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In vivo vascularization and islet function in a microwell device for pancreatic islet transplantation

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Supplementary material for this article is available [online](#)

Abstract

Islet encapsulation in membrane-based devices could allow for transplantation of donor islet tissue in the absence of immunosuppression. To achieve long-term survival of islets, the device should allow rapid exchange of essential nutrients and be vascularized to guarantee continued support of islet function. Recently, we have proposed a membrane-based macroencapsulation device consisting of a microwell membrane for islet separation covered by a micropatterned membrane lid. The device can prevent islet aggregation and support functional islet survival *in vitro*. Here, based on previous modeling studies, we develop an improved device with smaller microwell dimensions, decreased spacing between the microwells and reduced membrane thickness and investigate its performance *in vitro* and *in vivo*. This improved device allows for encapsulating higher islet numbers without islet aggregation and by applying an *in vivo* imaging system we demonstrate very good perfusion of the device when implanted intraperitoneally in mice. Besides, when it is implanted subcutaneously in mice, islet viability is maintained and a vascular network in close proximity to the device is developed. All these important findings demonstrate the potential of this device for islet transplantation.

1. Introduction

Type 1 diabetes (T1D) is characterized by the autoimmune destruction of the insulin-producing islets of Langerhans, which are located in the pancreas. This leads to an impaired glucose homeostasis. Therefore, the T1D patients rely on insulin administration to control their glucose levels, but this does not provide the precise control observed in non-diabetics. As a consequence, these patients may develop typical long-term complications associated with insulin therapy like retinopathy, nephropathy, and cardiovascular diseases later in their life [1].

Islet transplantation has emerged as treatment option for T1D patients since it could provide minute-to-minute control of the blood glucose levels [2] and thereby could prevent the development of the complications mentioned earlier. Clinically, islets are

infused into the liver but despite marked progress, islet transplantation lacks long-term success [3]. In fact, islets lose their function due to e.g. hypoxia [4], the instant blood-mediated inflammatory reaction [5] and high amounts of immunosuppressive drugs present in the liver [6].

To improve islet transplantation outcomes and prevent liver-associated limitations, the extrahepatic islet transplantation, as well as, immune isolating devices that potentially avoid the chronic use of immunosuppression have been investigated. These devices should meet specific requirements, including allowing long-term survival such as rapid revascularization, allowing exchange of nutrients, insulin and glucose, and accessible to allow transplantation with minor surgery, while at the same time accommodate high number of islets. In fact, several islet transplantation devices could restore the glucose homeostasis

in T1D animal models [7–10]. However, their geometry and characteristics limit their clinical success due to aggregation of islets and due to the relatively large diffusion distances of the islets from vascularization which limits transport of oxygen and nutrients to the islets. Within these devices, islets need to compete for nutrients and oxygen leading to the loss of a large numbers of islets. The islets in their native microenvironment are highly vascularized but unfortunately their own vascular network is disrupted during their isolation procedure [11, 12]. A functional vascular network in close proximity of the device is imperative for functional islet survival. In fact, blood vessels should be within 100–150 μm of the islets to adequately facilitate the exchange of nutrients, oxygen, and hormones across the membranes of the devices [13].

Recently, we have proposed a membrane-based macroencapsulation device consisting of a microwell membrane for islet separation covered by a membrane lid; both membranes were prepared using a low cell adhesive polymer blend of poly (ether sulfone)/polyvinyl pyrrolidone (PES/PVP). This device can prevent islet aggregation and support functional islet survival *in vitro* [14]. Besides, when a micropatterned lid is applied, the device can induce alignment of mesenchymal stem cells (MSCs) *in vitro* and contribute to improved vascularization *in vivo* [15]. Moreover, recently, based on modeling studies we proposed an improved device with optimal spatial distribution of oxygen gradient and insulin secretion [16]. This device should have smaller microwell (width: 180 μm , depth: 200 μm), decreased spacing between the microwells (10 μm) and reduced membrane thickness (50 μm). Here, we perform a systematic *in vitro* and *in vivo* study of this optimized device. We first investigate the membrane morphology by scanning electron microscope (SEM) and the membrane transport characteristics, followed by an *in vitro* study of islet functionality within the device. Subsequently, the vascularization of the device *in vivo* is evaluated at two different implantation sites, i.e. subcutaneous and intraperitoneal. Finally, to prove that this optimized device can support *in vivo* islet function, we perform an islet transplantation study in mice.

