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Successful Islet Transplantation Into a Subcutaneous Polycaprolactone Scaffold in Mice and Pigs

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Background. Islet transplantation is a promising treatment for type 1 diabetes. It has the potential to improve glycemic control, particularly in patients suffering from hypoglycemic unawareness and glycemic instability. As most islet grafts do not function permanently, efforts are needed to create an accessible and replaceable site, for islet grafts or for insulin-producing cells obtained from replenishable sources. To this end, we designed and tested an artificial, polymeric subcutaneous transplantation site that allows repeated transplantation of islets. **Methods.** In this study, we developed and compared scaffolds made of poly(D,L,-lactide-co-ε-caprolactone) (PDLLCL) and polycaprolactone (PCL). Efficacy was first tested in mice, and then, as a proof of principle for application in a large animal model, the scaffolds were tested in pigs, as their skin structure is similar to that of humans. **Results.** In mice, islet transplantation in a PCL scaffold expedited return to normoglycemia in comparison to PDLLCL (7.7 ± 3.7 versus 16.8 ± 6.5 d), but it took longer than the kidney capsule control group. PCL also supported porcine functional islet survival in vitro. Subcutaneous implantation of PDLLCL and PCL scaffolds in pigs revealed that PCL scaffolds were more stable and was associated with less infiltration by immune cells than PDLLCL scaffolds. Prevascularized PCL scaffolds were therefore used to demonstrate the functional survival of allogenic islets under the skin of pigs. **Conclusions.** To conclude, a novel PCL scaffold shows efficacy as a readily accessible and replaceable, subcutaneous transplantation site for islets in mice and demonstrated islet survival after a month in pigs.

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Islet transplantation is a promising approach for the treatment of type 1 diabetes (T1D). It has the potential for physiological glucose regulation, particularly in patients with severe hypoglycemic unawareness and glycemic instability. By optimal regulation of the glucose levels, it prevents long-term complications, comorbidities, and improves the quality of life.[1] However, a transplantation site that supports long-term islet function is still not available. Currently, islets are infused into the portal vein, but for multiple reasons, the liver does not efficiently support long-term functional islet survival.[2–4] For example, thrombosis or instant blood-mediated inflammatory reactions occur. A recent cohort study reported that the median graft survival is approximately only 5.9 y after multiple islet infusions.[5] This limits the potential of larger-scale application of the intraportal site. Islet transplantation will be more readily available for a larger group of patients when it can be performed in a readily accessible transplantation site where graft recovery and repeated grafting are possible. This will also support the potential use of replenishable cell sources, like insulin-producing cells derived from stem cells. These cells might show functional issues and need to be retrieved and replaced.[6]

To create a more readily accessible and replaceable site, we developed a subcutaneous islet transplantation procedure by using a polymeric scaffold.[7,8] Briefly, the scaffold is

placed under the skin to allow vascularization and dampening of tissue responses.[7,9] After 4 wk, islets are transplanted into the scaffold. In our development process, we have tested several polymer types. Poly(D,L,-lactide-co-ε-caprolactone) (PDLLCL) was selected as it successfully supported islet function in diabetic rodent studies.[7,8] Nevertheless, we observed signs of PDLLCL degradation (i.e., changes in the physical properties of the polymer, such as strength, color, shape, and weight) 12 wk post-transplantation in mice. Therefore, in this study, we compare PDLLCL with a polymer more resistant to biodegradation, that is, polycaprolactone (PCL). PCL is extensively used in clinical studies.[10,11] Its degradation profile and tissue response are well documented.[11] Furthermore, the characteristics of PCL make the polymer suitable for 3D printing, which would be beneficial for upscaling and standardizing the manufacturing process.

Here, we first compared the efficacy of these 2 polymers when incorporated into a scaffold for subcutaneous islet transplantation in diabetic mice. To test its applicability for potential use in humans, we also tested this scaffold in a large animal model with a skin structure similar to human's.[12,13] As a proof of principle and to demonstrate functional survival, we tested in vitro survival and function of porcine islets cultured on the 2 polymers and determined functional responses after 1-mo implantation in an allogeneic porcine transplantation model.

MATERIALS AND METHODS

Mice Scaffold Manufacturing

PCL (Mn 80.000; Sigma-Aldrich, Zwijndrecht, the Netherlands) and PDLLCL (Sigma-Aldrich) polymer particles were dissolved in chloroform (4% w/v) and mixed with 250–425-μm sodium chloride particles (10:1 w/w; Sigma-Aldrich) in a polytetrafluorethylene (PTFE) petri dish as described previously.[8] After drying, the sodium chloride particles were removed by washing with H_2O. This created a sheet with an interconnected network of pores with different sizes ranging from 250 to 425 μm. The sheet was resized in rectangle scaffolds of 1.0 by 1.5 cm with a thickness of 0.2 cm. Islet channels were created by a 25G needle (B. Braun Medical B.V., Oss, the Netherlands) and polyethylene tubing (diameter 0.5 mm; Brinkman, 's-Gravenzande, the Netherlands) was placed in the channels to prevent ingrowth of cells in the channels.

Before implantation, the pores of the scaffolds were filled with mouse fibrin by adding 1 μL mouse thrombin (100 U/mL in distilled water; Molecular Innovations, Novi) to 100 μL of fibrinogen solution (2 mg/mL in CMRL (Corning Cellgro, Manassas; Abcam, Waltham). After 1-h incubation at room temperature and 1 h at 37°C, the solution formed a fibrin gel.

Transplantation Efficacy in Mice

All animal procedures were approved by the University of California Irvine, Institutional Animal Care and Use Committee (#AUP-17-129/AUP-17-241). Islets were isolated from 250–300 g male Sprague-Dawley rats (Envigo, Livermore) as previously described by Smink et al.[8] Briefly, pancreata were distended with Collagenase V (Sigma-Aldrich) in Hank's Balanced Salt Solution (HBSS; Gibco; Thermo Fisher Scientific, Waltham) and dissected. After an 18 min of incubation at 37°C, islets were purified from the exocrine tissue using a Ficoll density gradient (Gradient stock solution; Corning Cellgro). The islets were cultured overnight in CMRL supplemented with 10% newborn calf serum (Equitech-Bio, Kerrville), 2 mM glutamine (Corning Cellgro), 10 mM HEPES (Corning Cellgro), and 1% penicillin/streptomycin (Fisher Scientific).

