Transactions on Additive Manufacturing Meets Medicine
Trans. AMMM, Vol 1, No 1, 2019, Article ID S07T02

Infinite Science | Publishing

PEOT-PBT and poly(ester urethane) microwell scaffolds for extrahepatic islet transplantation

Elahe Hadavi[1,2*], Ian Gibson[1,2]

[1] Department of Design, production and management, The university of Twente, Enschede, The Netherlands
[2] Fraunhofer project center at university of Twente, Enschede, The Netherlands
** Corresponding author, email: E.Hadavi@utwente.nl*

Abstract: Type 1 diabetes is an autoimmune disease that destroys insulin producing β-cells in the pancreas. Intrahepatic islet transplantation can prolong insulin independence. However, intravascular infusion, suboptimal microenvironment in transplanted site, and significant loss of mass remain elusive. In this study, PEOT-PBT and poly(ester urethane) microwell scaffolds were developed to provide a basis for additive manufacturing of extrahepatic islet transplantation to improve the survival and function of the islets.

I. Introduction

Intra-hepatic islet transplantation is a promising therapy to treat type 1 diabetes by infusion of donor islets into the portal vein with minimal side effects. However, a rapid decrease of transplanted islet mass, caused by multiple factors, limits wide-spread implementation of this therapy. Direct injection of islets can lead to a hemostasis chain reaction and instant blood mediated inflammatory reaction (IBMIR), resulting in islet loss. Moreover, mechanical stress and exposure to lower oxygen tension due to impaired vascularization will also negatively affect the survival and function of injected islets. Rapid islet loss is further complicated by limited availability of suitable pancreas donors. An alternative is to transplant islets to extrahepatic sites. Challenges to this approach are islet diffusion and weak vascularization upon implantation. We use a tissue engineering approach, involving the use of cells and materials to repair or generate specific tissues or organs, to create an efficient islet transplantation device.
Previously, we developed poly(ethyleneglycol-terephthalate-polybutylene-terephthalate)(PEOT-PBT) and poly(ester urethane) microwell scaffolds[1,2]. Results suggested creating biomimetic islet niches by functionalization of biomaterials may significantly improve endocrine function of β-cells. This leads to functional improvement by restoring part of the islets ECM[2]. However, the developed platform lacked immediate vascularization into the scaffolds to facilitate nutrient and hormonal exchange with islets. In addition, it had limited capacity to encapsulate islets. To address these limitations, we further improved the previous scaffolds by adding porosity, and enhancing their size and capacity [3].

II. Material and methods

Fabrication of polymer films: The polymer films were fabricated using PEOT-PBT copolymers that were composed of soft block of poly(ethylene oxide terephthalate) and hard block of poly(butylene terephthalate) with the composition 4000PEOT30PBT70 (Polyvation BV, Groningen, the Netherlands). The poly(ester urethane) was provided by Polyganics (Groningen, Netherlands). It was synthesized by reacting an α,ω-isocyanate end-functionalized prepolyester and an α,ω-hydroxy bicarbonate. 40μm thick polymer films were fabricated based on a previously reported process [2].

Laser drilling: Scaffolds were composed of a membrane containing microwells acting as islet containers and a flat film acted as a lid for the array. In both membranes, laser drilling was used to create holes. PEOT-PBT and poly(ester urethane) films were drilled in two different sizes for fabrication of microwells and lid of the scaffolds, respectively. PEOT-PBT films for microwell arrays were drilled with holes of 18μm using a 50μm pitch. PEOT-PBT films for microwell lids were drilled with a hole size of 38μm, 100μm pitch, and an outer dimension of 3mm. Poly(ester urethane) films for microwell arrays were drilled with a hole size of 15μm, a 50μm pitch, and an outer dimension of 3mm. Poly(ester urethane) films for microwell lids were drilled with a hole size of 40μm, a 100μm pitch, and an outer dimension of 3mm.

Microwell scaffolds: Scaffold layers, each with 4630 microwells, were manufactured by micro-back molding (Fig 1A). Micro-thermoforming was performed to create a 3D microwell structure. Drilled polymer films of 40μm were pressed into the mold backing material.

Primary human islet cultures: Human islets of Langerhans were isolated from donated pancreas tissue (Human Islet Isolation Lab., University Medical Center, Leiden, Netherlands). Isolated donor islets were cultured in supplemented CMRL-1066, as detailed in our previous study. Subsequently, human islets were handpicked for scaffold seeding. For the glucose stimulated insulin secretion test, Human islets were seeded on non-adherent plates, PEOT-PBT, and poly(ester urethane) microwell scaffolds.

Sealing the lid and layer of scaffolds: After cell seeding, the microwell and lid of the scaffolds were sealed using a soldering pen with a 2x0.5mm tip, coated with Teflon to protect from attaching to the polymers. The lid was placed on top of the scaffold and the border of the poly(ester urethane) and PEOT-PBT scaffolds were sealed at 85oC and 185oC respectively. Subsequently each layer of scaffold was sealed to the previous layer in 4 spots.

DOI: 10.18416/AMMM.2019.1909S07T02

III. Results and discussion

We have explored an approach that may mitigate several drawback of macro-encapsulation.