2. Materials and methods

2.1. Microwell device fabrication

The microwell device consists of two micropatterned porous membranes: a membrane with microwells and a membrane with intermittent lines used as a lid. A 15 wt% PES (Ultrason; BASF, Ludwigshafen, Germany), 5 wt% PVP (40.000 kDa; Sigma-Aldrich, Zwijndrecht, The Netherlands) polymer blend in N-methylpyrrolidone (Sigma-Aldrich) was used to fabricate both types of porous membranes via phase

separation micromolding, as previously described [14, 17]. Briefly, the membranes were prepared by casting the polymer solution on a custom made, silicon, micropatterned mold for the creation of the different micropatterns (figures 1(A) and (B)). A custom-made casting machine, with micrometric screws to regulate the casting thickness, was used to obtain 50 μm thick membranes. Membrane casting was followed by immersion into a coagulation bath, containing demineralized water. After the polymer precipitation, the membranes were carefully removed from the mold and rinsed with demineralized water. In order to increase the membrane porosity, the membranes were treated with 4000 ppm sodium hypochlorite aqueous solution (NaClO ; Fluka, Sigma-Aldrich) for 24 h to remove part of the PVP. Subsequently, the membranes were washed and stored in demineralized water.

The device was prepared by sealing the microwell membrane and the micropatterned membrane with the intermittent lines. For this, both membranes were placed between the two shaped molds of a custom-made sealing machine, following the protocol elsewhere [14] (figure 1(C)). The sealed microwell devices were sterilized with 70% ethanol, washed in PBS and pre-incubated in culture medium overnight before using them for *in vitro* and *in vivo* testing.

2.2. Micropatterned membrane characterization

The membrane surface morphology was analyzed using SEM. Briefly, the membranes were dried overnight at room temperature, placed on the SEM holders and sputter-coated with 2–5 nm thick gold layer prior to imaging. The membrane permeability was estimated via clean water flux measurements. Membranes with an effective surface area of 0.9 cm^2 were used there. These experiments were performed using nitrogen pressurized dead-end Amicon-type ultrafiltration cell and MilliQ water followed the procedure described in [14]. Firstly, the membranes were pre-pressurized for at least 30 min at transmembrane pressure (TMP) of 0.5 bar. Afterwards, the clean water flux through the membrane at various TMPs was measured for at least 1 h. The membrane water permeability was calculated from the slope of the linear part of the flux versus the TMP relation ($n = 3$).

2.3. Islet isolation

All animal procedures described here are approved by the Dutch Central Authority for Scientific Procedures on Animals and the Animal Welfare Body of the University of Groningen (AVD1050020185726). For the *in vitro* tests, islets were isolated from male HsdCpb:WU rats (Envigo, Horst, The Netherlands) weighing 300–350 g. For the *in vivo* transplantation experiments, islets were isolated from both male and female MIP-Luc-VU mice (21–28 g) obtained from

our own breeding colony. The islets were isolated as described previously [18]. Briefly, under anesthesia the pancreas was distended by injecting collagenase NB8 (Serva, Heidelberg, Germany)/DNase I (Roche; Sigma-Aldrich) solution into the bile duct. After dissection, the tissue was further digested by incubating the distended pancreas for 18 min in a 37 °C water bath, with continuous shaking. Subsequently, islets were separated from the exocrine tissue by a histopaque (1119, 1077-Sigma-Aldrich) density gradient. Islets with diameter of 30–150 μm were counted and handpicked before culturing them in CMRL (Life Technologies; Thermo Fisher Scientific) supplemented with 8.3 mM glucose (Sigma-Aldrich), 20 mM Hepes (Gibco; Thermo Fisher Scientific), 2 mM Glutamax (Gibco), 50 U ml⁻¹–50 $\mu\text{g ml}^{-1}$ penicillin streptomycin (Gibco), and 10% fetal calf serum (Thermo Fisher Scientific) at 37 °C and 5% CO₂.