Diabetes was induced in male athymic nude mice (Charles River, Wilmington) by intraperitoneal injection with 180 mg/kg streptozotocin (Sigma-Aldrich). After diabetes was confirmed (glucose > 350 mg/dL), insulin implants (LinBit; LinShin, Scarborough, Canada) were subcutaneously implanted to stabilize glycemic control. At the same time, the scaffolds were implanted under the skin on the back to allow vascularization of the scaffolds before islet transplantation.

Four weeks after subcutaneous implantation, an incision was made next to the scaffold to remove the tubing. Subsequently, 800 islet equivalent (IEQ) was injected into the scaffold using a 23G Hamilton syringe (SGE Analytical Science, Austin). The control mice received the same number of islets under the kidney capsule, as this is considered the gold standard for rodents. The insulin implants were removed directly after islet transplantation, and all mice received post-surgery analgesia (buprenorphine 0.05 mg/kg [Par Sterile Products, Rochester] and ibuprofen 0.2 mg/mL). After 65 d, the islet grafts were removed, and the blood glucose levels were measured for another 10 d to prove that the reduction in blood glucose levels was graft dependent and not caused by pancreas regeneration.

Porcine Islets

Porcine islets were isolated from 7- to 9-d -old pre-weaned Yorkshire pigs (Premier Biosource, Escondido) as described by Lamb et al.[14] (#AUP-17-129). Briefly, pancreata were dissected and stored in ice-cold HBSS. Subsequently, they were cut into small pieces (1 mm^3) and digested incubating with 2.5 mg/mL type V collagenase solution in HBSS (Sigma-Aldrich, St. Louis) at 37°C for 15 min while shaking (100 rpm). After digestion, the tissue was washed with HBSS containing 1% porcine serum (Gibco) and filtered using a 500-μm metal mesh.

As these islets were immature and required prolonged culture,[15] the islets were cultured for 7 d in maturation media (IMM) composed of Ham's F-12 medium (Corning Inc., Midland) supplemented with HEPES (Sigma-Aldrich), L-glutathione (Sigma-Aldrich), ITS+3 (Sigma-Aldrich), nicotinamide (Sigma-Aldrich), gentamycin sulfate (Corning Inc.), Trolox (Sigma-Aldrich), heparin (Sagent Pharmaceuticals, Schaumburg), pefabloc (Santa Cruz Biotechnology, Santa Cruz), L-glutamine (Alfa Aesar; Thermo Fisher Scientific), medium 199 (Corning Inc.), calcium chloride dihydrate (Thermo Fisher Scientific), Dnase (Sigma-Aldrich), 1X antibiotic/antimycotic solution (Corning Inc.), and 10% porcine serum (Sigma-Aldrich). On day 1, a 100% media change was performed with IMM. The same was done on day 3, but then the IMM was supplemented with 100 μM Necrostatin-1 (Abcam, Cambridge, UK). On day 5, a 50% media change with necrostatin-1 supplemented IMM was conducted, followed by a 100% media change on day 7 with IMM containing necrostatin-1.

In Vitro Culture

Before performing a porcine transplantation study, we investigated the direct consequences of these 2 polymers on the functional survival of porcine islets in vitro. To this end,

glass petri dishes (40 mm) were coated with 8% (w/v) PCL and 8% PDLLCL in chloroform (Merck Millipore, Amsterdam, the Netherlands) by using a Convac 1001S spin coater (ST 143 control unit; Convac, Wiernsheim, Germany). After 7 d of maturation, porcine islets were divided over the PCL and PDLLCL-coated petri dishes and control wells in a nontreated 6-well plate (Corning Inc.). On days 1, 3, and 7, islet viability and function were tested.

Islet viability was determined with a Calcein AM (Invitrogen; Thermo Fisher Scientific) and propidium iodide (PI; Invitrogen) staining. Briefly, islets were incubated with 50 µM Calcein AM and 0.025 mg/mL PI for 30 min at room temperature. Staining intensity was measured using a microplate reader (Tecan Infinite F200; Tecan, Mannedorf, Switzerland) and viability was calculated as Calcein AM-positive cells/(Calcein AM-positive cells + PI-positive cells) × 100.

To measure the in vitro porcine islet function, a glucose-stimulated insulin secretion assay was performed. Briefly, 3 batches of 100 IEQ per sample were incubated for 1 h at 37°C in low-glucose media (2.8 mM). Subsequently, the islets were incubated for 1 h at 37°C in high-glucose media (28 mM). Followed by a 1-h incubation with low-glucose media. A porcine insulin ELISA kit (Mercodia, Winston Salem) was used according to the manufacturer's instructions to quantify the insulin concentrations after each incubation.

Large Scaffold Manufacturing

PCL and PDLLCL scaffolds were obtained as described previously.[8] Briefly, polymer particles were dissolved in chloroform (4% w/v) and mixed with 250–425-µm sodium chloride particles (10:1 w/w) in a PTFE petri dish. After stirring, a custom-made PTFE ring with 6 16G needles was placed in the solution to form the islet channels. The sheet was washed, removed from the petri dish, and the ring with the needles was removed, resulting in a scaffold with a 6.7-cm diameter and 0.4-cm thickness. The channels were kept free of cell ingrowth during the prevascularization period by placing polyethylene tubing (diameter 1.5 mm) in the channels. Before implantation, the pores of the PCL and PDLLCL scaffold were filled with porcine fibrin by using porcine fibrinogen (2 mg/mL; Molecular Innovations) and porcine thrombin (100 U/mL; Molecular Innovations) as described above.

Tissue Responses In Vivo

To study the in vivo tissue responses against the polymers, the scaffolds were implanted in subcutaneous pockets on the back of 4-mo-old Yucatan minipigs (Premier Biosource). Each pig received a PCL and a PDLLCL scaffold (n = 4). After 12 wk, the scaffolds were processed for histology and gene expression analysis.

For histological analysis of the tissue responses, the scaffolds were cut in half, fixated in either 2% paraformaldehyde (PFA) or 10% formalin, and processed for paraffin embedding. PFA-fixated sections were used for a myeloperoxidase (MPO) staining, indicating granulocytes and neutrophils. To this end, antigen retrieval was applied using a Tris-EDTA solution. Nonspecific binding was blocked by a 30-min incubation with 10% normal goat serum (Sigma-Aldrich). Subsequently, sections were incubated for 24 h with the primary MPO antibody (1:50; Abcam, Cambridge, UK). A goat antirabbit alkaline phosphatase-conjugated antibody (1:100; Abcam) was used as secondary antibody. The alkaline phosphatase activity was demonstrated by incubating the sections for 10 min with SIGMAFAST Fast Red (Sigma-Aldrich) and a 20-s incubation with hematoxylin.

