision was made at the side of the scaffold. A 25 G needle was used to create channels in the scaffold for the islets. Immediately after islet isolation from AO rats, an islet graft of 9.9 ± 1.6 μL (3456 ± 236 islets) was divided over the channels with a Hamilton syringe (VWR International B.V., Amsterdam, The Netherlands).

To compare the performance of the scaffold with a well-established islet transplantation site in rodents, islets were transplanted under the kidney capsule of diabetic rats ($n = 6$).[25] Briefly, a small incision was made in the skin and the peritoneum, just above the kidney, to access the kidney capsule. With a needle an opening in the kidney capsule was created, subsequently a glass rod was used to obtain a subcapsular pocket under the upper pole. Islets were carefully placed in the subcapsular pocket with a Hamilton syringe. Control rats were transplanted with an islet graft of 11.3 ± 1.5 μL (3313 ± 253 islets).

Blood glucose concentrations were determined using the Accu-Chek Sensor system (Roche, Mannheim, Germany). Transplantation was considered successful when nonfasting blood glucose concentrations reached levels below 10 mmol/L. At the end of the experiment, scaffolds containing the islet grafts were removed for histological examination. In all animals pancreas regeneration was excluded by histology. In some, selected animals blood glucose levels were measured for 1 week after removal of the islet graft to confirm graft dependency of normoglycemia.

Histology

Polymer sheets or islet-containing scaffolds were fixed in 2% paraformaldehyde and were embedded in glycol methacrylate (GMA, Technovit 7100; Heraeus Kulzer GmbH, Wehrheim, Germany) for histological analysis.[26]

GMA blocks were sectioned at 2 μm and stained with 1% (w/v) aqueous toluidine blue for 10 seconds. Quantification of the degree of inflammation was done using a similar scoring system to those previously described.[27,28] All sections were viewed first and subsequently graded, using a semiquantitative scoring system with 5 grades by 3 technicians: 0 (no positive cells) to 4. The severity of inflammation was scored based on the number of cells with morphological characteristics of monocytes/macrophages, lymphocytes, granulocytes, fibroblasts, basophils, erythrocytes, and multinucleated giant cells. When no significant cell adhesion was observed, the inflammation was scored as 0. Vascularization was expressed quantitatively by measuring the median vessel diameter and semiquantitatively based on the density of blood vessels. Blood

vessels can be easily recognized in GMA-embedded sections as the morphology is well conserved by this method of embedding. Blood vessels are recognized by the presence of erythrocytes in the lumen and by the characteristic morphology of endothelial cells of the vessel wall. The density of blood vessels was graded based on the quantity of observed large and small blood vessels in the proximity of the polymer using a scoring system with 5 grades: 0 (no blood vessels) to 4.

For insulin staining sections were incubated for 2 hours at 37°C with the primary anti-insulin antibody (Sigma-Aldrich, Mouse-anti-Rat-IgG1) in a 1:300 dilution (PBS + 1% BSA). After several washes, 10% normal rabbit serum was used to block nonspecific binding of the secondary antibody. Sections were incubated for 45 minutes at 20°C with the secondary rabbit antimouse alkaline phosphatase-conjugated antibody (Dako, Heverlee, Belgium) in a 1:100 dilution (PBS + 1% BSA). Alkaline phosphatase activity was demonstrated by incubating the sections for 10 minutes with SIGMAFAST Fast Red (Sigma-Aldrich). Nuclei were counterstained with hematoxylin. Sections were analyzed using a Leica DM 2000 LED microscope with a Leica DFC 450 camera (Leica Microsystems B.V.).

Statistical Analysis

Data were tested for normality by the Shapiro-Wilk normality test ($P < 0.05$). For statistical analysis of the contact angles, an unpaired t test with Welch's correction ($P < 0.05$) was applied. Values are expressed as mean ± SD. As the insulin secretion, the inflammation score, blood vessel score, and the diameter of the blood vessels were not normally distributed, a Mann-Whitney U test ($P < 0.05$) was performed to test statistical significant differences. The cell death data were also not normally distributed; statistical significant differences were tested with a Kurskal-Wallis test with a Dunn's post hoc test ($P < 0.05$). All the nonparametric values are expressed as median ± interquartile range.