2.4. *In vitro* glucose-stimulated insulin secretion (GSIS) test

To determine the effect of the microwell polymer and thickness on islet function, rat islets were cultured for seven days in the microwell devices and subsequently a GSIS test was performed ($n = 3$). To this end, 150 rat islets were placed in the devices with a Hamilton syringe and compared to conventional-cultured control islets (free floating, not in a device). After seven days of culture, islets were removed from the devices and divided in three batches for the GSIS test. Briefly, the islets were first incubated in a 2.75 mM glucose solution consisting of Krebs-Ringer-Hepes (KRH; pH 7.4; 133 mM NaCl, 4.69 mM KCl, 1.18 mM KH₂PO₄, 1.18 mM MgSO₄·7H₂O, 25 mM HEPES, 2.52 mM CaCl₂·2H₂O) for 45 min at 37 °C in a shaking water bath. Subsequently, the islets were placed in a 16.5 mM glucose KRH solution for 45 min at 37 °C in a shaking water bath followed by a

second incubation in 2.75 mM glucose solution. After each incubation period samples were taken to measure the insulin concentration with an ultra-sensitive rat insulin ELISA kit (CrystalChem, Zaandam, The Netherlands). To correct the GSIS data for differences in islet size and number, the DNA content of the islets used for the GSIS was measured with the Quanti-iT[™] PicoGreen[®] dsDNA assay kit (Invitrogen; Thermo Fisher Scientific). Briefly, islets were lysed using a 1 M Ammonia/Triton X100 solution and sonication (Bandelin electronic, Berlin, Germany). Afterwards, the DNA content was measured according to manufacturer's instructions with the Varioskan microplate reader (Thermo Fisher Scientific).

2.5. *In vitro* vascularization

To investigate the vascularization close to the microwell device at different implantation sites, the device was subcutaneous or intraperitoneally implanted in immunocompetent 4–8 week-old C57BL/6BrdCrHsd-Tyrc mice (Envigo). The angiogenic process was measured non-invasively using a *in vivo* imaging system (IVIS) spectrum (PerkinElmer, Groningen, The Netherlands) at day 0, 7, and 14. For this, the mice received 24 h prior to imaging an intravenous, retro orbital injection of AngioSense 750 EX (2 nmol; PerkinElmer) under anesthesia. AngioSense is a near-infrared labeled fluorescent macromolecule that remains localized in the vasculature for extended periods of time and enables imaging of blood vessels and angiogenesis. The following fluorescent imaging setting were used: 1 s exposure time, medium binning, and FStop 2. The Living Image software (PerkinElmer) was used to analyze the obtained IVIS data.

At day 7, 14, and 28, the mice were sacrificed for histological analysis. To this end, explanted microwell devices were fixed in 4% paraformaldehyde and further processed for paraffin embedding. The paraffin sections were used for staining the blood vessels with the endothelial marker CD31. Briefly, sections were deparaffinized and antigen retrieval was applied by a 15 min incubation in 10 mM citrate buffer (pH 6.0) at 100 °C. Subsequently, blocking with 5% donkey serum (Sigma-Aldrich) was performed and an overnight incubation with the primary CD31 antibody (1:200; R&D Systems, Abingdon, UK) at 4 °C. The next day, sections were incubated for 45 min with the secondary donkey anti-goat alkaline phosphatase conjugated antibody (1:100; Abcam, Cambridge, United Kingdom) at room temperature. SIGMAFAST[™] Fast Red with a hematoxylin counterstain was used to show the alkaline phosphatase activity. All sections were analyzed with a Leica DM 2000 LED microscope with Leica DFC 450 camera (Leica Microsystems B.V., Rijswijk, The Netherlands) and scanned with a Hamamatsu Nanoxoomer slide scanner (Hamamatsu, Almere, The Netherlands) for measuring number of blood vessels

per mm of device and the distance between the device and the blood vessels in Aperio ImageScope (Leica Microsystems B.V.).