A similar protocol was followed for staining of macrophages, CD3, and CD31 on formalin-fixated sections. Briefly, for antigen retrieval, citrate buffer, EDTA, and Tris-HCL, respectively, were used. Nonspecific binding was blocked by a 30-min incubation with 10% normal goat serum (Sigma-Aldrich). Subsequently, sections were incubated either 1 h with the primary macrophages (prediluted; Abcam) and CD3 (prediluted; Abcam) antibodies or overnight with the CD31 antibody (1:50; Abcam). The macrophage staining included an avidine and biotin block, after which the sections were incubated for 30 min first with the biotin-labeled secondary antibody (1:100; Agilent, Amstelveen, the Netherlands) and second with a streptavidin-alkaline phosphatase (1:100, SouthernBiotech, Uden, the Netherlands). For the CD3 and CD31 sections, a goat anti-rabbit alkaline phosphatase-conjugated antibody (1:100; Abcam) was used as secondary antibody. For all 3 stains, the alkaline phosphatase activity was demonstrated as described above for the MPO staining. Images of all stains were obtained using a Leica DM2000 LED microscope with a Leica DFC 450 camera (Leica Microsystems B.V., Amsterdam, the Netherlands).

For gene expression analysis, samples were processed for real-time reverse-transcription polymerase chain reaction (RT-PCR). First, RNA was isolated using Trizol (Invitrogen) according to manufacturer's instructions. Subsequently, cDNA was reverse transcribed by using a SuperScript III Reverse Transcriptase kit (Life Technologies; Thermo Fisher Scientific, Groningen, the Netherlands). After which RT-PCR was conducted using the ViiATM Real Time PCR system (Life Technologies) with Taqman primer/probes (TaqMan Gene Expression Assays) (Thermo Scientific, Table 1) and qPCR Mastermix Plus (Eurogentec, Seraing, Belgium). Reactions were performed at 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and 60°C for 60 s, repeating these last 2 steps for 40 cycles. Delta Ct values were calculated and normalized against the expression of the housekeeping gene GAPDH.

Porcine Transplantation Study

As proof of principle, islets were transplanted into preimplanted scaffolds in nondiabetic pigs. For this, 1 scaffold was implanted under the skin of a 4-mo-old Yucatan pig for 12 wk (n = 2). Islets were isolated from 7–9-d-old preweaned

TABLE 1.

Assay IDs of RT-PCR genes

Gene	Assay ID
CD31 (PECAM1)	Ss03392600_u1
VEGFa	Ss03393993_m1
CD105 (Endoglin)	Ss03391353_m1
aSMA (ACTA2)	Ss04245588_m1
CSF1	Ss03373560_g1
Col1a1	Ss03373340_m1
TGFb	Ss03382325_u1
GAPDH	Ss03375629_u1

RT-PCR, reverse-transcription polymerase chain reaction.

Yorkshire pigs and cultured for 7 d to allow maturation. After 12 wk, an incision was made next to the scaffold to remove the tubing. Subsequently, 5000 IEQ was injected into each channel. Four channels were filled with islets; therefore, each pig received a total of 20 000 IEQ. To prevent rejection, the pigs received 1500 mg oral cyclosporine (Teva Czech Industries/Teva Pharmaceuticals, North Wales) per day until the end of the study. Cyclosporine was started 1 wk before transplantation with a gradual increase up to the final dosage.[16]

After 1 mo, a local glucose tolerance test was performed. Briefly, a subcutaneous pouch surrounding the scaffold was carefully created by keeping the tissue covering the scaffold intact. Subsequently, a 15 mmol glucose solution was injected into this pouch, and samples were taken from this solution at 0, 5, 30, 60, 90, 120, 140, and 180 min. The same was done for a control sham site to be able to distinguish between insulin produced by the pig itself and that of the graft. The in vivo function test was followed by the removal of the scaffold and an in vitro function test with the whole scaffold and processing for histological analysis as both described above.

Insulin Staining

For histological analysis of insulin-positive cells within the PCL scaffold 1 mo after transplantation, the scaffold was fixated in 2% PFA and processed for paraffin embedding. Briefly, sections were incubated for 1 h with the primary insulin antibody (1:300; Sigma-Aldrich). Nonspecific binding was blocked by a 15-min incubation with 10% normal goat serum, followed by a 45-min incubation with the biotin-labeled secondary antibody (1:100; Agilent, Amstelveen, the Netherlands) and second incubation with a streptavidin-alkaline phosphatase (1:100, SouthernBiotech, Uden, the Netherlands). The alkaline phosphatase activity was demonstrated using SIGMAFAST Fast Red (Sigma-Aldrich) and hematoxylin counterstain.

Statistical Analysis

Statistical analysis of the data was conducted in GraphPad Prism (version 9.2.0; GraphPad Software, Inc., La Jolla). Data were tested for normality by a Shapiro–Wilk normality test. The in vitro viability, functionality, and the gene expression data were analyzed with respectively a 1-way analysis of variance with a Tukey post hoc test, a 2-way analysis of variance, and a Kruskal–Wallis with a Dunn's post-hoc test. P values <0.05 were considered significant.

RESULTS

Expedited Reversal of Hyperglycemia by Islets Within PCL Scaffolds

To compare the efficacy of the prevascularized PCL and PDLLCL scaffolds, 800 rat islets were transplanted into subcutaneous scaffolds in diabetic nude mice. In agreement with our previous study,[8] mice that received islets in the PDLLCL scaffold became normoglycemic (<150 mg/dL) within 16.8 ± 6.5 d after transplantation (Figure 1). Recipients of islets in the PCL scaffold became normoglycemic within 7.7 ± 3.7 d. This is 2 times faster than mice with PDLLCL scaffolds, and the blood glucose levels were also more stable. The time to become normoglycemic was longer for the scaffold-carrying mice than for the grafts under the kidney capsule (2.7 ± 1.1 d). This was statistically significant for PDLLCL ($P < 0.05$). All animals with a successful transplant remained normoglycemic until the end of the study (65 d). After removing the grafts, all animals became hyperglycemic again, indicating graft-dependent normoglycemia.