RESULTS

Only PDLLCL Does Not Disturb Islet Function During Culture

First, the effect of polysulfone, PDLLCL, and PEOT/PBT 4000 on the function of rat islets was studied by means of a GSIS test (Fig. 1, left side). Islets cultured in nontreated culture plates served as control. At the first day after isolation, no statistical significant differences could be observed between the control islets and islets cultured on the polymers. This changed on the third day with PEOT/PBT 4000; rat islets cultured on PEOT/PBT 4000 lost their ability to respond adequately during a GSIS. The rat islets cultured on PEOT/PBT 4000 demonstrated a disturbed, higher insulin response ($P < 0.001$). On day 7, the functional impairment of the rat islets cultured on PEOT/PBT 4000 was even more pronounced ($P < 0.05$). Also, rat islets cultured on polysulfone showed statistical significant functional impairment ($P < 0.05$). Islets cultured on PDLLCL retained adequate islet function during the whole culture period of 7 days.

Hydrophilicity of the Polymer Only Impacts Rat Islets on Polysulfone

The hydrophilicity of the polymers was determined by measuring the contact angle. The contact angle of PDLLCL was 85.7° ± 4.8; therefore, PDLLCL can be considered hydrophobic (Table 1). Polysulfone was also hydrophobic because the contact angle was 78.0° ± 1.4. The contact angle of the hydrophilic PEOT/PBT 4000 was 53.0° ± 0.8. It might be argued that the differences in GSIS are caused by differences in hydrophilicity of the applied polymers rather than by differences in chemical structure and interfere with functionality and morphology.[15] During culturing, islet morphology also indicated differences between PEOT/PBT 4000 and PDLLCL (Fig. 2).

To exclude that the differences in hydrophilicity does influence the observed morphology and GSIS anomalies, the above-described experiment was repeated with polymers with adjusted hydrophilicities (Table 1). The hydrophilicity of the surface was adjusted by adding PEG in the casting process. This resulted for PDLLCL and polysulfone in a reduction of the contact angle to, respectively, 43.3° ± 2.9 ($P < 0.001$) and 45.5° ± 2.4 ($P < 0.001$), which are both in the hydrophilic range. An exception was PEOT/PBT; PEOT/PBT 150 was applied to repeat the experiment with a different hydrophilicity. The contact angle of PEOT/PBT 150 was 82.3° ± 2.6.

The repeated GSIS tests showed that the change in hydrophilicity had no effect on the functionality of the rat islets when cultured on PDLLCL-PEG (Fig. 1, right side). Only with polysulfone, an improvement was observed when the hydrophilicity was adjusted.

Cell Loss in Rat Islets Cultured on Polymers

As of day 1, a statistically significant ($P < 0.05$) higher percentage of dead cells was observed in islets cultured on PEOT/PBT 4000, 6.2% (4.8–12.0) compared with 1.2% (0.6–1.3) at day 1 of control islets (Fig. 3). After 3 days of culture, this effect was still present; islets cultured on PEOT/PBT 4000 showed 7.1% (2.4–10.4) dead cells, whereas in control islets 0.3% (0.3 – 0.4) dead cells were found. At day 7, this effect was diminished. PDLLCL, PDLLCL-PEG, PEOT/PBT 150, polysulfone, and polysulfone-PEG did not enhance cell death when compared with the control conditions.

Tissue Responses Against the Polymers

As shown above, PDLLCL maintained the ability of rat islets to normally respond to a glucose load, whereas islets on other polymers lost this ability. However, another important prerequisite for application is that the tissue responses against the polymers are not too severe as islet cells are very sensitive for inflammation.[29] Vascularization should also be optimal to allow immediate revascularization and minimal loss of islets after implantation.

Tissue responses were studied at days 14 and 28 after implantation under the skin. Both the conventional polymers and the polymers with adjusted hydrophilicity were tested as hydrophilicity might influence the cell adhesion[15] and vascularization. As shown in Figure 4, all polymers induced a tissue response. However, there were clear differences in the severity of the response between the polymers. At day 14, at the interface of the polymer and the subcutaneous tissue, PDLLCL showed a statistical significant ($P < 0.05$) lower inflammation score of 2 (0–2) than the polymers PDLLCL-PEG, PEOT/PBT 4000, PEOT/PBT 150, and polysulfone-PEG (Fig. 4A–G). In some cases, cells did not even attach to PDLLCL. Addition of PEG to PDLLCL to create a more hydrophilic polymer resulted in a more severe tissue reaction. After 28 days of implantation, the response around the polymer interface was diminished (Fig. 4A). Only fibroblasts were found and virtually no immune cells. The tissue response to PDLLCL of 0.5 (0–2.5) was statistically significantly ($P < 0.05$) less severe than the response against PEOT/PBT 4000 [4 (3–4)].