2.6. Islet transplantation

To investigate the *in vivo* survival of islets, the microwell device was first implanted empty (no islets) in 4–8 week-old immunodeficient athymic nude mice (Envigo; $n = 5$) and 28 days later 460–575 bioluminescent MIP-Luc-VU mouse islets were seeded in the device. To this end, a small incision was made in the skin next to the device. The port of the device was gently opened after which the islets were injected with a Hamilton syringe. After seeding the islets, a surgical clip was used to close the device and the skin was sutured. At day 0, 5, and 7 after transplantation IVIS was performed to measure the bioluminescence of the islets. Briefly, 150 mg kg⁻¹ body weight beetle (D)-luciferin dissolved in 3 g D-glucose/kg body weight solution was subcutaneously injected in close proximity of the device to induce bioluminescence. Within 5 min after injection, the IVIS images were acquired (auto exposure, medium binning, and F/Stop 2). One week after transplantation, mice were sacrificed and the device was explanted for CD31 staining. Nondiabetic and immunodeficient mice were used in order to study the survival without the discomfort of being diabetic and the need for immunosuppression.

2.7. Statistical analysis

All data was analyzed using GraphPad Prism (version 8.4.0; GraphPad Software, Inc., La Jolla, USA). A Shapiro–Wilk normality test was performed to test the data for normality. To test the effect of device thickness on islet function and implantation site on biocompatibility and vascularization a two-way ANOVA was applied. For the transplantation study a Friedman test was used, for both tests p -values < 0.05 were considered significant. The data are presented in mean $\pm$ standard error of mean.

3. Results

3.1. Micropatterned membranes characterization

We used phase separation micromolding to fabricate the highly porous, microstructured membranes for our device. Figures 2(A) and (B) present SEM images of developed flat PES/PVP membranes with microwells and figure 2(C) presents the SEM image of the lid with the intermittent lines. The membranes have high quality micropatterns with defined shape and size, closely resembling the designed topography of the silicon molds used for the membrane fabrication. The microwell membrane allows separation of the islets, preventing aggregation and avoids oxygen and nutrient transport limitations. The lid membrane has equally spaced intermittent lines to facilitate pre-vascularization of the device surface. We observed a typical membrane shrinkage following the phase

Figure 2. Membrane characteristics. SEM images of micropatterned membranes with microwells (A: top and B: section) and intermittent lines (C). The clean water flux versus TMP was measured for membranes with thickness of 50 μm (D).

separation process, which facilitated the release of the micropatterned membranes from the silicon wafer. As a result, the microwell diameter and the length of the intermittent lines, was about 18% smaller compared to the designed features on the silicon mold. The fabricated membranes were highly porous with a pore size of up to 2 μm on the patterned surface and 1–5 μm pores on the bottom flat surface.

Figure 2(D) presents the clean water flux of the two membranes (microwell and lid) membranes at various TMPs. In both cases, the water transport across the membranes is very high. Actually, the water permeability of membranes with microwells and intermittent lines was $3856 \pm 275 \text{ l m}^{-2} \text{ bar}^{-1} \text{ h}^{-1}$ and $2937 \pm 1901 \text{ m}^{-2} \text{ bar}^{-1} \text{ h}^{-1}$ respectively. Besides, the flux versus TMP is linear indicating high mechanical stability of the membranes in this pressure range.

