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Smink, Alexandra M; de Haan, Bart J; Paredes-Juarez, Genaro A; Wolters, Anouk H G; Kuipers, Jeroen; Giepmans, Ben N G; Schwab, Leendert; Engelse, Marten A; van Apeldoorn, Aart A; de Koning, Eelco

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PAPER

Selection of polymers for application in scaffolds applicable for human pancreatic islet transplantation

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Alexandra M Smink¹, Bart J de Haan¹, Genaro A Paredes-Juarez¹, Anouk H G Wolters², Jeroen Kuipers², Ben N G Giepmans², Leendert Schwab³, Marten A Engelse⁴, Aart A van Apeldoorn⁵, Eelco de Koning⁶, Marijke M Faas¹ and Paul de Vos¹

¹ Department of Pathology and Medical Biology, Section of Immunoendocrinology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, EA11, 9700 GZ, Groningen, The Netherlands

² Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

³ Polyganics, Groningen, The Netherlands

⁴ Laboratory for Pancreatic Islet Isolation, Leiden University Medical Center, Leiden, The Netherlands

⁵ Department of Developmental BioEngineering, Faculty of Science and Technology, University of Twente, Enschede, The Netherlands

⁶ Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

E-mail: A.M.SMINK@UMCG.NL

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Abstract

The liver is currently the site for transplantation of islets in humans. This is not optimal for islets, but alternative sites in humans are not available. Polymeric scaffolds in surgically accessible areas are a solution. As human donors are rare, the polymers should not interfere with functional survival of human-islets. We applied a novel platform to test the adequacy of polymers for application in scaffolds for human-islet transplantation. Viability, functionality, and immune parameters were included to test poly(D,L-lactide-co- ϵ -caprolactone) (PDLLCL), poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer, and polysulfone.

The type of polymer influenced the functional survival of human islets. In islets cultured on PDLLCL the glucagon-producing α -cells and insulin-producing β -cells contained more hormone granules than in islets in contact with PEOT/PBT or polysulfone. This was studied with ultrastructural analysis by electron microscopy (nanotomy) during 7 d of culture. PDLLCL was also associated with statistically significant lower release of double-stranded DNA (dsDNA, a so called danger-associate molecular pattern (DAMP)) from islets on PDLLCL when compared to the other polymers. DAMPs support undesired immune responses. Hydrophilicity of the polymers did not influence dsDNA release. Islets on PDLLCL also showed less cellular outgrowth. These outgrowing cells were mainly fibroblast and some β -cells undergoing epithelial to mesenchymal cell transition. None of the polymers influenced the glucose-stimulated insulin secretion. As PDLLCL was associated with less release of DAMPs, it is a promising candidate for creating a scaffold for human islets. Our study demonstrates that for sensitive, rare cadaveric donor tissue such as pancreatic islets it might be necessary to first select materials that do not influence functionality before proposing the biomaterial for *in vivo* application. Our presented platform may facilitate this selection of biomaterials.

Introduction

Pancreatic islet transplantation into patients with type 1 diabetes is a promising strategy to restore glucose homeostasis and prevent life-threatening hypoglycemic events [1]. The first year success rate of islet-transplantation is rather high. About 80% of the patients, who undergo this procedure, become

insulin independent but the long-term success is still low [2]. Only 10% of the patients are still insulin independent at five years after transplantation. The lack of long-term success is considered multifactorial. Not only factors such as reoccurrence of autoimmunity, alloimmunity, and high metabolic pressure but also the transplantation site have been shown to influence long-term survival of the grafts [3, 4]. Up to now islets are

usually infused via the portal vein into the liver. During recent years it has been shown that the liver might not be an optimal environment for islet transplantation as it is associated with several issues such as insufficient vascularization, low oxygen tension, and the occurrence of the instant blood-mediated inflammatory reaction (IBMIR) [5, 6]. A number of alternative sites, such as pancreas [7], striated muscle [8], peritoneum [9], kidney capsule [10], and spleen [11], have been tested as alternative for the intrahepatic transplantation site. Up to now, none of these sites provided an optimal environment for pancreatic islets. Therefore, creating a novel custom-made artificial transplantation site for islets, using engineered polymer scaffolds, may be a preferred option [5, 12, 13].

A transplantation site for islets should meet a number of requirements. It should not interfere with function of the islets, support fast revascularization, cause minimal or preferably no activation of the immune system, and should be associated with minor surgery [13–15]. A polymer scaffold can be designed to meet these requirements by providing a support structure and mimicking the pancreatic environment [13]. Moreover, a polymer scaffold could prevent the direct exposure of islets to blood and therefore prevent the enormous islet loss associated with IBMIR [16]. Crucial in the engineering of this artificial transplantation site is the polymer. The polymer should not induce severe inflammation, toxicity, or a severe foreign body response, and simultaneously support vascularization. These are very pertinent considerations for islet transplantation as human cadaveric donors are scarce. Therefore, loss of functionality or viability should be reduced to a minimum.

In this study we designed a novel platform to test and select possible applicable polymers for production of a scaffold for pancreatic islets. We combined a number of assessment technologies that are rather new for the pancreatic islet transplantation field. In addition to general parameters such as glucose-stimulated insulin secretion (GSIS) and viability staining [17, 18], we applied assays to determine the release of danger-associate molecular patterns (DAMPs) and nanotomy. DAMPs released by pancreatic islets have been shown to be associated with several cell-death processes such as necrosis, necroptosis, autophagy, and secondary apoptosis [19]. DAMPs from islets are also immunostimulatory as they stimulate Toll-like receptors on immune cells [19]. Furthermore, we applied transmission electron microscopy and created large-scale high-resolution images of a cross section of the whole islet [20–22]. The technology, called nanotomy [20–22], allows discrimination of β -cells from other endocrine cells, insulin content and location as well as cell-cell contacts, cell-biomaterial interfaces, β -cell condition, endoplasmic reticulum (ER) stress, abnormalities in mitochondria, and fibrosis [20–22].

