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Micro-fabricated scaffolds lead to efficient remission of diabetes in mice

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ABSTRACT

Despite the clinical success of intrahepatic islet transplantation in treating type 1 diabetes, factors specific to this transplantation site hinder long-term insulin independence. The adoption of alternative, extravascular sites likely improve islet survival and function, but few locations are able to sufficiently confine islets in order to facilitate engraftment. This work describes a porous microwell scaffold with a well-defined pore size and spacing designed to guarantee islet retention at an extrahepatic transplantation site and facilitate islet revascularization. Three techniques to introduce pores were characterized: particulate leaching; solvent casting on pillared wafers; and laser drilling. Our criteria of a maximum pore diameter of 40 μm were best achieved via laser drilling. Transplantation studies in the epididymal fat of diabetic mice elucidated the potential of this porous scaffold platform to restore blood glucose levels and facilitate islet engraftment. Six out of eight mice reverted to stable normoglycemia with a mean time to remission of 6.2 ± 3.2 days, which was comparable to that of the gold standard of renal subcapsular islet grafts. In contrast, when islets were transplanted in the epididymal fat pad without a microwell scaffold, only two out of seven mice reverted to stable normoglycemia. Detailed histological evaluation four weeks after transplantation found a comparable vascular density in scaffold-seeded islets, renal subcapsular islets and native pancreatic islets. However, the vascularization pattern in scaffold-seeded islets was more inhomogeneous compared to native pancreatic islets with a higher vascular density in the outer shell of the islets compared to the inner core. We also observed a corresponding decrease in the beta-cell density in the islet core. Despite this, our data indicated that islets transplanted in the microwell scaffold platform were able to maintain a viable beta-cell population and restore glycemic control. Furthermore, we demonstrated that the microwell scaffold platform facilitated detailed analysis at a subcellular level to correlate design parameters with functional physiological observations.

1. Introduction

The transplantation of islets of Langerhans from donor pancreata into the liver has become an established therapy for a subpopulation of patients with type 1 diabetes and labile glycemic

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control [1]. Although the clinical outcome of this islet transplantation approach has improved over the last decade, progressive islet loss in the post-transplantation period often prevents long-term insulin independence to only a subset of patients [2]. One advantage of the intrahepatic site has been the ease of islet delivery, achieved by islet infusion into the portal vein. However, this exposes islets directly to blood and elicits the instant blood-mediated inflammatory reaction (IBMIR). This has been shown to cause islet destruction and islet loss [3,4]. In addition, the hepatic environment imposes additional challenges and stress to islet engraftment and survival, such as the exposure to reduced oxygen tension [5,6] and high concentrations of immunosuppressants [7].

While these limitations can be partly overcome by employing extrahepatic extravascular transplantation sites, these sites are typically challenged by limited space to transplant sufficient amount of islets, the inability to restrict islet migration, or poor vascularization [8]. These challenges have driven the field towards engineering efficient and tailored transplant microenvironments using biomaterials that ensure optimal islet spatial distribution and promote revascularization [9–11].

Pancreatic islets are highly vascularized by a dense network of capillaries. This capillary network is not only crucial for an adequate nutrient supply, but it is also critical for glucose sensing and the rapid secretion of islet hormones into the blood stream [12]; islets with vascular defects do not regulate blood glucose levels properly [12,13]. These requirements dictate that an islet tissue engineered construct should allow for high mass transport and good vascularization, implying the creation of a porous implantable construct that permits blood vessel infiltration. However, recent findings report that it is a narrow range of pore size, 30–40 μm, that maximizes vascularization [14], suggesting that having precise control over pore size would greatly facilitate revascularization of an implantable scaffold.

Recently, we have developed a novel polymer film-based microwell scaffold platform for islet transplantation that provides a mechanically protective environment while maintaining islet morphology and islet functionality *in vitro* [15]. In the current study, we sought to further optimize this scaffold platform to promote islet engraftment *in vivo* by facilitating vascular tissue ingrowth via introduction of well-defined pores with a maximum diameter of 40 μm. Therefore, we have evaluated the feasibility of three process sequences based on substrate modification and replication by (micro)thermoforming (SMART) [16] in conjunction with the following methods to introduce pores with controlled dimensions and spacing in polymer films: 1) particulate leaching using salt crystals; 2) solvent casting on patterned silicon wafers; and 3) laser drilling using an ultra-short pulsed laser source, of which laser drilling best matched our design criteria. As a first step in identifying the *in vivo* potential of this porous microwell scaffold for extrahepatic islet transplantation, we have investigated whether this highly flexible and tunable platform can support islet function and engraftment in a diabetic mouse model.