3.2. Microwell device supports *in vitro* islet function

To test whether islets can survive in the device and if the device influences islet function, we cultured rat islets for seven days. Conventionally cultured islets, free floating, were served as control. After seven days of culture, no statistical difference could be observed in the glucose-stimulated insulin response of the

control islets and islets cultured in the microwell device (figure 3). In fact, during the first low glucose incubation the control and device islets secreted respectively 17.6 ± 5.9 and $8.4 \pm 4.2 \text{ ng ml}^{-1}$ insulin μg^{-1} DNA. As expected for normal islet function, approximately a two-fold increase of the amount of insulin was secreted during the high glucose incubation compared to the first low glucose incubation. The control and the microwell device with islets secreted respectively 31 ± 5 and $32 \pm 2 \text{ ng ml}^{-1}$ insulin μg^{-1} DNA. Both the control and the device with islets showed a reduction in insulin secretion when incubating a second time with low glucose after the high glucose incubation (17 ± 7 and $18 \pm 5 \text{ ng ml}^{-1}$ insulin μg^{-1} DNA, respectively). In the end of the experiments, we opened the device to access the positioning of the islets within the device. Due to opening, it was not always possible to keep all islets in the wells, nevertheless, we observed that the islets were not aggregated and mainly one islet was seeded per well, consistent to earlier findings with MIN6 aggregates [16].

3.3. The implantation site determines vascularization degree

To investigate the vascularization of the device, we implanted it subcutaneously or intraperitoneally

in immunocompetent C57BL/6BrdCrHsd-Tyrc mice. The development of blood vessels in the proximity of the device was followed with AngioSense injections (figure 4). A higher AngioSense signal there indicates higher perfusion and blood volume [19–21]. Overall, we found a statistically significant effect of implantation time, implantation site, and interaction between implantation time and site ($p < 0.05$). In fact, the signal was significantly higher at the intraperitoneal site than at the subcutaneous site ($p < 0.05$), see figure 4. The average radiance was $6.4 \pm 0.6 \times 10^{11}$ and $3.4 \pm 0.3 \times 10^{11} \text{ p s cm}^{-2} \text{ sr}^{-1}$, respectively.

At day 7, 14, and 28, the mice were sacrificed to determine the vascularization degree via histology (see photograph in the supplement (available online at stacks.iop.org/BMM/16/035036/mmedia)). Figures 5(A)–(D) present histological sections of the device implanted subcutaneously or intraperitoneally. At both sites, a minor foreign body response was observed around the devices, characterized by a thin layer of fibroblasts. A CD31 staining was performed to visualize blood vessels and their proximity to the device. We observed that the blood vessels did not penetrate the membranes and were mainly present at the side of the intermitted lines membrane. After seven days of implantation, the devices implanted subcutaneously contained 2.5 ± 0.9 blood vessels per mm device, whereas almost no blood vessels were found around the devices implanted at the intraperitoneal site (0.1 ± 0.1 blood vessels per mm device; figure 5(E)). At day 14, the number of blood vessels was significantly lower at the intraperitoneal site compared to the subcutaneous site (respectively 6.3 ± 1.9 and 15.4 ± 4.0 blood vessels per mm device; $p < 0.05$). However, this difference was no longer observed after 28 days of implantation at both sites. Regression of blood vessels, in absence of transplanted islets, has also been observed by others [22].

The distance from the CD31-positive blood vessels to the device was also quantified (figures 5(F)–(H)). Our results show that after seven days of implantation in the subcutaneous site most blood vessels ($57 \pm 18\%$) are positioned more than $100 \mu\text{m}$ away from the microwell device. At day 14, the percentage of blood vessels at $50 \mu\text{m}$ from the device increases to $32 \pm 8\%$ (compared to approximately 10% at day 7), whereas the percentage of vessels at a distance of more than $100 \mu\text{m}$ from the device, decreases to $41 \pm 13\%$ (compared to approximately 57% at day 7). From day 14, the distance of blood vessels at the peritoneal site was also measured, showing the highest percentage at a distance of $50 \mu\text{m}$ of the device ($46 \pm 9\%$). After 28 days of implantation; most blood vessels were found at $50 \mu\text{m}$ for both implantation sites (subcutaneous site $45 \pm 5\%$ and intraperitoneal site $52 \pm 16\%$).