The following polymers were tested: poly(D,L-lactide-co- ϵ -caprolactone) (PDLLCL), poly(ethylene

oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer, and polysulfone. PDLCL is a random copolyester of lactide and ϵ -caprolactone synthesized by Polyganics (Groningen, The Netherlands) to produce an anti-adhesive sheet Vivosorb[®] and a biodegradable nerve guide Neurolac[®] [23]. PolyActive is an example of a commercial PEOT/PBT copolymer; it is made of the soft, amorphous hydrophilic PEOT block and the hard, crystalline PBT [24]. Due to this structure the PEOT/PBT copolymer is very suitable for cement restriction or bone filling in orthopedics [25]. Polysulfone is used as dialysis membrane for chronic kidney diseases [26]. All three polymers are already used in the clinic and are FDA approved. In this study we investigated whether these polymers also meet the other requirements described above in order to be applied in a scaffold for human islet transplantation. The polymers are exclusively tested for application in a scaffold. The platform was not designed for polymers applicable for immunoisolation.

Materials and methods

Polymers

PDLLCL (poly(68/32[15/85D/L-lactide]-co- ϵ -caprolactone)) was provided by Polyganics. 150PEOT50PBT50 (PEOT/PBT 150) was purchased from PolyVation (Groningen, The Netherlands). Polysulfone was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). The hydrophilicity of PDLCL and polysulfone was modulated by adding polyethylene glycol (PEG; Merck Millipore, Amsterdam, The Netherlands) with a molecular weight of 20000 g mol⁻¹ resulting in PDLCL-PEG (50/50 w/w) and polysulfone-PEG (87.5/12.5 w/w). The hydrophilicity of PEOT/PBT 150 was adjusted by using PEG of another molecular weight (4000 g mol⁻¹ instead of 150 g mol⁻¹) and by changing the ratio of the copolymer (50/50 w/w to 30/70 w/w).

Solvent casting

Glass petri dishes (40 mm) were spin-coated with the polymer solutions. A 13% (w/w) PEOT/PBT 150 and a 10% (w/w) PEOT/PBT 4000 solution was prepared in 23% (w/w) hexafluoroisopropanol (Sigma-Aldrich) and 77% (w/w) chloroform (Merck). A 3% (w/w) polysulfone solution and a 2% (w/w) polysulfone solution with 0.3% (w/w) PEG was prepared in 100% (w/w) chloroform. These polymer solutions were casted in glass petri dishes. Petri dishes were rotated at high speed using a Convac 1001S spin coater (ST 143 control unit; Convac, Wiernsheim, Germany) in order to create a thin (0.5–1.5 mm) film on the bottom of the petri dishes. Polymer dishes were left overnight to allow evaporation of the solvents. PDLCL and PDLCL-PEG blend (50/50 w/w) films were casted at Polyganics from a 2% (w/w) polymer solution in chloroform. Residual solvents were removed by drying under reduced pressure overnight. All polymer dishes were stored at 4 °C. Before use, the dishes were incubated

Table 1. Donor characteristics at time of shipment.

Age (years)	BMI	Gender (F/M)	HB/NHB	Purity (%)
52 (34–60)	26 (23–31)	9/9	12/6	70 (40–80)

Note: Data is plotted in median (interquartile range). Heart beating (HB) and non-heart beating donors (NHB) were used, both female (F) and male (M).

overnight in CMRL (Gibco; Life Technologies, Bleiswijk, The Netherlands) to remove solvent residues.

Hydrophilicity of the polymers

The hydrophilicity of the polymers was determined by measuring the contact angle using the static sessile drop method. A drop of Milli-Q water was placed onto the surface through a micro syringe [27]. A photograph for quantification of the angle was taken after water drop stabilization. The contact angle of the droplet was determined with a custom-made camera-goniometer system [27]. On each sample, five droplets were placed on different spots. Three samples of each polymer type were measured.

Human pancreatic islets

Human islets were isolated at the pancreatic islet isolation laboratory of the Leiden University Medical Center (Leiden, The Netherlands), as previously described [28]. The Dutch Government has approved human islet isolation by this institution for clinical and research purposes. In this experiment we used human islets which were not eligible for clinical transplantation. Only islets of donors with a signed informed consent were used. All anonymous donors were coded in accordance with the Code of Proper Secondary Use of Human Tissue in The Netherlands, which is formulated by the Dutch Federation of Medical Scientific Societies. In some cases, human islets were ordered from Prodo Laboratories Inc. (Irvine, USA).

Table 1 presents donor characteristics at the time of islet shipment. A dithizone staining was performed before shipment to determine the purity. Islets were shipped to the Groningen University Medical Center (Groningen, The Netherlands) in CMRL 1066 supplemented with 2 mg ml⁻¹ Ciproxin (Sigma-Aldrich), 50 mg ml⁻¹ Gentamicin (Sigma-Aldrich), 200 mM L-Glutamine (Sigma-Aldrich), 250 µg ml⁻¹ Fungizone (Sigma-Aldrich), 1 M Hepes (Gibco), 300 mg ml⁻¹ Nicotinamide (Sigma-Aldrich), and 10% human serum (Thermo Scientific, Breda, The Netherlands). After shipment islets were handpicked and washed five times with CMRL before culture.

Islet culture

Human islets (50–80 per ml) were cultured in the polymer-coated petri dishes in CMRL containing 8.3 mM glucose (Sigma-Aldrich), 20 mM Hepes, 2 mM Glutamax (Gibco), 50 U ml⁻¹–50 µg ml⁻¹ Penicillin-Streptomycin (Gibco), and 10% fetal calf serum (Thermo Scientific) at 37 °C and 5% CO₂. Control

Table 2. PCR primer sequences (Sigma-Aldrich).