Scaffold structure: Laser drilling effectively endowed the poly(ester urethane) and PEOT-PBT polymers films with arrays of well-defined microholes. SEM showed homogeneously shaped microwells throughout the scaffold. The process was reproducible and the shape of both formatted microwell scaffolds remained stable upon handling. The upper well diameters were 380±5 and 425±5 µm, the lower well diameters were 290±5 and 170±5 µm, and the depths of each well were 280±10 and 210±10 µm, for PEOT-PBT and poly(ester urethane) microwells respectively. The wall thickness of the upper and bottom part of the PEOT-PBT wells was 15±2 and 26±3 µm respectively. The thickness of the upper and bottom part of the poly(ester urethane) wells was 40±3 and 15±4 µm, respectively. The hole sizes of both PEOT-PBT and poly(ester urethane) microwells varied depending on location, ranging from 15µm at the upper part of the film to 38µm at the bottom of the microwell. Each scaffold layer consisted of 4630 microwells. A human requires about 11000 Islet Equivalent (IEQ) per Kg. Based on the patient's weight more layers can be added to the 3D scaffold.

Function of primary human islets: To assess effect of the substrate material (i.e. non-treated culture plastic, PEOT-PBT, and poly(ester urethane) and topography (i.e. flat films vs. microwells), islet functionality was studied. Human islets were cultured on different substrates and topographies and subjected to a glucose stimulation insulin secretion test at days 3 and 7. Islets cultured in the both microwell scaffolds released significantly more insulin than islets cultured on ultra-low adhesive plates (Fig. 2). Moreover, the increase in insulin secretion showed a consistent yet discrete tendency to be highest in islets cultured on PEOT-PBT groups. Also, islets seeded in microwells secreted more insulin than islets seeded on flat membranes of the same materials. Furthermore, while islets on a flat surface showed consistent decline in insulin release over time, islets cultured in microwells increased their insulin secretion over time. It is important to provide islets with sufficient nutrients. Cell nourishment cannot be achieved if the islets form big aggregates or are fused. A drawback of macroencapsulated islets and intrahepatic transplanted islets is fusion and adhesion of islets. Fusion affects the islet due to lack of oxygen and nutrients causing necrosis in its core. This can be addressed by seeding islets in microwells, preserving its round shape and preventing fusion. This change in islet function and morphology might relate to the design of additively manufactured scaffolds. A hemispherical structure can support and preserve the round shape. In addition, each microwell can act as a barrier to avoid fusion of neighboring islets. Thus, the scaffold's design might have a positive influence on islet behavior. Another potentially positive influence on islets relates to the properties of the used substrates. We believe that more pores per microwell can increase the rate of vascularization. Moreover, functionalization of the scaffold with ECM molecules, discussed in our previous study[2], have a potential to further improve the outcome of islet transplantation.

IV. Conclusions

The current study presents the PEOT-PBT and poly(ester urethane) porous microwell scaffolds as a suitable carrier for extrahepatic islets transplantation. This design can be adapted to suit additive manufacturing based scaffold construction in the future for a variety of cell types. The use of polymer films with symmetric holes using laser cutting is a novel means to develop scaffolds of various depths and designs that can be adapted for a variety of cells of various shapes and densities. This may lead to more uniform organ replacements due to the reproducibility of the laser cuts.

ACKNOWLEDGMENTS

The author acknowledge the infrastructure and financial support of the supervisors and colleagues during her PhD at the department of Developmental Bioengineering (DBE) at the university of Twente.

AUTHOR'S STATEMENT

Research funding: This research was financially supported by the Diabetes Cell Therapy Initiative (DCTI) FES 2009 program LSH-DCTI, including the Dutch Diabetes Research Foundation (DF).

REFERENCES

[1] Hadavi, Elahe, et al. "Fibronectin and Collagen IV Microcontact Printing Improves Insulin Secretion by INS1E Cells." Tissue Engineering Part C: Methods 24.11 (2018): 628-636.

[2] Hadavi, Elahe, et al. "Microwell scaffolds using collagen-IV and laminin-111 lead to improved insulin secretion of human islets." Tissue Engineering Part C: Methods 25.2 (2019): 71-81.

[3] Hadavi, Elahe. "A tissue engineering approach towards treatment of type 1 diabetes (Doctoral thesis) (2018), ISBN: 978-94-6361-087-2

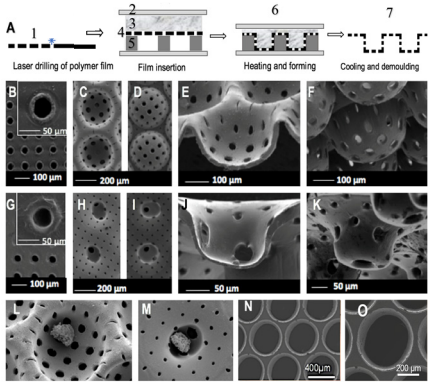

Figure 1. Microwell scaffold structure. A) Representation of the fabrication of microwell scaffold using micro-back molding. A1) Laser drilling of the polymer film, A2) Press device A3) Backing material A4) PEOT-PBT or poly(ester urethane) film A5) mold A6) Micro-thermoformed scaffold. SEM images of B) Laser drilled PEOT-PBT lid C-F) PEOT-PBT microwell scaffold G) Laser drilled Poly(ester urethane) lid H-K) poly(ester urethane) microwell scaffold L) seeded islet in PEOT-PBT microwells M) Seeded islet in poly(ester urethane) microwell, N-O) mold structure for applying thermoforming on polymer films.

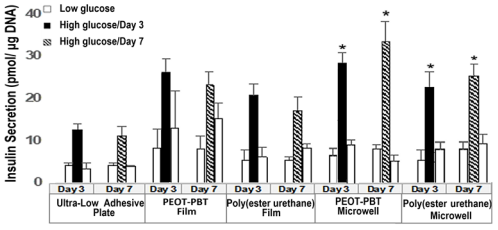

*Figure 2. Glucose stimulated insulin secretion test of human islets on ultra-low adhesive plate (control), PEOT-PBT and poly(ester urethane) film and microwell scaffold at days 3, 7. * = $p < 0.05$.*