3.4. Microwell devices support islet survival after transplantation

Since histology showed that faster vascularization and higher number of blood vessels are present when the device is implanted subcutaneously, islet transplantation was only performed there. To monitor islet survival and study whether islets influence the vascularization of the device, bioluminescent islets were transplanted subcutaneously in the microwell devices after 28 days of prevascularization followed via IVIS for 7 days (figure 6). The bioluminescent signal at the day of transplantation was $2.0 \pm 1.1 \times 10^9 \text{ p s cm}^{-2} \text{ sr}^{-1}$. This decreased to $3.8 \pm 3.0 \times 10^8 \text{ p s cm}^{-2} \text{ sr}^{-1}$, at day 5 and $9.8 \pm 7.4 \times 10^8 \text{ p s cm}^{-2} \text{ sr}^{-1}$, at day 7 indicating that the functional survival of the islets decreases directly after transplantation and thereafter stabilizes as of day 7, consistent to earlier studies [13].

Figure 4. *In vivo* angiogenesis within microwell devices during 14 days of implantation at either the subcutaneous or intraperitoneal site. Microwell devices were subcutaneous or intraperitoneal implanted in immunocompetent C57BL/6BrdCrHsd-Tyrc mice. The angiogenesis process was followed *in vivo* using AngioSense injections and IVIS imaging at day 0, 7, and 14. The average radiance was corrected for background signal. Mean and standard error of the mean are plotted ($n = 5$), statistical analysis was applied by a two-way ANOVA with Bonferroni's post hoc test, $p < 0.05$ (*).

Histology studies show that implanting the microwell device under the skin in nude mice resulted in a higher amount of CD31-positive blood vessels per mm device compared to the results from the immunocompetent mice. After 35 days of subcutaneous implantation, we found $91 \pm 37\%$ blood vessels per mm device as well as high numbers of vessels near the device wall ($46 \pm 9\%$ of the total number of blood vessels) (figure 7).

4. Discussion

The development of a device that supports long-term functional survival of pancreatic islets is a promising treatment option for T1D. There, it is extremely important that islets are in close proximity of a functional vascular network to ensure adequate

diffusion of oxygen, nutrients, and hormones [23]. Another prerequisite is that the device is mechanical stable and supports survival of the encapsulated islets. Here, we show that the optimized device consisting of a microwell membrane and a membrane lid with intermittent lines can support functional islet survival both *in vitro* and *in vivo*. This microwell system provides some pertinent advantages over other, conventional encapsulation systems. In fact, in many macroencapsulation devices, islets are enveloped without avoidance of clumping and fusion of islets which could lead to necrosis and necroptosis of islets and significant loss of function, as it increases the oxygen diffusion distance [24]. In our microwell system, high number of islets can be encapsulated, by each islet individually settling within a microwell. This avoids islet clumping and also allows the islets to maintain their architecture, which is important for

Figure 5. Staining of CD31-positive blood vessels. Devices were implanted in immunocompetent mice and blood vessels were stained with the endothelial marker CD31 (pink) at 28 days after implantation. (A) and (B) show respectively the microwell and intermittent lines membranes after subcutaneous implantation. (C) and (D) show respectively the microwell and intermittent lines membranes after intraperitoneal implantation. The number of CD31-positive blood vessels per mm of device were counted after 7, 14, and 28 days of implantation at both sites (E). In addition, the distance between the device and the blood vessels was measured and expressed as percentage from the total number of blood vessels (F)–(H). Mean and standard error of the mean are plotted ($n = 5$), statistical analysis was applied by a two-way ANOVA with Bonferroni's post hoc test, $p < 0.05$ (*).

long-term function [25]. This approach of individual packing of islets is expected to allow higher seeding densities of islets. The microwell membranes used in this study have a microwell diameter optimized for small size islets (50–150 μ m), which generally show higher viability and function compared to large size islets and are more suitable for clinical application [26]. In addition to the smaller microwell diameter, the optimized device also has smaller spacing between the wells, which allows seeding of 8 times higher

number of wells per membrane surface in comparison to the previous microwell membrane [14]. Moreover, the thickness of the microwell membranes decreased to 50 μ m. Based on our modeling studies, all these optimizations should result to improvements in oxygen, nutrient, and insulin transport across the device [16].