Genes	Forward	Reverse
TWIST1	CCGGAGACCTA-GATGTCATTG	CACGCCCT-GTTTCTTTGAAT
SNAI1	GCTGCAG-GACTCTAATCCAGA	ATCTCCGGAG-GTGGGATG
SNAI2	TGGTTGCTTCAA-GGACACAT	GTTGCAGT-GAGGGCAAGAA
β-Actin	CCAACCGCGA-GAAGATGA	CCAGAGGCGTA-CAGGGATAG

islets were cultured in conventional conditions in non-treated 6-well plates (Corning; Sigma-Aldrich) to maintain suspension culture. Islets were cultured for 7 d. At day 1, 3, and 7 functional islet survival was analysed.

Islet viability

Islet viability was determined with an ethidium bromide staining (Molecular Probes; Life Technologies) according to the manufacturer's protocol. Briefly, islets were handpicked from the polymers by gentle aspiration with a 100 µl capillary and incubated with 2 mM ethidium bromide for 30 min at room temperature. Islets were studied by confocal microscopy (Leica SP2; Leica Microsystems B.V., Rijswijk, The Netherlands). The percentage of dead cells was determined using Image J (Version 1.47f; Rasband, W.S., ImageJ, U.S.A. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997–2012).

Electron microscopy

Cellular structures were analysed by embedding islets for electron microscopy. Briefly, islets were fixed in 2% glutaraldehyde and 0.5% PFA in 0.1 M cacodylate buffer for minimally 1 h and embedded in 2% low melting point Agarose (VWR, Amsterdam, The Netherlands). Agarose-embedded islets were post-fixed for minimally 2 h at 4 °C with 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M sodiumcacodylate and rinsed twice with Milli-Q. After dehydration with a graded series of ethanol (30%, 50%, 70%, 100%) and subsequently 30 min incubation with acetone, islets were left overnight in 1:1 acetone and EPON. The acetone/EPON was replaced by pure EPON and islets were incubated for minimally 3 h. After 15 min at 58 °C and 1 h at room temperature at reduced pressure (200 mbar), islets were embedded in beemcapsules. Polymerisation was completed in 1–2 nights at 58 °C. Ultrathin sections (60 nm) were cut on an ultramicrotome (Leica UC7; Leica Microsystems B.V.) with a diamond knife (Diatome Inc., Biel, Switzerland) and contrasted using 2% uranylacetate in methanol for 2 min followed by 2 min of Reynolds leadcitrate. Images were taken with a Zeiss Supra55 in STEM mode at 29 KV using an external scan generator (Fibics, Ottawa, Canada) yielding mosaics of large area scans at 2 nm pixel resolution [21, 22]. These large-scale

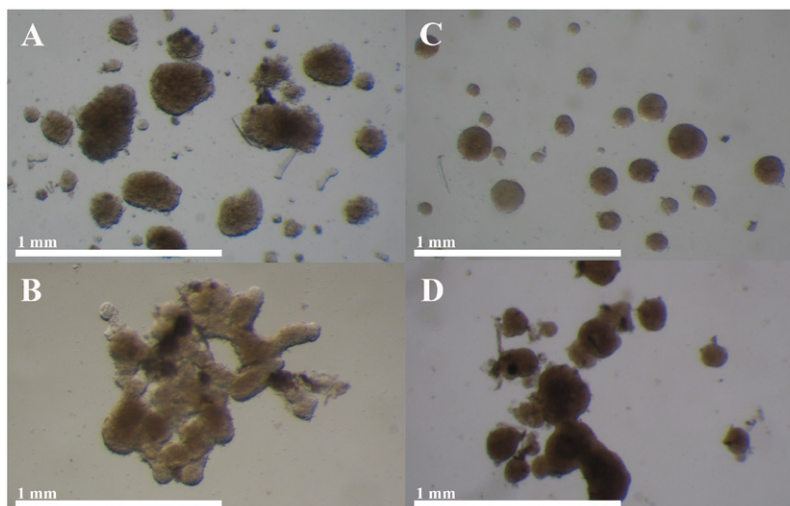


Figure 1. Morphology of islets cultured on the various polymers. The morphology of islets at day 1 of culture on PEOT/PBT 150 (B), PDLLCL (C), and polysulfone (D) compared with conventional islet culture (control; (A)).

TIF images were stitched and converted to html files using VE Viewer (Fibics) and are available through www.nanotomy.org. After exporting the stitched tiles as a single TIF file (downscaled to 10 nm pixel^{-1}), annotation has been performed manually using Adobe Photoshop CS6 (Adobe Systems Benelux BV, Amsterdam, The Netherlands).

Quantification necrosis

To quantify the amount of necrosis in islets cultured on the different polymers, the presence of unmethylated double-stranded DNA (dsDNA) was quantified with a Human CpG oligodeoxynucleotide (CpG-ODN) ELISA kit (Qayee-Bio, Shanghai, China). Samples of the supernatant were taken on day 1 and 7 of culture in order to measure dsDNA according to manufactures instructions. All incubations were performed at 37°C and $5\% \text{ CO}_2$. The optical density was measured at 450 nm immediately after adding the stop solution using a microplate reader (VersaMax; Molecular Devices, Berkshire, United Kingdom).

Phenotyping of cellular outgrowth

Islets tend to adhere to hydrophobic surfaces after which cellular outgrowth can occur. In a series of experiments it was determined whether these outgrowing cells are fibroblasts or endocrine cells undergoing epithelial to mesenchymal cell transition (EMT). In one of the series of experiments 5 mM 2-deoxyglucose (Sigma-Aldrich) was added to the cultures. The 2-deoxyglucose is selectively toxic for fibroblasts and was used to delete fibroblasts from the culture [29, 30]. After 7 d of culture in CMRL containing 2-deoxyglucose, cells were processed for Real Time reverse transcription polymerase chain reaction (RT-PCR) for the EMT markers TWIST1, SNAI1, and SNAI2. Fetal lung fibroblasts (flf92, passage 7) were used as positive control. RNA was isolated using the RNeasy plus micro kit (Qiagen, Venlo, The Netherlands) according to the

Table 3. Contact angles of the various polymer compositions.