The vascularization degree of the polymers was determined by measuring the diameters of the blood vessels (Fig. 5A) and scoring the density of blood vessels (Fig. 5B). After 14 days vascularization had already occurred and was similar in and around the polymers. Only the diameter of blood vessels from polysulfone-PEG increased significantly ($P < 0.05$) from day 14 to day 28 of implantation. The density of blood vessels doubled over time from day 14 until

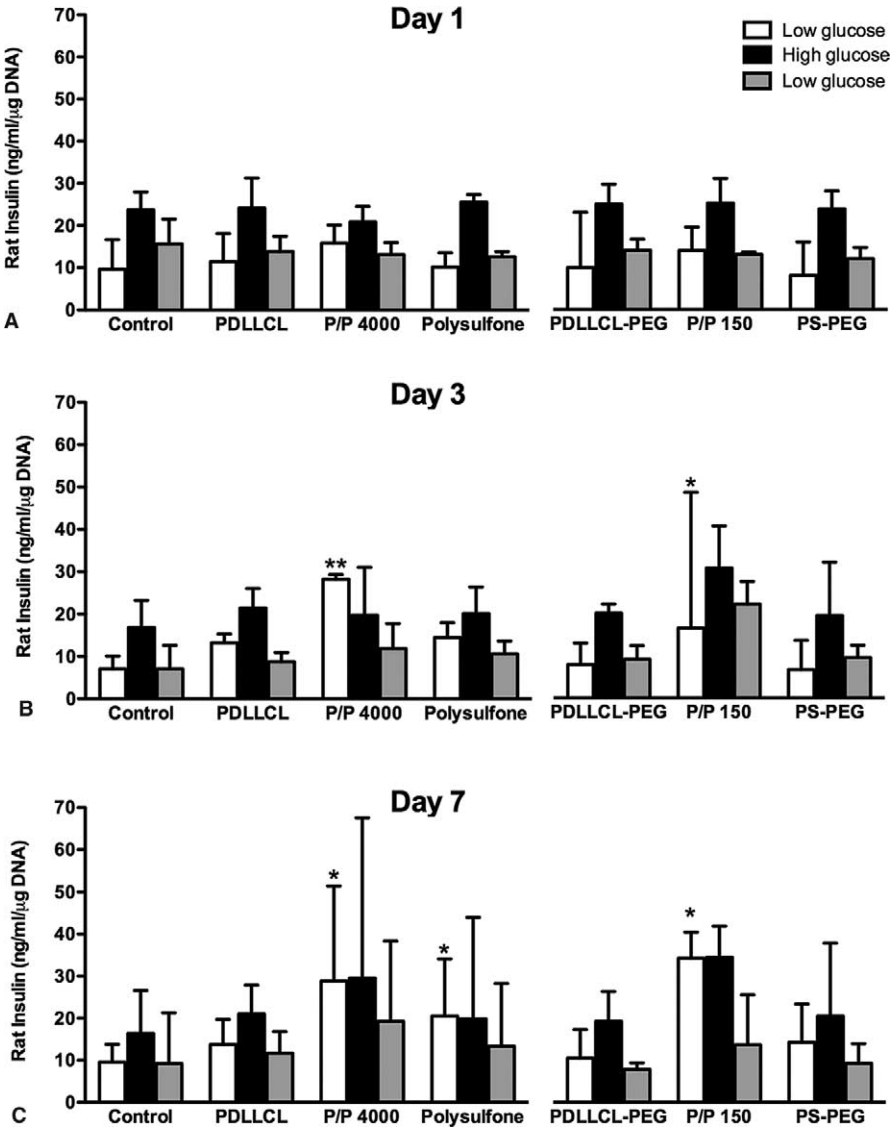

FIGURE 1. Glucose-stimulated insulin secretion of rat islets after culturing on PDLLCL, PEOT/PBT, and polysulfone. Glucose-stimulated insulin secretion of rat islets (ng/mL per μg DNA) after 1 (A), 3 (B), and 7 (C) days of culture on PDLLCL, PEOT/PBT 4000 (P/P 4000), and polysulfone compared with conventional culturing (control) (left side of the graph). The white bars indicate the amount of insulin secreted in the first low glucose (2.75 mM) incubation, the black bars the amount of insulin secreted during high glucose (16.5 mM) incubation, and the grey bars the return to basal insulin secretion during a second low glucose (2.75 mM) incubation. The right side of the figure depicts insulin secretion when cultured on polymers with adjusted hydrophilicity; PDLLCL-PEG, PEOT/PBT 150 (P/P 150), and polysulfone-PEG (PS-PEG). Median and interquartile range of insulin secretion are plotted (n = 5), a statistical analysis was carried out using a Mann-Whitney *U* test, $^{*}P < 0.05$ and $^{**}P < 0.001$. The amount of insulin produced in the low glucose incubation of the control condition was compared with the low glucose incubations of the different polymers; the same was done for the high glucose incubations.