In Vitro Porcine Islet Viability Decreased With PDLLCL

To investigate if with porcine islets also differences occur between PDLLCL and PCL, porcine islets were cultured on the polymers and compared with islets cultured in control conditions to determine the effect of the polymers on islet viability (Figure 2). At day 1, no differences were observed in viability between the control (94.3% ± 2.1%), PDLLCL (92.0% ± 2.4%), and PCL (94.0 ± 0.6%). The same applies for day 3, the viability was, respectively, 96.8% ± 0.8%, 86.3% ± 6.5%, and 89.8% ± 4.7%. However, after 7 d, islets cultured on PDLLCL showed significantly reduced viability compared with the control ($P < 0.05$). The viability of the control and PCL islets was still 94.8% ± 2.1% and 93.8% ± 2.7%, whereas the viability of the PDLLCL islets decreased to 70.3% ± 9.9%.

To determine if the viability reduction was associated with an impaired function, a glucose-stimulated insulin secretion test was performed on days 1, 3, and 7 of culture (Figure 3). Neither of the 2 polymers significantly impaired insulin release. In all 3 groups, the islets respond properly to changes in glucose concentrations on days 1 and 3. However, on day 7, the function of all groups decreased.

Porcine Tissue Response

Next, the in vivo performance of the subcutaneous scaffolds was studied in pigs. This preclinical model resembles the skin anatomy of humans.[12,13] Macroscopically, differences could be observed between the explanted polymers. The PDLLCL device was reduced in size and showed degradation signs (small fragments), while the PCL maintained its size and shape during the 12 wk of implantation (Figure 4). By histology, we found fewer CD3-positive cells and macrophages in both scaffolds. However, a major difference was observed in the MPO-positive cells, which represent granulocytes and neutrophils. Barely any MPO-positive cells were found in the PCL scaffolds, whereas the PDLLCL contained a high abundance of these cells (Figure 4). Furthermore, no differences were observed in vascularization. PCR analysis of several vascularization and tissue response genes (Figure 5) showed no significant differences between the devices.

Successful Pig Islet Transplantation in PCL Scaffold

Based on the reduced viability of pig islets when cultured on PDLLCL and the degradation of PDLLCL after 12 wk of implantation, a transplantation study was conducted using the PCL scaffold (Figure 6). To show proof of principle, 20 000 IEQ piglet islets were transplanted into the PCL scaffold after 12 wk of prevascularization of the device.

One month after islet transplantation into the scaffolds, a local in vivo glucose tolerance test was performed by injecting a glucose solution into the subcutaneous pocket (Figure 6C).