Polymers	Contact angles	Significance ^a
PDLLCL	$85.7^\circ \pm 4.8$	$p < 0.001$
PDLLCL-PEG 50/50	$43.3^\circ \pm 2.9$	
150PEOT50PBT50	$82.3^\circ \pm 2.6$	$p < 0.001$
4000PEOT30PBT70	$53.0^\circ \pm 0.8$	
Polysulfone	$78.0^\circ \pm 1.4$	$p < 0.001$
Polysulfone-PEG 87.5/12.5	$45.5^\circ \pm 2.4$	

Note: Contact angles (degrees) and standard deviation measured with static sessile drop method. The contact angle quantifies the wettability of the polymer surface by a water drop via the Young equation.

^a A statistical analysis was carried out using an unpaired t test with Welch's correction, $p < 0.05$

manufacturer instructions. The RNA concentration was determined using a NanoDrop 1000 spectrophotometer (NanoDrop products, Wilmington, USA). cDNA was reverse transcribed using a SuperScript[®] III Reverse Transcriptase kit according to the instructions of the manufacturer (Life technologies). Real Time RT-PCR was conducted using ViiA[™] Real Time PCR system (Life technologies). It was performed in a final reaction volume of $10 \mu\text{l}$, consisting of SYBR Green Supermix (Bio-Rad, Hercules, USA), $6 \mu\text{M}$ primer-mix (table 2) and 10 ng cDNA. Reactions were performed at 95°C for 10 min, 95°C for 15 s repeating in 40 cycles, 60°C for 30 s, and 72°C for another 30 s. PCR was performed in triplicate. Delta Ct values were calculated and normalized against the expression of the housekeeping gene β -Actin.

Glucose-stimulated insulin secretion of isolated pancreatic islets

Islet function was determined with a GSIS test. Briefly, three batches of 25 islets were handpicked and incubated with low glucose (2.75 mM) Krebs–Ringer–Hepes (KRH; pH 7.4, 133 mM NaCl, 4.69 mM KCl,