2. Materials and methods

2.1. PEOT/PBT thin film fabrication

Scaffolds were fabricated from a poly(ethylene oxide terephtalate)/poly(butylene terephthalate) block copolymer with composition 4000PEOT30PBT70 (PolayActive™, IsoTis Orthobiologics S.A., Irvine, USA) as previously described [15]. A 15% (w/w) polymer solution was prepared in 35% (w/w) 1,1,1,3,3,3-hexafluoro-2-isopropanol (Biosolve, Valkenswaard, The Netherlands) and 65% (w/w) chloroform (Merck, Darmstadt, Germany). The polymer solution was cast on silicon wafers at a height of 200 μm, finally resulting in ~15 μm thick films. The films were placed under nitrogen-flow for 12 h, incubated in ethanol overnight to remove solvent residue, and dried in a vacuum oven (Heraeus, Hanau, Germany) at 30 °C for 3 days.

2.2. Microwell scaffold fabrication and characterization

Microwell scaffolds consisted of a thin PEOT/PBT polymer film patterned with microwells and a porous lid with ~40 μm pores. Microthermoforming [17] was employed to create the microwell structure into PEOT/PBT thin polymer films as described previously [15]. In short, a heated polymer film was pressed into a stainless steel mold (produced by Lightmotif BV, Enschede, The Netherlands) using polyethylene films as backing material. Molding temperature and pressure were 85 °C and 45 kN, respectively. Pore geometry and architecture were characterized by scanning electron microscopy (SEM, Philips XL 30 ESEM-FEG, Eindhoven, The Netherlands). Scaffolds were gold sputter coated (Cressington, UK) prior to SEM analysis. Pore dimensions were measured in three different samples per type of scaffold at the side, rim and bottom of the microwells using ImageJ software (http://rsb.info.nih.gov/ij/).

2.3. Particulate leaching

To fabricate porous microwell scaffolds by particulate leaching, sodium chloride crystals were added to the polymer solution just before solvent casting. The crystals were manually grinded and sieved on an automatic shaker (sieve range 38–63 μm). Three different salt-to-polymer ratios were tested: 1:2, 1:1.5, and 1:1. After scaffold fabrication by microthermoforming, the scaffolds were leached in demi-water at 50 °C for 4 days. To assess whether residual sodium chloride crystals were present, C and O (the polymer) and Na and Cl atoms were mapped using a SEM integrated energy dispersive spectrometer (EDS, EDAX, AMETEK Materials Analysis Division, Mahwah, USA).

2.4. Pillared wafer

To fabricate porous microwell scaffolds using microfabrication techniques, silicon wafers patterned with micropillars were produced. For this purpose, a chromium-glass mask was designed for patterning photoresist on the wafers. The photomask design contained circular features to produce a grid pattern of pillars with a diameter of ~20, 30, and 40 μm, spaced at 200 μm. Process flow design, lithography and etching were done by ThermoFisher (ThermoFisher Scientific Inc., Enschede, The Netherlands). Briefly, single side-polished wafers (100 mm diameter, 525 μm thick) were oxidized to a thickness of 1 μm. Photoresist OiR 907–17 was patterned using the abovementioned mask using an EVG®620 Automated Mask Alignment System (EV Group, St. Florian am Inn, Austria). The oxide masking layer was etched by a reactive ion etching (RIE) step. To form pillars of a height of 75 μm, a deep RIE (DRIE) step was performed. The photoresist and passivation layer were stripped and the wafer was cleaned in a Piranha solution (H_2SO_4: H_2O_2 = 3:1 (v/v)). To improve demolding of the thin PEOT/PBT films, pillared wafers were coated with trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (Sigma Aldrich, USA). The polymer solution was cast at a height of 90 μm, 15 μm above the pillars, to ensure perforation of the film during solvent evaporation and subsequent thinning of the polymer film. The porous polymer films were used for microwell scaffold fabrication.

2.5. Laser drilling

To produce porous microwell scaffolds by laser drilling, an