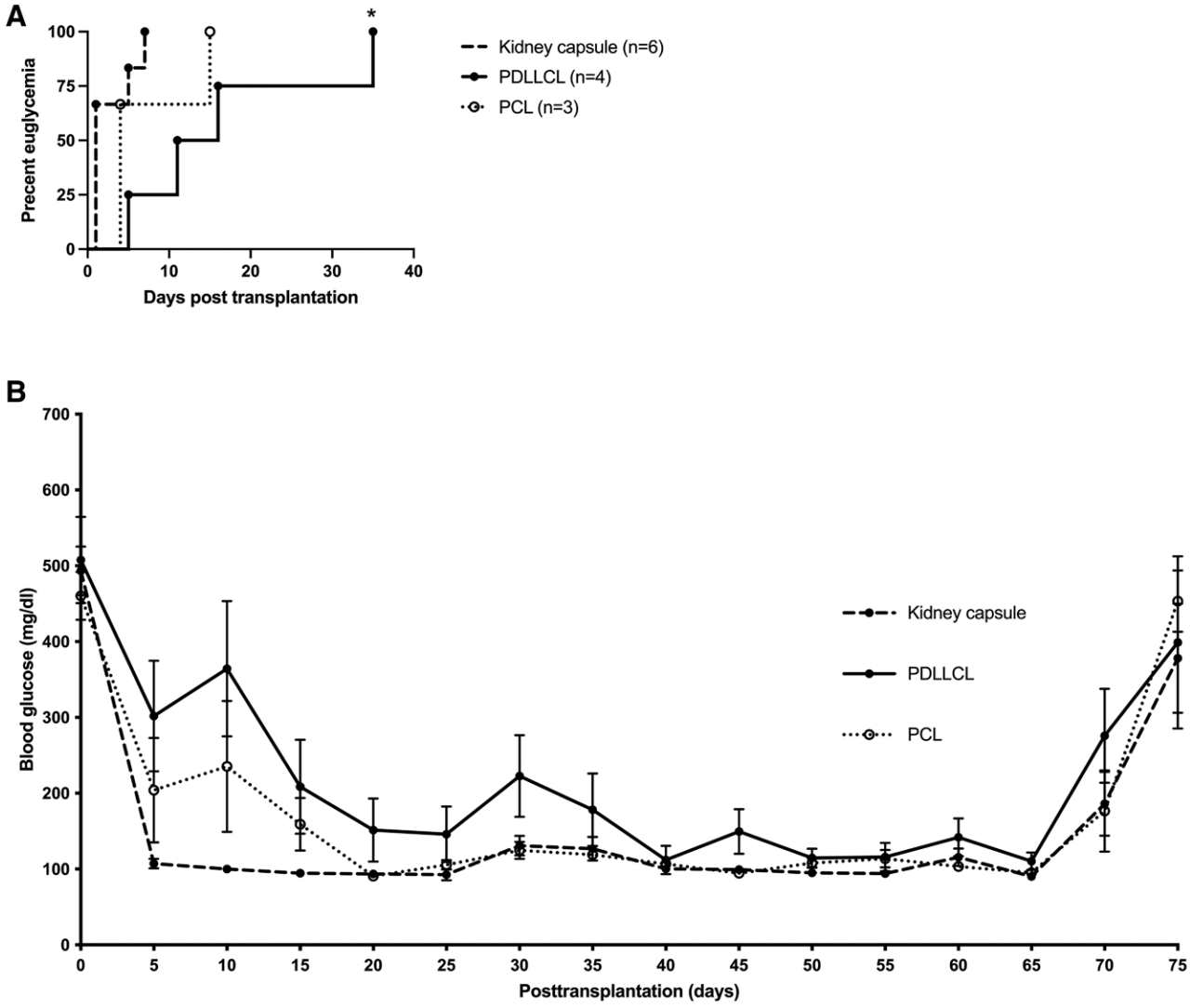

FIGURE 1. Transplantation outcome of mouse study. Kaplan–Meier graph for rates of diabetes reversal (<150 mg/dL) after transplantation of 800 rat islets into prevascularized subcutaneous PDLLCL (n = 4) and PCL (n = 3) scaffolds in diabetic nude mice (A). Transplantation of the same islet dosage under the kidney capsule was used as control (n = 6). Statistical analysis was carried out using a log-rank (Mantel–Cox) test with multiple comparison correction (*<0.05 compared with the kidney capsule control). In addition, the long-term nonfasting blood glucose levels of these mice are depicted (B). After 65 d, the islet grafts were removed to prove that the reduction in blood glucose levels was graft dependent and not caused by pancreas regeneration. The mean and standard error of the mean are plotted. PCL, polycaprolactone; PDLLCL, poly(D,L,-lactide-co-ε-caprolactone).

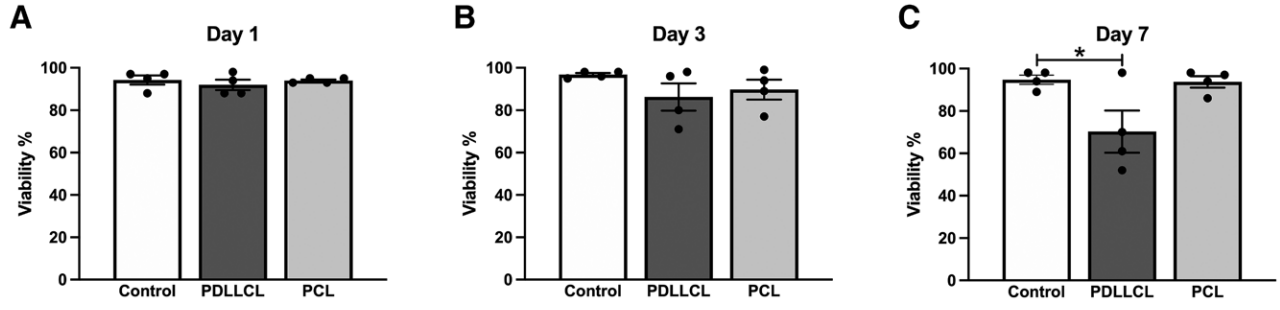

FIGURE 2. In vitro viability of porcine islets after culturing on PDLLCL and PCL. The viability of porcine islets was measured by calcein AM/propidium iodide staining after 1 (A), 3 (B), and 7 (C) d of culture. Mean and standard error of mean are plotted (n = 4), statistical analysis was carried out using a 1-way ANOVA with a Tukey posthoc test (*P < 0.05). ANOVA, analysis of variance; PCL, polycaprolactone; PDLLCL, poly(D,L,-lactide-co-ε-caprolactone).

Because the pigs are nondiabetic and thus not allowing distinction between insulin produced by the animal itself or by the transplanted islets, the test was also done at a sham control site. One of the pigs demonstrates a peak in insulin production within half an hour after adding the glucose solution, whereas this peak was not seen at the sham site, indicating in