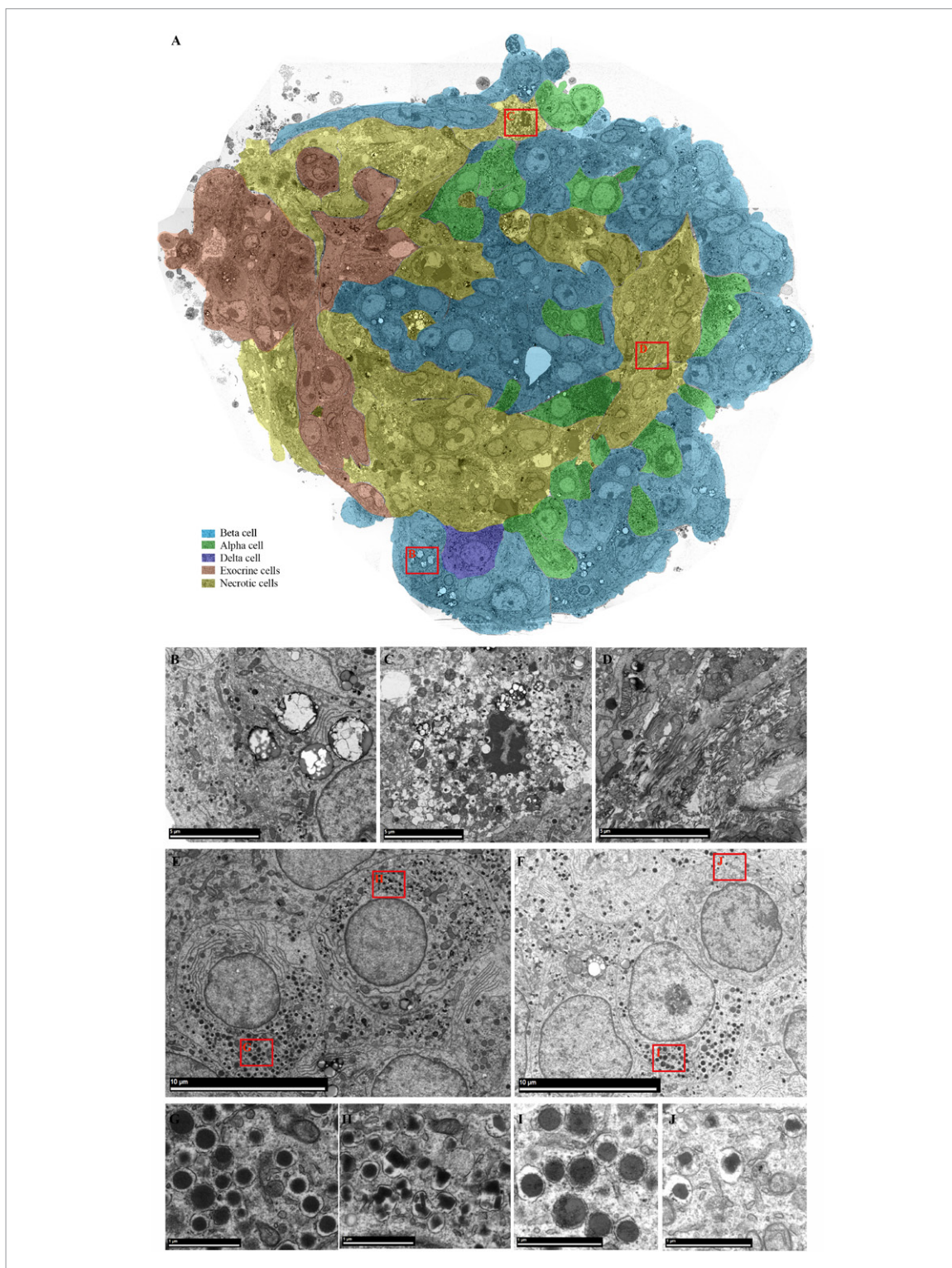
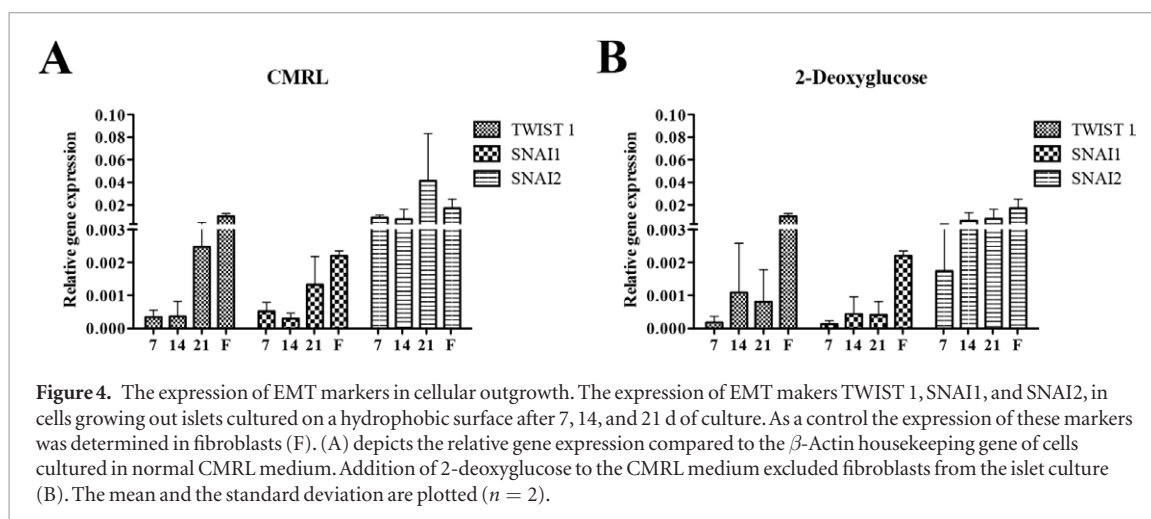
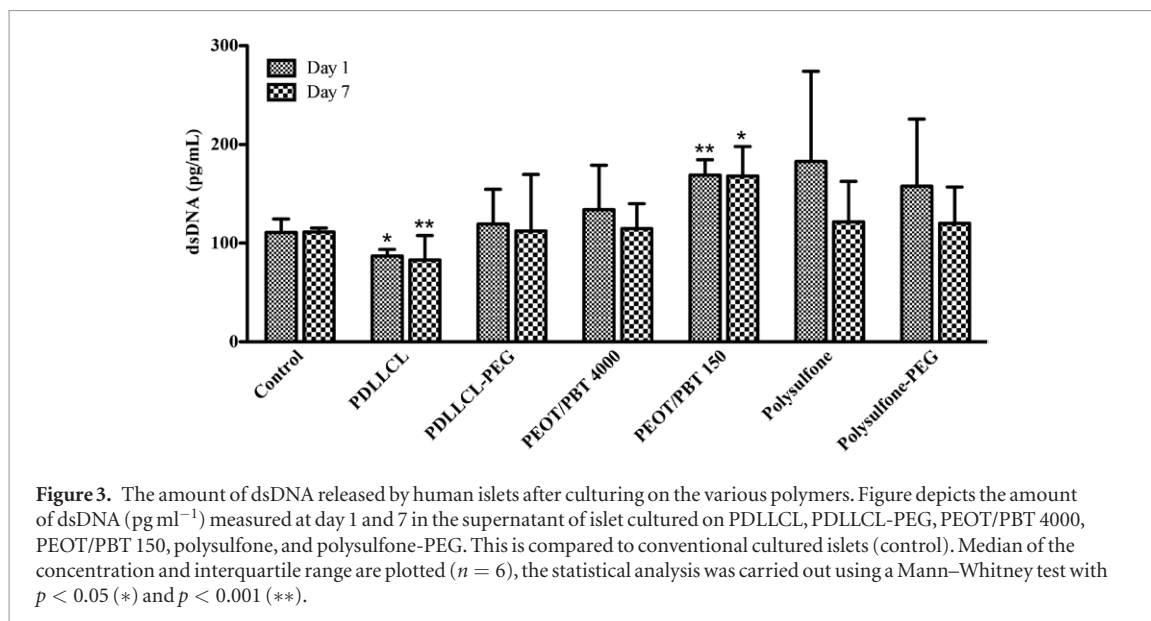


Figure 2. The effect of PDDLCL and PEOT/PBT 400 on a subcellular level demonstrated by electron microscopy. Control islets (A) contained β -cells (blue), α -cells (green), δ -cells (purple), exocrine cells (orange), and necrotic cells (yellow). The hormone granules of β -cells show a high degree of variation in their ultrastructure, whereas α and δ granules appear round, dense, and homogeneous. The granules of δ -cells are larger than the α -cell granules. The endoplasmic reticulum is more prominent in exocrine cells. Necrotic cells show disruption of the cell membrane and loss of organelles. A high number of lysosomes (B), cell type heterogeneity, and necrosis (C) were observed in similar degree in control and polymer culture conditions. In necrotic areas collagen deposition was found (D). The α and β -cells of islets cultured on PDDLCL (E) contained more hormone granules (glucagon G and I, insulin H and J) than the islets cultured on the other polymers, such as PEOT/PBT 400 (F).

1.18 mM KH_2PO_4 , 1.18 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 25 mM HEPES, 2.52 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution for 1 h at 37 °C with continuous shaking. Subsequently, islets were incubated in high glucose (16.5 mM) KRH solution for 1 h at 37 °C with continuous shaking. Finally, another low glucose incubation followed. After each

incubation period, supernatant was sampled for quantification of the insulin concentration. Insulin concentrations were determined with a human insulin ELISA kit (Merckodia, Uppsala, Sweden) according to manufactures instruction. The optical density was measured at 450 nm within 30 min after adding the



stop solution using a microplate reader (VersaMax; Molecular Devices). Normal islet function was defined as at least a two-fold increase in insulin levels in the high glucose incubation compared to the first low glucose incubation. The function of islets cultured on the polymers was compared to the function of control islets that were cultured under conventional conditions.