28 (Fig. 5B). This increase was observed for all polymers, but was statistically significant ($P < 0.05$) only for polysulfone. Because after 28 days the majority of infiltrating immune cells were gone and many blood vessels were found, it was decided to take 28 days of engraftment for the scaffolds before implanting islets in the subsequent proof-of-principle experiment.

Implantation of Rat Islets in PDLLCL Scaffold

To obtain a final proof of concept, we subcutaneously implanted rat islets in PDLLCL scaffolds. Transplantation of rat islets in the PDLLCL scaffold and under the kidney capsule induced normoglycemia within 3 days. The rats remained normoglycemic up to 4 months after transplantation, which was the end point of this study (Fig. 6A). At the end of the experiment, grafts were retrieved and histologically examined. Many insulin positive islets were found in the polymeric scaffolds (Fig. 6B). The islets maintained their normal spherical shape and were found scattered throughout the device and in close contact with the polymers. Also, we found smaller aggregates of 2 to 5 insulin-positive cells in close proximity of the polymers. We did not find any inflammatory cells near or in the islets. Nor did we find any signs of an ongoing foreign body response

TABLE 1. The Contact Angles of PDLLCL, PEOT/PBT, and Polysulfone Measured With Static Sessile Drop Method

Polymers	Contact Angle, °	Standard Deviation	Significance *t* Test
PDLLCL	85.7	4.8	$P < 0.001$
PDLLCL-PEG	43.3	2.9	
PEOT/PBT 150	82.3	2.6	$P < 0.001$
PEOT/PBT 4000	53.0	0.8	
Polysulfone	78.0	1.4	$P < 0.001$
Polysulfone-PEG	45.5	2.4	

Differences between the contact angles were analyzed using an unpaired *t* test with Welch's correction ($P < 0.05$).

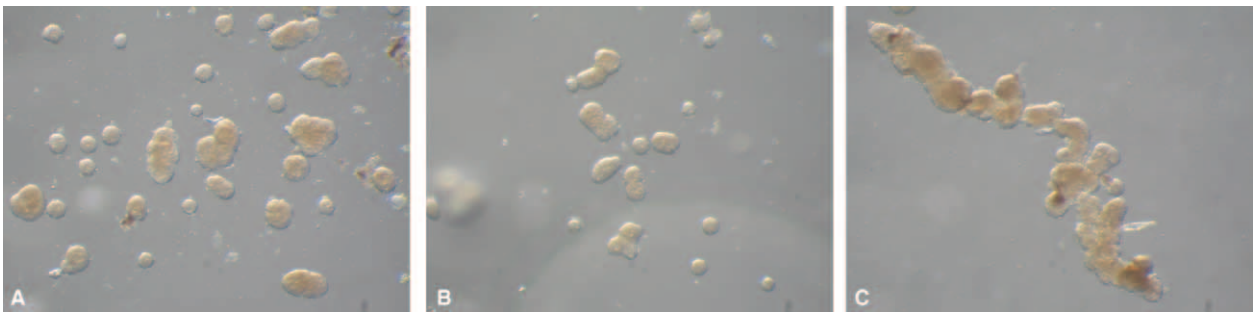

FIGURE 2. Islet morphology during 7 days of culturing. The morphology of conventional cultured islets (A) was different from islets cultured on PDLLCL (B) and PEOT/PBT 4000 (C). Islets morphology of PDLLCL was comparable to the conventional cultured islets. Islets of PDLLCL were spherical and showed minor aggregation, whereas islets cultured on PEOT/PBT 4000 formed large aggregates.

against the scaffold. After removal of the islet grafts, rats returned to their pretransplant hyperglycemic state within 3 days.

DISCUSSION

Here we show a profound polymer chemistry-dependent effect on the functional survival of rat islets. Islets are extremely sensitive organelles equipped with many types of integrins and pattern recognition receptors,[30] and need interaction with the surrounding microenvironment to function optimally. These interactions should not lead to any dysregulation of cellular processes[30,31] associated with loss of islet viability or function, as islet tissue is isolated from relatively rare cadaveric donors. The crucial factors contributing to optimal biomaterial-islet cell interactions remain to be identified, but it can be explained by the fact that every integrin receptor and pattern recognition receptor needs a different ionic and/or hydrophobic interaction.[32]

Islet transplantation into an unmodified subcutaneous site has never reversed diabetes in animals or humans, as the microenvironment is inhospitable to cell survival owing to poor oxygen tension and inadequate vascularization. Stimulation of angiogenesis is critical to successful subcutaneous islet transplantation.[9] Here we used polymeric scaffold to induce vascularization and to