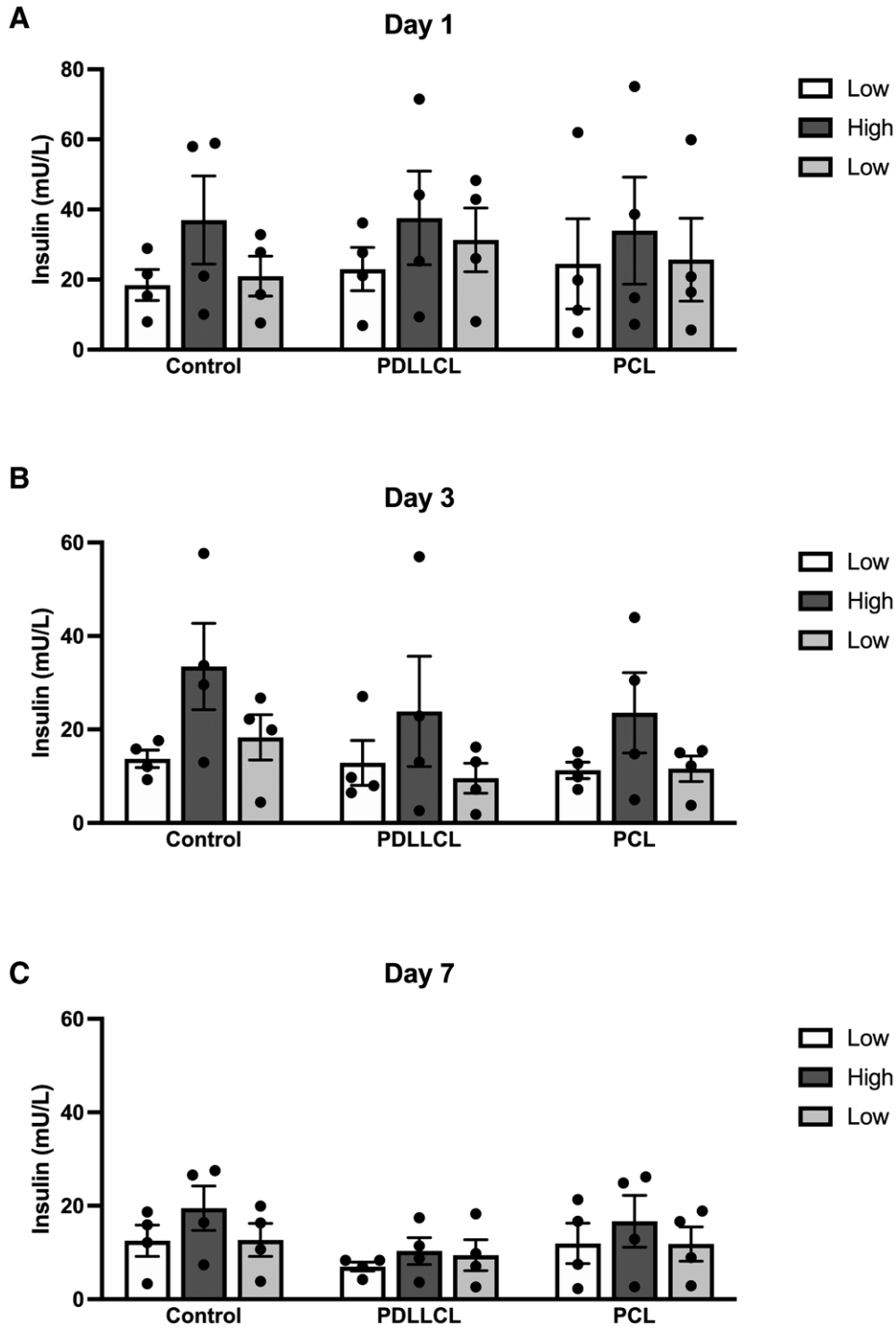

FIGURE 3. In vitro functionality of porcine islets after culturing on PDLLCL and PCL. Glucose-stimulated insulin secretion of porcine islets after 1 (A), 3 (B), and 7 (C) d of culture. The white bars indicate the amount of insulin secreted during the first low-glucose (2.8 mM) incubation, the black bars represent the amount of insulin produced during high-glucose (28 mM) incubation, and the grey bars show the return to basal insulin secretion during the second low-glucose (2.8 mM) incubation. The mean and standard error of the mean are plotted (n = 4), statistical analysis was carried out using a 2-way ANOVA ($P < 0.05$). ANOVA, analysis of variance; PCL, polycaprolactone; PDLLCL, poly(D,L,-lactide-co-ε-caprolactone).

vivo responsiveness of the islets in the scaffold (Figure 6D). After doing the in vivo function test, the whole scaffold was explanted, and an in vitro function test was performed as well. Figure 6D shows that after 1 mo in the scaffold, the pig islets are still capable of responding to glucose challenges. Also, histology showed insulin-positive cells present in the scaffolds (Figure 6E). Together, this indicates that our transplantation procedure supports islet functional survival.

DISCUSSION

There is a need for the replacement of deficient insulin-producing cells to improve glycemic control and quality of life in T1D. Besides finding a replenishable insulin-producing cell source and avoiding the need for life-long immunosuppression,[17] designing an accessible and replaceable transplantation site is essential for the success of islet transplantation.[18,19] In this study, we focused on developing an artificial transplantation

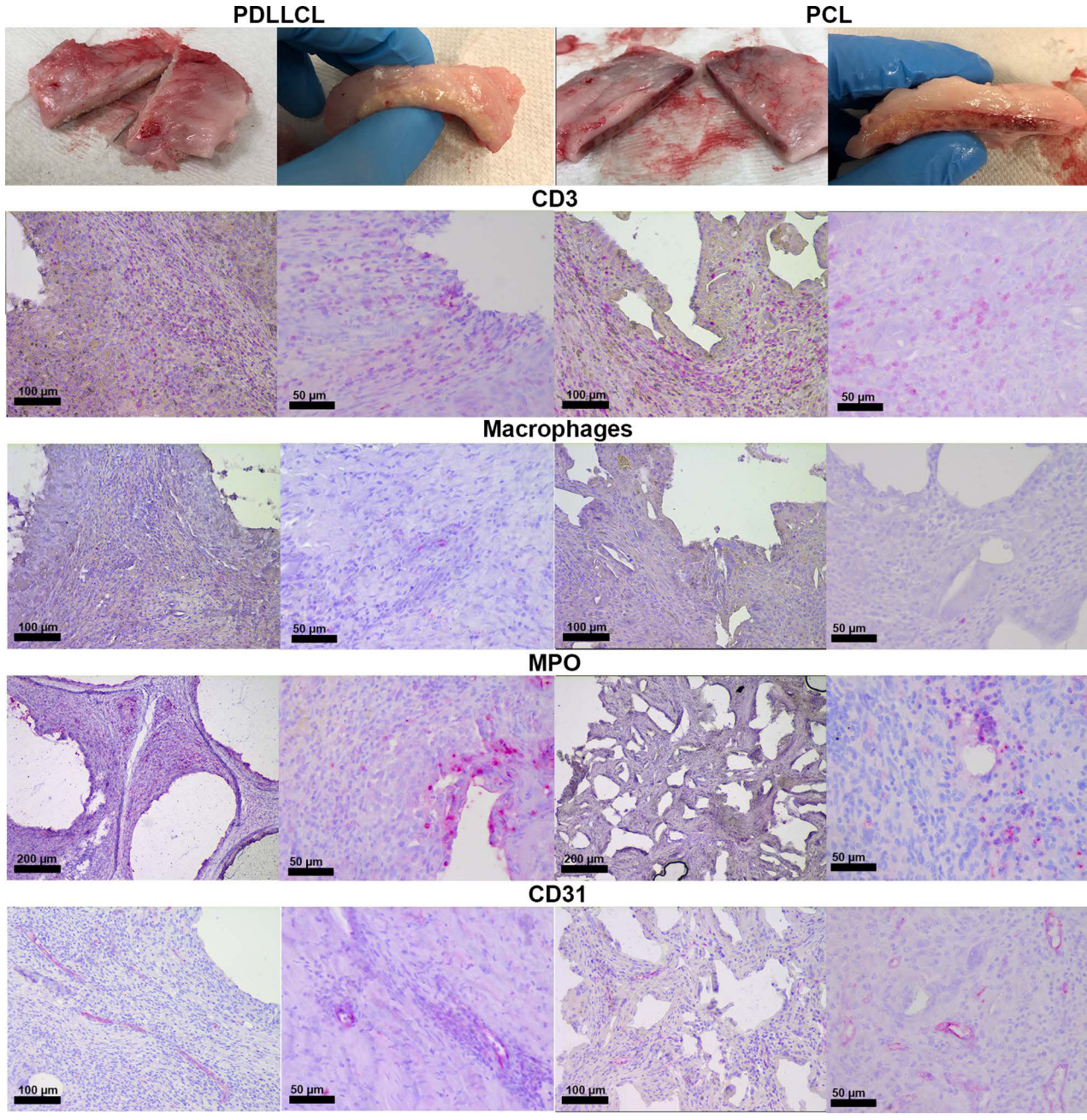

FIGURE 4. Histology of PDLLCL and PCL scaffolds after 12 wk implantation in pigs. Sections were stained (pink) for CD3-positive cells, macrophages, MPO, and CD31. MPO, myeloperoxidase; PCL, polycaprolactone; PDLLCL, poly(D,L,-lactide-co-ε-caprolactone).

site using a subcutaneous polymer scaffold, where we directly compare PCL and PDLLCL. We showed successful islet transplantation in both subcutaneous PCL and PDLLCL scaffolds in diabetic mice, but mice became normoglycemic faster when using a PCL scaffold. Previously, we have shown normoglycemia is not obtained when transplanting islets under unmodified skin,[8] illustrating that the prevascularized PCL scaffold makes the skin a more hospitable site for islets.