DNA content of the islets used for the GSIS test was determined using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen; Life Technologies). Islets were sonicated (Bandelin electronic, Berlin, Germany), and DNA content was quantified according to the manufacturer's instructions. The insulin concentration of each islet batch was divided by the corresponding DNA content in order to correct for size differences between the islets.

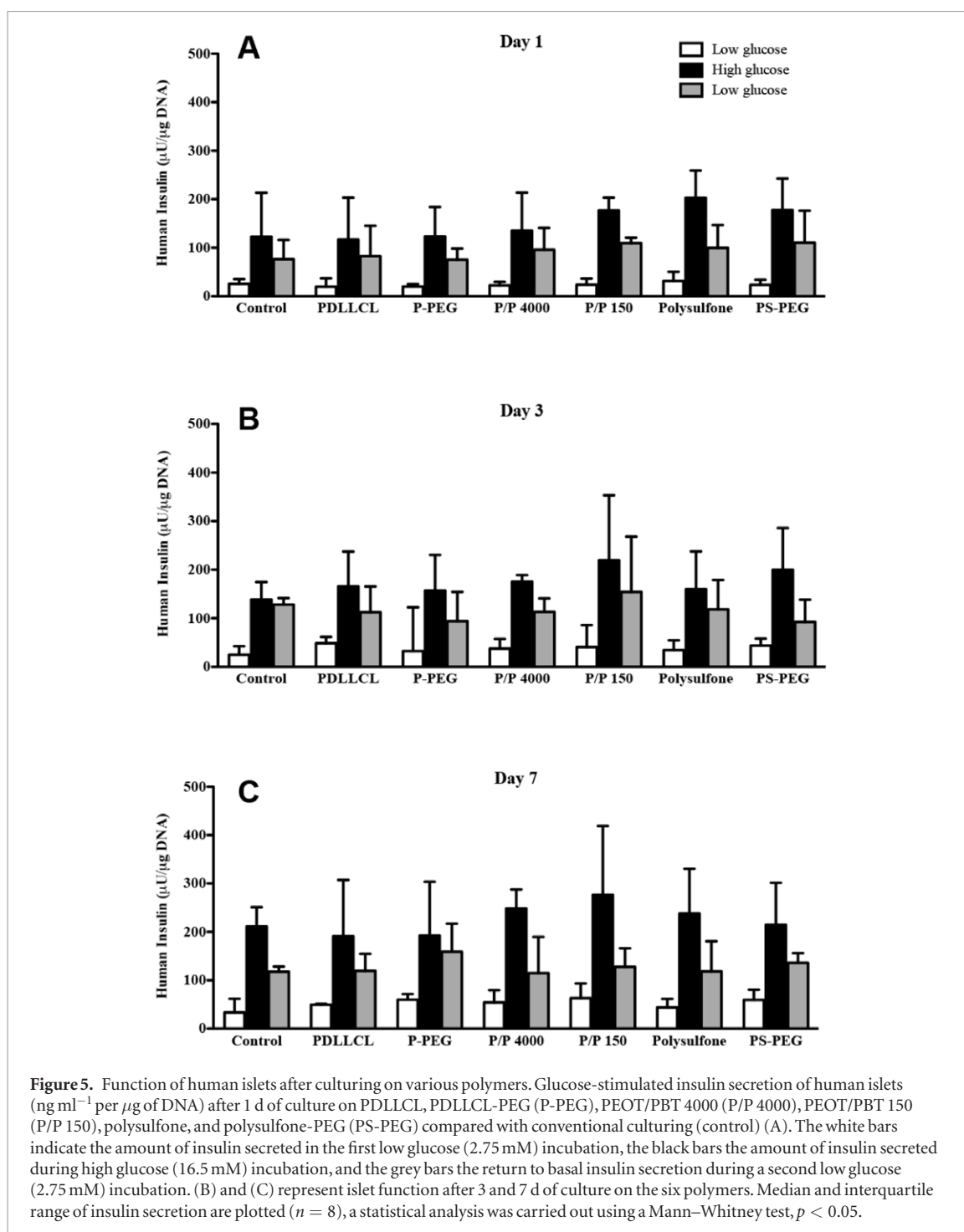
Statistical analysis

For statistical analysis of the contact angles an unpaired t test with Welch's correction ($p < 0.05$) was applied. Data was tested for normality by the Shapiro–Wilk normality test ($p < 0.05$). For testing differences in

contact angle, insulin secretion, cell death, and release of dsDNA a Mann–Whitney U test ($p < 0.05$) was performed. The effect of hydrophilicity on dsDNA release was tested with a two-way ANOVA ($p < 0.05$).

Results

Human islets were cultured on PDLCL, PEOT/PBT 150, and polysulfone, i.e. three polymers that potentially qualify for application as scaffold for transplanted islets [5]. As islets are extremely sensitive cells and isolated from relative rare cadaveric donors, interaction with a polymer should not lead to dysregulation of islet viability or function [31, 32]. Therefore, it is important to study this islet-polymer interaction. On the first day of culture, there were already clear differences in the morphology of the seeded islets (figure 1). Compared to controls, the morphology of islets cultured on PDLCL was more spherical with only minor aggregation. However, islets cultured on PEOT/PBT 150 and polysulfone performed worse as the islets clustered and formed large aggregates. Furthermore, we observed significant outgrowth of cells on all the polymers.