Polymers	Day 1	Day 3	Day 7
Control	1.2 (0.6 – 1.3)	0.3 (0.3 – 0.4)	2.8 (1.0 – 4.1)
PDLLCL	2.4 (1.9 – 2.5)	1.6 (0.9 – 2.0)	1.7 (1.2 – 4.0)
PDLLCL-PEG	1.4 (0.3 – 3.2)	0.7 (0.4 – 2.1)	2.2 (2.0 – 2.2)
PEOT/PBT 4000	6.2 (4.8 – 12.0) *	7.1 (2.4 – 10.4) *	4.3 (3.8 – 8.1)
PEOT/PBT 150	2.7 (1.6 – 3.0)	3.2 (1.4 – 4.6)	4.8 (2.1 – 13.1)
Polysulfone	3.9 (2.5 – 4.6)	2.3 (2.0 – 7.1)	3.7 (1.5 – 4.5)
Polysulfone-PEG	3.0 (2.4 – 3.6)	1.0 (0.7 – 2.5)	2.0 (2.0 – 4.1)

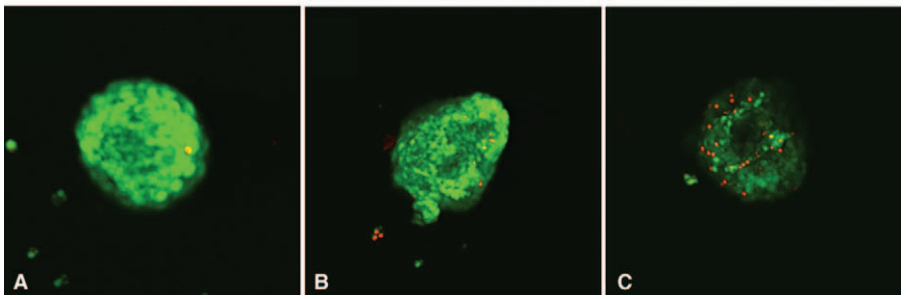

FIGURE 3. Cell death in rat islets after culturing on PDLLCL, PEOT/PBT, and polysulfone. The percentage of dead cells in rat islets during 7 days of culture on PDLLCL, PDLLCL-PEG, PEOT/PBT 4000, PEOT/PBT 150, polysulfone, and polysulfone-PEG compared with conventional culturing (control). Median of the percentage and interquartile range are shown in the table (n = 5); a statistical analysis was carried out to compare the control with the polymers using a Kruskal-Wallis test with a Dunn's post hoc test, $^{*}P < 0.05$. The images (10×) represent the cell death percentages of a control islet (A), islets cultured for 1 day on PDLLCL (B), and on PEOT/PBT 4000 (C). Viable cells are stained green, dead cells are stained red.