To be able to apply this concept in a clinical setting, the scaffold had to be enlarged and tested in a clinically relevant and larger animal model. To this end, we selected a pig model for these experiments. As islets are sensitive organoids and have been shown to undergo functional impairment when in contact with specific polymers,[7,20] we first tested the cell survival and functionality of porcine islets when cultured for prolonged periods with the polymers. We found clear differences in cell survival in favor of PCL. Although this did not translate into differences in glucose-induced insulin responses, it is likely caused by compensation in insulin production rates of the insulin-producing cells. Another reason to abandon PDLLCL for further pig experiments was the finding that the scaffold disintegrated and had increased infiltration of neutrophilic granulocytes. Our subsequent in vivo study was then designed to show functional survival of islets in the PCL scaffold by in vivo and in vitro function tests and histology.

Several studies have confirmed the versatility of PCL for the development of a scaffold for beta cell replacement. Subcutaneous implanted PCL devices are well vascularized and associated with minor foreign body responses.[21–23] Furthermore, it supports islet functional survival[21,22] and

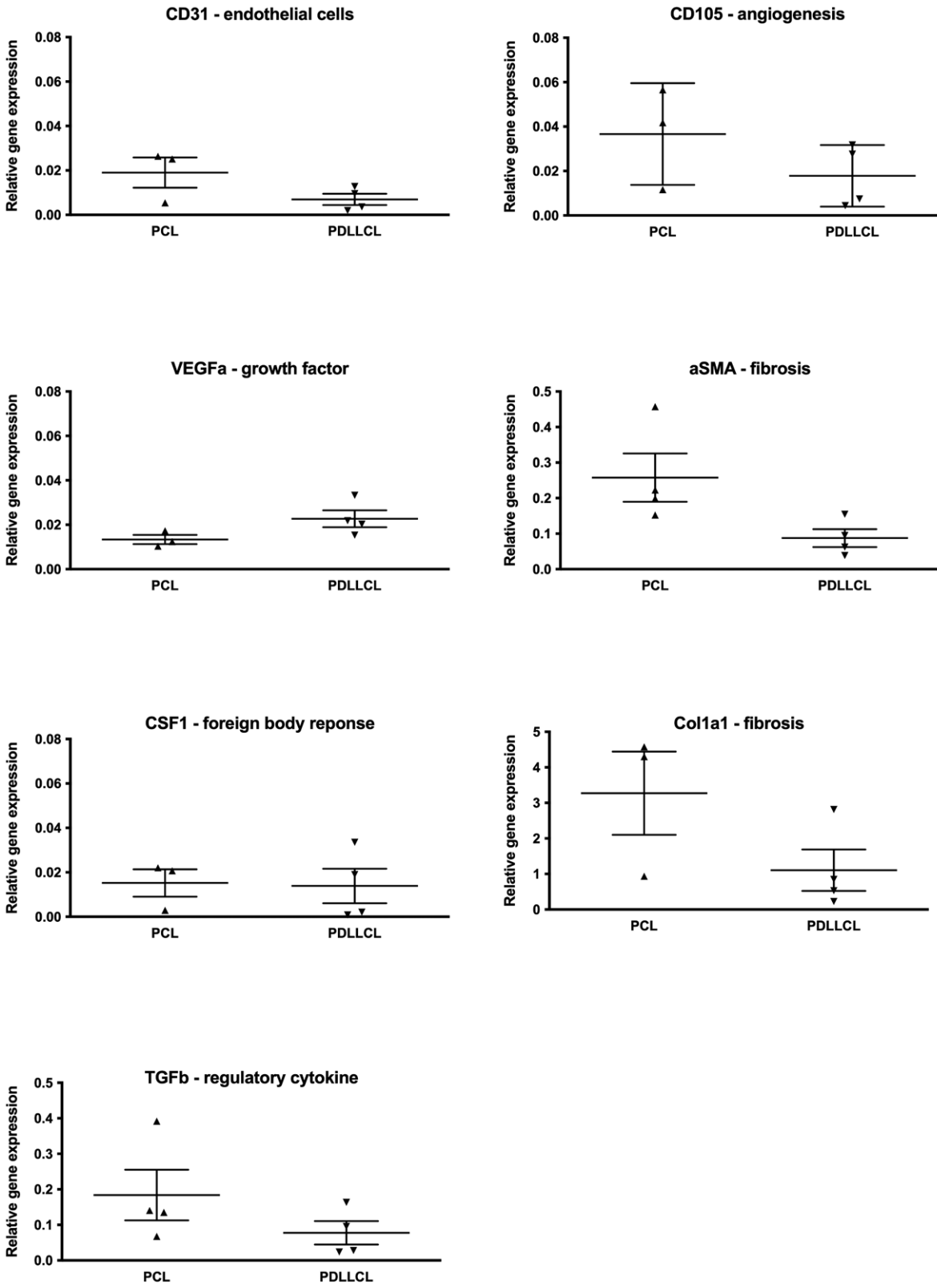

FIGURE 5. Relative gene expression of several vascular and tissue response genes. The mean and standard error of the mean are plotted (n = 4), statistical analysis was carried out using the Kruskal–Wallis test with Dunn's post hoc test (*$P < 0.05$). aSMA = alpha-smooth muscle actin; Col1a1 = collagen 1 alpha 1; CSF1 = colony-stimulating factor 1; TGFb = transforming growth factor beta; VEGFa = vascular endothelial growth factor A.

seems valuable for directing the differentiation of less mature insulin-producing cells.[24,25] In addition, the properties of PCL allow relatively easy addition of amino acids, growth factors, or immunomodulatory components.[26–28]

The transition from rodent models to large preclinical models is always challenging. In addition to upscaling the device, it is essential to use a model that resembles the skin structure of humans. Here we used immunocompetent pigs and found