Adequate vascularization is also a very important consideration for successful application of the microwell devices. Here, we show that our

device allows rapid vascularization. We applied the intermittent microstructured lid which can guide vascularization *in vivo*, as shown in an earlier study using

MSCs [15]. To allow this degree of vascularization, we applied a distance between the lines of 100 μ m, since it has been shown that cell alignment occurs in

patterns between 20 and 130 μm [27]. Earlier studies also showed that cells respond better to topographies at the cell scale, rather than to rough surfaces and topographies above the cell scale ($>100\text{ }\mu\text{m}$) [28–30], therefore we developed membranes with patterns of 40 μm in height and 20 μm wide. We do have to apply a pre-implantation period for the device before introducing the islets. This allows vascularization to develop and dampens the inflammatory events which are associated with the vascularization process and might damage islets.

Besides the application of suitable micropatterned lid membranes, the implantation site played an important role in the vascularization; significantly more blood vessels were observed after subcutaneous implantation compared to intraperitoneal implantation. After 14 days of implantation under the skin more than 50% of the blood vessels was observed within only 50 μm of the device. This is a very important result for achieving optimal transport of oxygen and nutrients to the encapsulated islets. For example, for the beta- O_2 device it has been shown that a distance of 50 μm of the device from vascularization is required for achieving appropriate function of islets in the devices [31]. Although histology did not show more blood vessels for the peritoneally implanted devices compared to the subcutaneously implanted ones, the AngioSense signal was significantly higher in the devices implanted peritoneally for 14 days. This could be explained by the fact that AngioSense is more a perfusion measure and quantifies the neovascular leakage area rather an indicator of ongoing vascularization processes [32]. The AngioSense data indicates that the intraperitoneal device is better perfused and provokes stronger vascularization processes in the intraperitoneal than subcutaneous site, however, the subcutaneous implanted device attracts more blood vessels.

Our study also demonstrated a reduction in cell viability in the first five days after transplantation. This should not be interpreted as an indication that the device cannot support survival of islets. Similar loss of islets in encapsulation devices has been reported before and may be up to 40% in the first week's post transplantation [23]. This is the consequence of adaptation of islets to the new *in vivo* microenvironment combined with damage done by inflammatory events associated with the implantation surgery [33]. We consider that the decrease of islet viability in the first week after transplantation into the device implanted subcutaneously to have a similar cause. Nevertheless, the islets are capable of recovering once revascularization starts and the immune responses associated with implantation surgery fade away. Vascularization is a complex process and takes time, histology showed optimal vascularization 14 days after implantation, therefore it can be expected that some islets are lost during this first phase when the device is not yet fully vascularized.

In conclusion, the micropatterned device proposed here can achieve good islet viability and function *in vitro* and *in vivo*. Due to its unique characteristics, the device can provide a functional vascular network in close proximity to the device, can prevent islet clustering and minimize islet loss in the immediate period after transplantation. Thereby, the device can possibly provide improved long-term outcomes of islet transplantation as treatment for T1D.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization with (non-) financial interest in the subject matter or materials discussed in this manuscript.

Author contributions

A M Smink, K Skrzypek, J A L Liefers-Visser, performed literature search, experimental design, data collection, data analysis, data interpretation and manuscript writing. B J de Haan and R Kuwabara contributed to experimental design, data collection, data analysis and manuscript writing. P de Vos and D Stamatialis contributed to experimental design, data interpretation and manuscript writing.

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