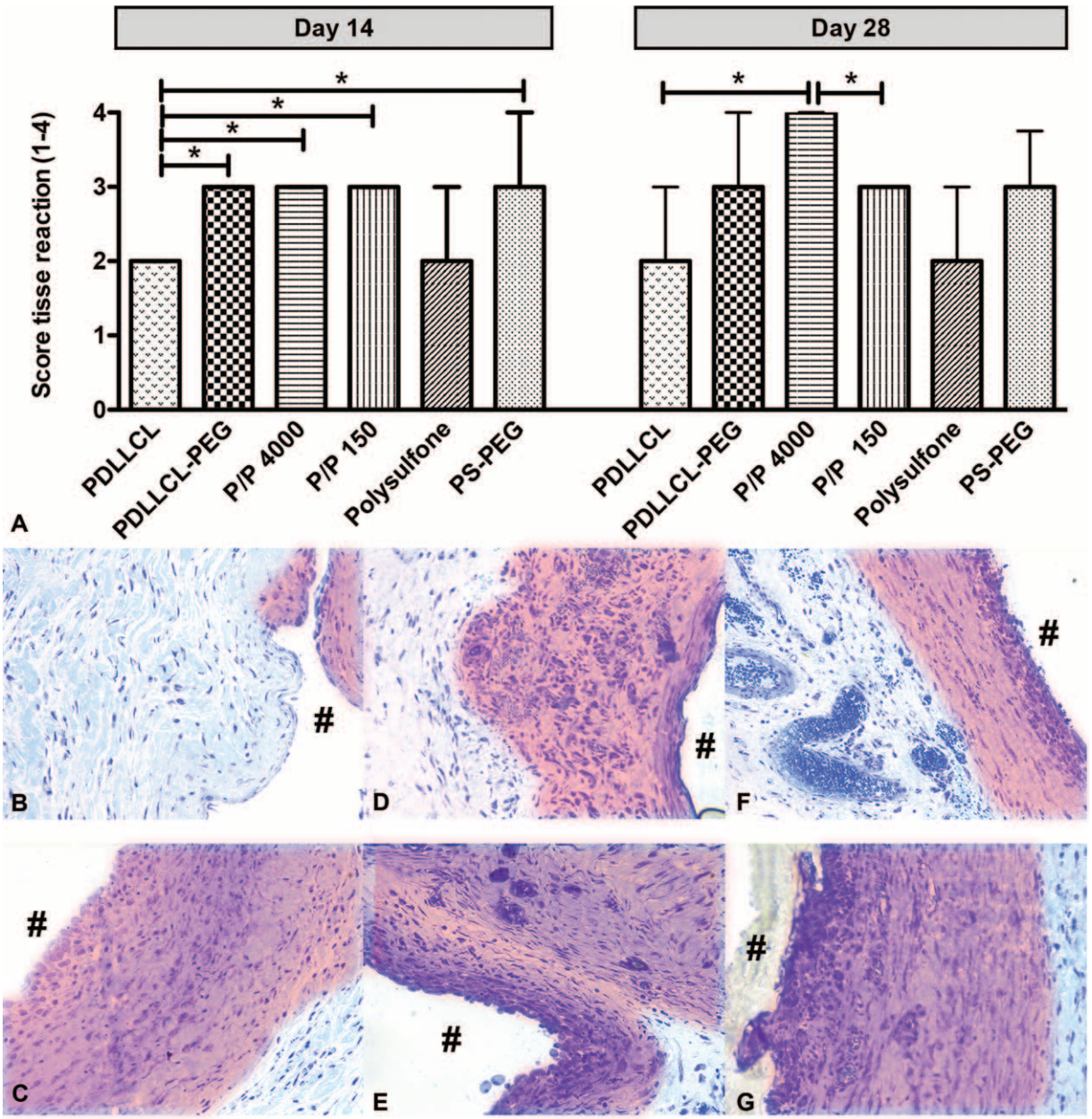

FIGURE 4. Tissue responses against PDLLCL, PEOT/PBT, and polysulfone in AO rats. The severity of the tissue reaction at the interface with the polymers PDLLCL, PDLLCL-PEG, PEOT/PBT 4000 (P/P 4000), PEOT/PBT 150 (P/P 150), polysulfone, and polysulfone-PEG (PS-PEG) scored on a scale from 0 (no reaction) to 4 (severe reaction) after 14 and 28 days of implantation (A). Low magnification images (20×) of toluidine blue staining to depict the inflammation score at the polymer interface from PDLLCL (B), PDLLCL-PEG (C), P/P 4000 (D), P/P 150 (E), polysulfone (F), and PS-PEG (G) at day 14. The tissue response is marked by the red area and the place of the polymer with the hash sign (#). Median and interquartile range are plotted, statistical analysis was carried out using a Mann-Whitney *U* test (n = 6), $^{*}P < 0.05$.

create an islet-friendly environment in the subcutaneous site. The subcutaneous site is readily accessible and will be associated with minor surgery,[33] and is therefore to our opinion not only applicable for islet tissue but also for other emerging insulin-producing cell populations such as embryonic stem cells.[34] The use of these alternative insulin-producing cell populations is associated with potential teratoma formation,[35] malignant transformation,[36] or disturbed hormone release.[37] Our design allows immobilization of the cells, is retrievable, and allows replacement if required.