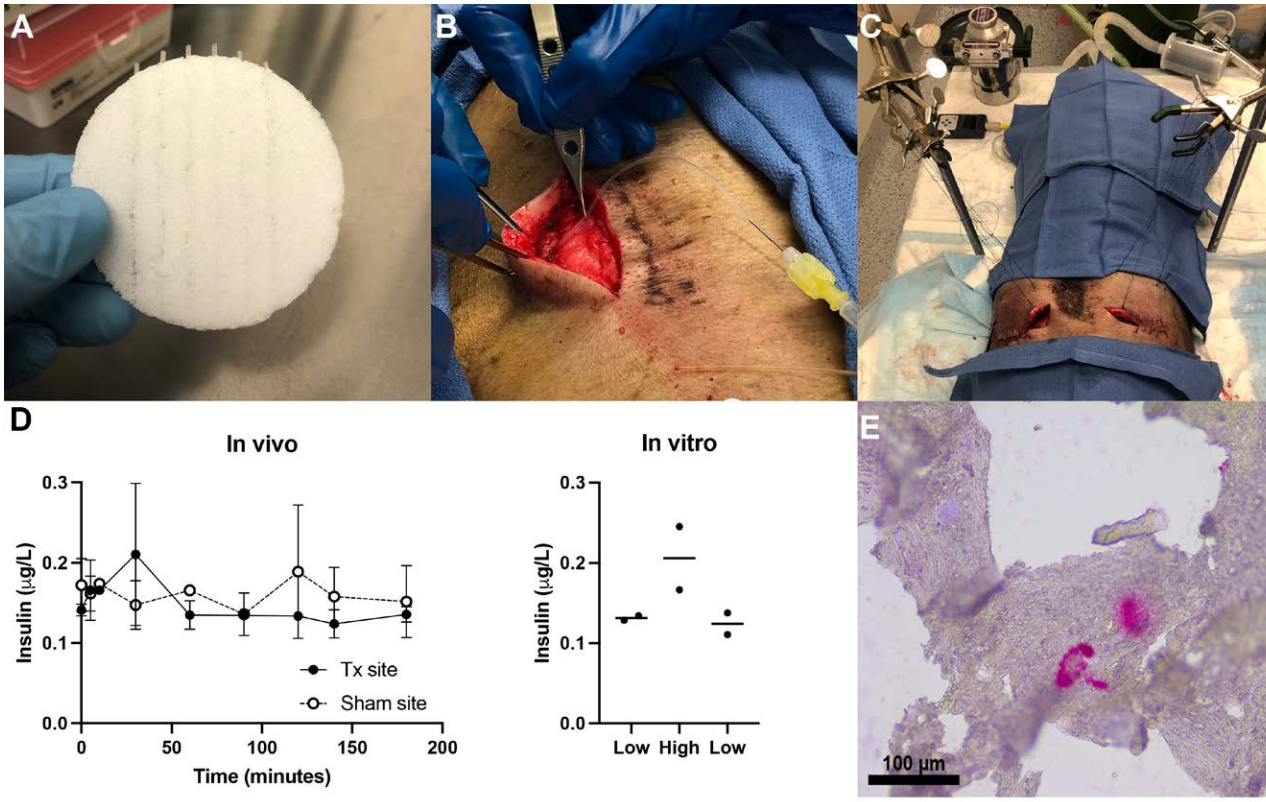

FIGURE 6. The transplantation procedures. A large (polycaprolactone) PCL scaffold with 6 islet channels was developed using the salt leaching technique (A), which was implanted subcutaneously. After 12 wk of prevascularization, an incision was made next to the implanted scaffold to remove the tubing from the islet channels, and islets were injected into the channels (B). One month after the islet transplantation, an in vivo glucose tolerance test was performed by injecting glucose into a subcutaneous pocket around the scaffold and at a sham site (C) while the animal was anesthetized. The mean insulin concentration (± standard error of the mean) of the in vivo and in vitro glucose tolerance tests are plotted (D; n = 2). Histology shows insulin-positive cells (pink) within the scaffold after 1 mo (E).

that the device had to fulfill additional requirements compared with the mouse model. For example, the skin structure of pigs is more rigid, which forced us to test different polymers. T1D curative studies in pigs are difficult due to the need for immunosuppression and issues with housing and care. Therefore, we used a standard immunosuppression model using cyclosporine to prevent allograft rejection and were able to show successful islet survival in this model for a month. The devices were well vascularized, and islets had normal morphology. Safety and functional survival were shown in this study, and the next step will be efficacy studies in diabetic pigs.

The major difference between our procedure and other biomaterial-based approaches[28–33] is the prevascularization period. During this prevascularization time, the device is implanted under the skin without the presence of islets, allowing the material-induced inflammatory response to dampen and providing the opportunity for blood vessels to invade. This leads to improved nutrient delivery and less loss of islet cells due to hypoxia and necrosis. Furthermore, it supports the glycemic control of the grafted islets in the scaffold, as also shown with a similar approach by Patikova et al.[34] A possible disadvantage is the need for 2 separate surgical procedures. However, these are simple procedures that might even be done under local anesthesia. Our pig study shows fast recovery and wound healing after implantation and transplantation. There is always some degree of tissue response due to the surgery and the placement of a "foreign" material. We observed minor macroscopic fibrotic reaction to both types of scaffolds. This tissue response is, however, required and is advantageous for adequate vascularization of our type of scaffold.[7] The vascularization period for our pig transplantation study was 12 wk, which was based on the degree of vascularization observed in a pilot study (data not shown). This vascularization period might be shortened by the development of a scaffold-vascularization strategy. This can be achieved, for example, by the addition of extracellular matrix molecules,[35,36] vascular growth factors,[37,38] or vascular cells.[39–41] Such an approach may also lead to the possibility of increasing the islet load as more vessels imply better nutritional supply after transplantation and expedited graft revascularization. However, this should be investigated in a T1D preclinical model before the translation to the clinic can be made.

To conclude, PCL is suitable for engineering a readily accessible and replaceable, subcutaneous transplantation site for islets in pigs. It successfully supports both in vitro and in vivo functional islet survival, indicating that this might be a potential alternative site for the current clinical intraportal transplantations, and also providing an excellent site to transplant insulin-producing cells derived from stem cells due to the possibility to monitor and retrieve the cells in case of complications.[42]

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