Influence of hydrophilicity of the polymer

As hydrophilicity of the polymers might influence the aggregation and outgrowth of cells, we also cultured human islets on polymers with an altered hydrophilicity (table 3). These modulations reduced the contact angle, which is a measure for hydrophilicity. The contact angles of PDLCL, PEOT/PBT 150, and polysulfone were in the hydrophobic range. The contact angles were significantly reduced ($p < 0.001$) after modulation and therefore, PDLCL-PEG, PEOT/PBT 4000, and polysulfone-PEG are classified as hydrophilic. To investigate whether the change in hydrophilicity results in increased cell death, we compared cell survival by performing confocal studies on the islets. The

alterations of hydrophilicity did not result in changed cell-death frequencies as studied after ethidium bromide staining (data not shown). Notably, ethidium bromide only stains cells in apoptosis. Necrotic cells cannot be quantified by this staining procedure. To determine if the polymers influence necrosis, we applied nanotomography and DAMP measurements.

Nanotomography and necrosis

Nanotomography showed high heterogeneity in quality of islet-tissue. Nanotomography revealed that all islets contained a number of cells with features of necrosis. We found collagen deposition, dilatation of the ER, and some cells were lacking a membrane (figure 2). However, these

features were also found in controls. Under all culture conditions a high amount of lysosomes was observed. In islets cultured on PDLLCL α and β -cells were having more hormone granules than in the other conditions.

As nanotomography is a qualitative measure and not a quantitative technique to identify necrosis, we also determined the release of dsDNA by the islets. Released dsDNA is a damage associated molecular pattern (DAMP). DAMPs are used in many disciplines of research to quantify necrosis of cells [33]. As shown in figure 3, there was a statistically significant difference in DAMP release ($p < 0.05$) from the islets under the different culture conditions beginning as of day 1 of culture. Islets cultured for 1 and 7 d on PDLLCL released approximately 25% less dsDNA compared to the control cultured islets ($p < 0.05$ for day 1, and $p < 0.01$ for day 7). In the supernatant of islets cultured on PEOT/PBT and polysulfone, the amount of dsDNA was 75% higher than in the control-cultured islets. The amount of dsDNA released by islets cultured on PEOT/PBT 150 was significantly higher than released by the control islets at day 1 ($p < 0.01$) and day 7 ($p < 0.05$). Hydrophilicity did not influence differences in dsDNA release. A two-way ANOVA ($p < 0.05$) showed no significant differences between the hydrophilic and hydrophobic counterpart of each polymer (data not shown).

Outgrowing cells are mainly fibroblasts

The observed outgrowing cells could be islet cells undergoing EMT or it could be proliferating fibroblasts. We observed that islets easily attached to a hydrophobic surface, and cells rapidly proliferated from the islets. To investigate the phenotype of these cells, we harvested the outgrowing cells and determined the expression of the EMT markers SNAI1, SNAI2, and TWIST 1 was determined with Real Time RT-PCR.

Our analysis demonstrated that the cellular outgrowth of islets consisted mainly of fibroblasts. Phenotyping of the outgrowing cells showed that the expression of EMT markers SNAI1, SNAI2, and TWIST 1 increased during culture on a hydrophobic surface (figure 4(A)). However, these expression levels were not high. The relative gene expression compared to β -Actin at day 21 of culture was 0.0025 (Twist 1), 0.0015 (SNAI1), and 0.02 (SNAI2). The fibroblasts, the positive control, also expressed these levels. To exclude the fibroblasts from the proliferating cells, islets were cultured in the presence of 2-deoxyglucose (figure 4(B)). The 2-deoxyglucose selectively kills fibroblasts [29, 30]. The expression of the EMT markers decreased approximately 50% in the 2-deoxyglucose after 21 d in cultures. This low expression of EMT markers indicates that there was some EMT involved, but the main phenotype of the cells was fibroblast.

Undisturbed glucose-stimulated insulin secretion

To determine whether the cell loss due to necrosis and cellular outgrowth are also associated with impairment of the functionality of the islets, a glucose-stimulated insulin

secretion test was performed. None of the polymers statistically significantly disturbed functionality of the human pancreatic islets. Up to day 7 of culture, islets cultured on the various polymers retained their ability to respond properly to changes in glucose concentrations during the GSIS test (figure 5). The glucose-stimulated insulin release showed a four-fold increase when islets were incubated in 16.7 mM glucose compared to the first incubation with 2.75 mM glucose. The subsequent 2.75 mM glucose incubation after the 16.7 mM incubation caused a return of insulin to a lower level, indicating that islets can recover from the high glucose exposure. However, this level was still three-fold higher than the insulin concentration in the first low glucose incubation. The hydrophilicity of the polymers did not cause differences in functionality during the 7 d of culture.

Discussion

Many biomaterials have been applied during the past two decades for enveloping and seeding islets for either immunoprotection or for supporting islet function in recipients [13, 34]. A comparison study on compatibility of biomaterials with human islets has not been done up to now, although maintaining the functional cell-viability is an important prerequisite when using human cadaveric islets. Here we applied a well-defined set of specific analyses; GSIS, viability staining, DAMP-release and nanotomography, for a systematic selection of suitable islet supporting biomaterials, and demonstrate that not all biomaterials have the same degree of compatibility with human pancreatic islets. As shown, PDLLCL does not interfere with functionality and was not associated with significant loss of islet cells or DAMPs released. PDLLCL was associated with higher granulation of α and β -cells. PEOT/PBT and polysulfone did not meet these requirements. Despite a non-disturbed glucose-stimulated insulin release the increased release of dsDNA suggests more islet loss by (secondary) necrosis and necroptosis when cultured on PEOT/PBT and polysulfone [19].