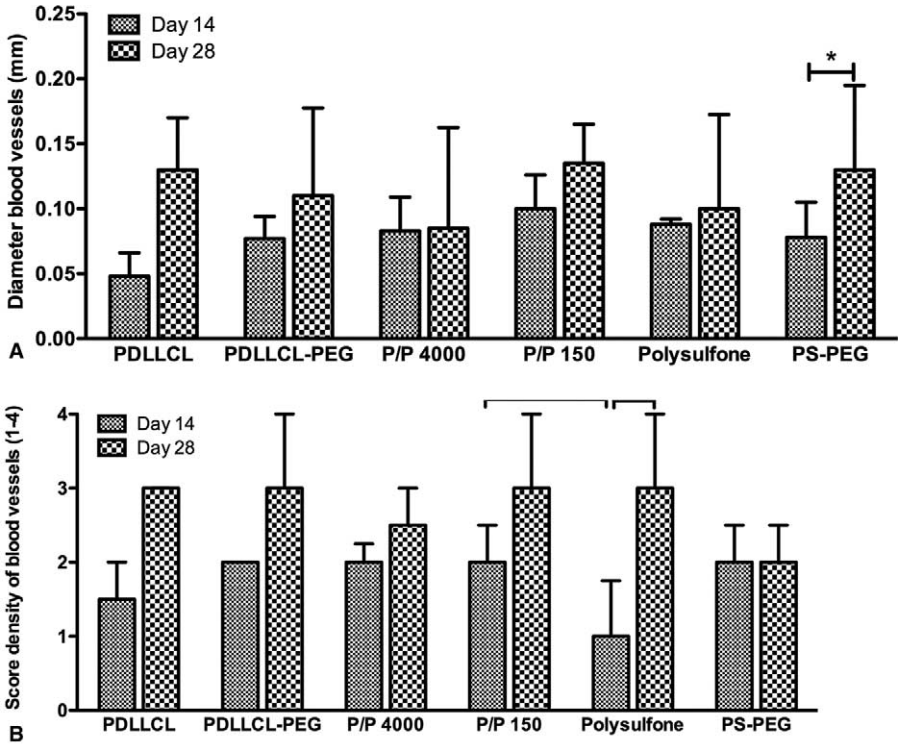

FIGURE 5. Vascularization of PDLLCL, PEOT/PBT, and polysulfone in vivo. The diameter of blood vessels in millimeters (A) after subcutaneous implantation of PDLLCL, PDLLCL-PEG, PEOT/PBT 4000 (P/P 4000), PEOT/PBT 150 (P/P 150), polysulfone, and polysulfone-PEG (PS-PEG) for 14 and 28 days. The density of blood vessels (B) scored on a scale from 0 (no blood vessels) to 4 after 14 and 28 days of implantation. Median and interquartile range are plotted; statistical analysis for the inflammation score was carried out using a Mann-Whitney *U* test (n = 6), $^{*}P < 0.05$.

In this study, rat islets were used instead of human islets because we were able to isolate a more standardized quality of islets from rats with much less interindividual differences than with human islets.[38] In this way, we could investigate the effect of the polymers on functional islet survival in a standardized manner. As shown, only PDLLCL did not interfere with function or survival of pancreatic rat islets; PEOT/PBT impaired functional islet survival to the largest extend, though polysulfone had intermediate effects. The basal insulin levels were disturbed, and the number of dead cells was elevated with PEOT/PBT 4000 even after 7 days illustrating long-term incompatibility of islets cells to interact with this biomaterial. The rearrangements in islets induced by the biomaterials were also visible in the morphology of the islets. Islets were having a more loose structure, and increased clumping of islets was observed when cultured on PEOT/PBT polymers. This clumping is undesired as it is associated with cell death induced by necrosis and release of danger-associated molecular patterns[24] that, as recently shown, induce rejection of islets.[39]

A similar study was performed with human islets.[40] Human islets also performed better on PDLLCL when compared with PEOT/PBT and polysulfone. The alpha- and beta-cells of islets cultured for 7 days in contact with PDLLCL contained more glucagon and insulin granules than the islets cultured on the other polymers as shown with electron microscopy. Furthermore, islets cultured on PDLLCL released statistically significant lower amounts of double-stranded DNA (dsDNA) when compared with the other polymers. The release of dsDNA is not only associated with cell loss, but dsDNA is also a danger-associated molecular pattern that provokes immune activation.[24,41]

Not only the polymer-islet interaction determines the success of a scaffold but also the immune responses against the polymers. In previous studies we have repeatedly found that the tissue responses associated with implantation of biomaterials are associated with significant loss of islets and early failure of the grafts.[42–44] This is the main rational for our group to focus on preimplanted, prevascularized grafts in which immune responses either are minimal or have disappeared at the moment of transplantation of pancreatic islets. The subcutaneous site is, compared with other sites such as the peritoneal cavity, an exceptionally cumbersome site for islets as it is characterized by a low vascularization degree and high proinflammatory responses.[45–47] Therefore, all polymers tested for compatibility with islet survival and functionality in vitro were tested for in vivo inflammatory responses. The degree and dynamics of vascularization in vivo were also investigated.

We showed that PDLLCL does not interfere with functional survival of islets and that a pretransplant period of 28 days suffices for dampening of inflammatory responses and to allow prevascularization. We applied a dose of 10 μL of islet tissue, which is the equivalent of the endocrine volume of the rat pancreas.[48] Animals became readily normoglycemic and remained so until the end of the study period of 4 months. Large numbers of islets were found in the scaffold. By the stepwise approach, we underpin the need for careful selection of suitable polymers for the formation of scaffolds for islet transplantation. Several properties of the polymer, such as their effect on functional survival and biocompatibility, influence their applicability as scaffold material.

PDLLCL met all the required properties for human application of a polymer scaffold for islet transplantation, whereas PEOT/PBT and polysulfone did not meet the preset requirements for such a scaffold. PDLLCL seems to be a suitable scaffold candidate for the treatment of type 1 diabetes. Upscaling and testing the efficacy for application in large mammals and humans is warranted for future clinical application. The PDLLCL scaffold can be easily enlarged to transplant a sufficient islet mass under the skin. However, with increasing the size detailed studies on vascularization have to be repeated. It can be predicted that the dynamics of ingrowth of blood vessels will be different and that

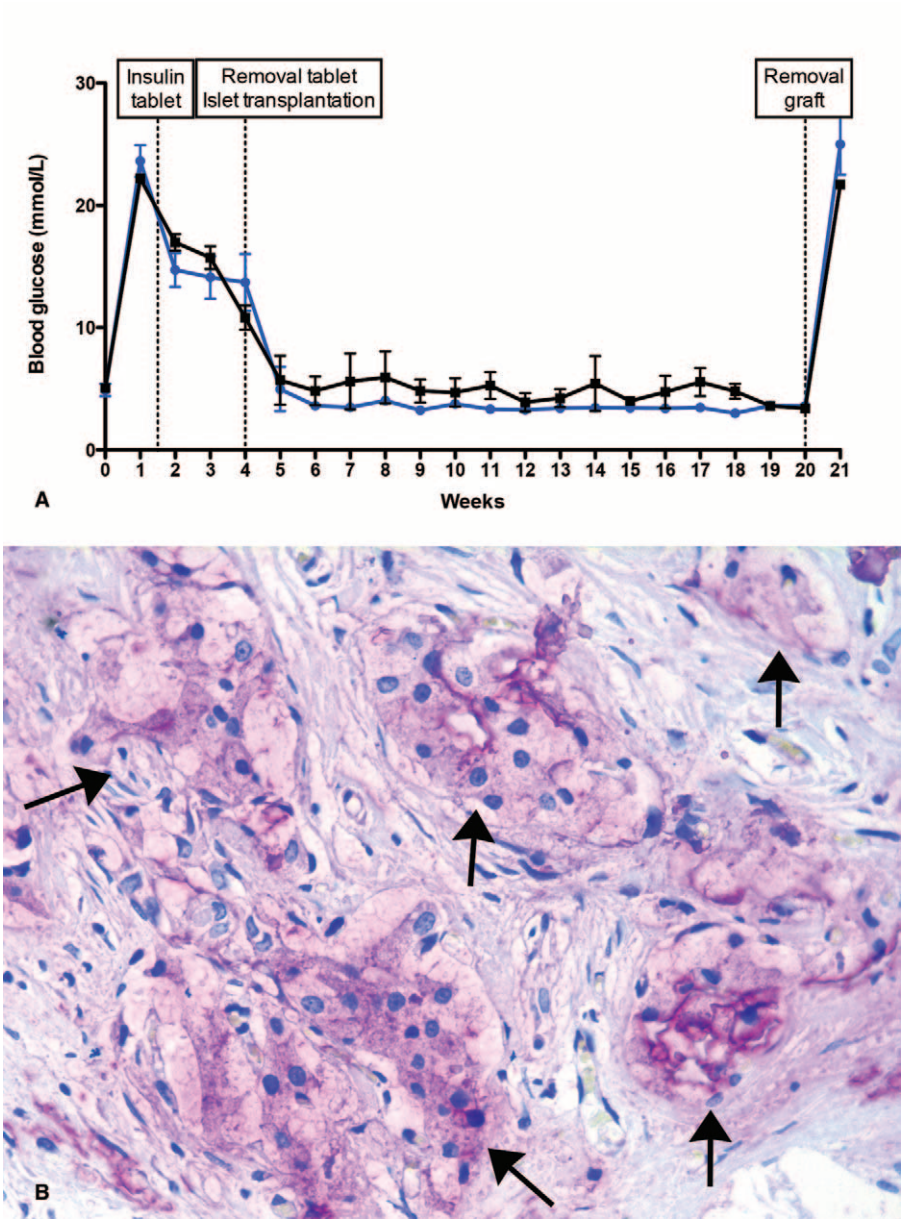

FIGURE 6. Blood glucose levels after islet transplantation in scaffold. The blood glucose levels (mmol/L) after transplantation (A) of rat islets subcutaneously in a preimplanted PDLLCL scaffold (n = 4, black line) and under the kidney capsule (n = 6, blue line). Mean and standard error of mean are plotted. In addition to excluding pancreas regeneration by histology, we removed from 1 recipient of the scaffold group and 2 of the kidney capsule group the graft and followed the animals for another week to confirm that the normoglycemia was dependent on the islet graft. Insulin positive islets (40×, SIGMAFAST Fast Red, arrows) were found in the scaffold pores after 4 months of transplantation (B).

longer time periods are required to come to a similar vascularization degree similar as observed in this study.

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