The release of dsDNA requires some further consideration as it is not only associated with cell-loss, but dsDNA is also a DAMP that provokes immune activation [19, 35]. DsDNA is released when necrotic cells lose the integrity of their plasma membrane and release their intracellular content. The intracellular substances will serve as alarm molecules or DAMPs. DAMPs, such as dsDNA, activate antigen-presenting cells via toll-like receptors (TLRs) and thereby stimulate an inflammatory response via the innate and adaptive immune system contributing to failure of islet-cells [19, 33]. Therefore, the increased dsDNA release *in vitro* is a strong indication that PEOT/PBT and polysulfone might provoke increased DAMP production and associated immune responses. Therefore, we conclude it is not advisable to use PEOT/PBT and polysulfone for islet-scaffolds. Polysulfone has been applied as enveloping,

tubing material for islet grafts in immunoprotection applications [36], with varying degrees of success [37–39]. Our data contribute to the understanding of the limited survival time of islets in these tubes.

The polymers have an immediate effect on islet-biology as illustrated by the fact that as of day one in culture islets tended to aggregate on PEOT/PBT and polysulfone and to a lesser extent on PDLCL. Islets are very sensitive organ structures that demonstrate disturbed function when the architecture is changed when for example cells adhere to the biomaterials or grow out of the islets. As hydrophilicity of a material is influencing dynamics of cells such as cell adhesion, proliferation, and morphology, we investigated whether the differences in hydrophilicity had to be held responsible for the observed differences. By adding PEG and by changing the ratio of the PEOT/PBT copolymer, all three polymers were tested at a similar hydrophilicity. Comparison of the polymers at similar hydrophilicity revealed that hydrophilicity does influence the amount of cellular outgrowth; on the hydrophobic polymers an increase in cellular outgrowth was found. However, the hydrophilicity does not influence dsDNA release, the amount of dead cells in islets, and the glucose-stimulated insulin release. Therefore, it is concluded that it is predominantly the chemical structure that influences DAMP release and functional survival of islet-cells.

We studied the origin and fate of the cellular outgrowth on the polymers as it was postulated that this could be islet-cells in the process of EMT or another proliferating cell type such as fibroblasts. The outgrowth of cells from islets was polymer chemistry dependent and was more severe in islets cultured on PDLCL and PEOT/PBT than on islets cultured on polysulfone. Furthermore, the outgrowth seemed to be polymer hydrophilicity dependent as it was more pronounced on the hydrophobic counterpart of each polymer. During EMT epithelial cells lose their epithelial characteristics, such as tight junctions and cell polarity, and acquire mesenchymal cell characteristics, such as migration, expression of E-cadherin, and Vimentin [40]. After EMT, the endocrine-cells do not contribute to graft function anymore.

Here we show by a combined strategy of quantifying EMT in islet cultures in which fibroblast were eliminated with 2-deoxyglucose or were maintained, that both processes occur in polymer-cultured islets, but that it are mainly fibroblasts that grow out of the islets. However, it might be argued that these fibroblast-like cells are still dedifferentiated β -cells as it has been shown that β -cells can differentiate into fibroblast-like cells by EMT [41, 42]. However, our results with 2-deoxyglucose and the fact that we never observed high expression of EMT markers in combination with genetic-based lineage tracing of others [43] support our argumentation that these cells originate from contamination of still present mesenchymal cells after islet isolation [43]. Our data show that this outgrowth of fibroblasts did not affect the function of islets.

A disadvantage of using islet-tissue derived from human donors is the high variation in GSIS due to difference in donor genetics, diet habits, age, and weight [44, 45]. It is virtually impossible to control all these GSIS parameters with the low availability of donors. This heterogeneity in quality of human islets was also observed in our nanotomy analysis. In many islets we found high amounts of lysosomes indicating that insulin is being degraded [46]. To increase the sensitivity of our platform more tests for islet function has been included such as DAMP (dsDNA) release, which has impact on both survival and immunological responses against islets, as well as nanotomy, and cellular survival. Our results that GSIS was not different between islets cultured on the three polymers support our suspicion that taking only GSIS with all its donor associated variability may lead to underestimation of impact of polymers on human islet function. This technology platform may also be applicable for more than just human islets. Also in tissue engineering and cellular delivery in which rare, cadaveric donors are applied the platform may lead to selection of adequate polymers [47].

An advantage of PDLCL in addition to its optimal compatibility with islets is that it is biodegradable. Recently it has been shown that after implantation around a scaffold a local environment is developed facilitating continuous support and survival of the cells even after removal of the biomaterial [48]. PDLCL degrades in 4 till 24 months by hydrolysis [49]. The degradation products of this polymer are non-acidic, so will not cause damage to the surrounding tissue [50]. PDLCL has already been successfully applied for repairing nerve defects in humans [51]. It supports wound repair and has more advantages for the patient such as prevention of scar formation [51, 52]. However, some studies demonstrated the presence of small fragments after 24 months of implantation that nourished a mild secondary immune response [52], which require some further, long term safety evaluations.

Our current study was designed to select a polymer applicable for creating a transplantation site for free pancreatic islets as alternative for the liver as transplantation site. The polymer scaffold will be used as an islet-friendly environment, with an open structure in which islets are readily revascularized. However, our findings also have implications for another field in islet transplantation where biomaterials are applied, i.e. immunisolation or encapsulation of islets. This field is faced with a frustrating lab-to-lab variation in longevity of the encapsulated islets [53]. Many factors contributing to this variation in survival of islets has been described [53–56], but effects of the biomaterials on the islets in the capsules have not been studied nor implicated as explanation for low functionality of islets up to now. Our data demonstrate that the effects of the polymer on islet-survival and the effect on release of islet-derived pro-inflammatory factors such as DAMPs might be an additional factor responsible for the lab-to-lab variations of encapsulated islet grafts.

In conclusion, we have shown that the type of polymer influences function and cellular homeostasis of human pancreatic islets. Islets cultured on PDLCL performed better than islets on two other commonly applied polymers for creating scaffolds [15, 36, 57]. PDLCL seems to be a promising candidate for creating an artificial transplantation site for human pancreatic islets. A PDLCL scaffold can offer advantages as minimal loss of tissue and maintenance of islet function. Thereby improving the long-term success of islet transplantation. However, further human *in vivo* studies are warranted.

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