Lonnie Shea ORCID iD: 0000-0002-9296-9673

Evaluation of Encapsulating and Microporous Non-degradable Hydrogel Scaffold Designs on Islet Engraftment in Rodent Models of Diabetes

Rios, Peter Daniel [a**], Skoumal, Michael [b**], Liu, Jeffrey [a,c], Youngblood, R.[b], Kniazeva, Ekaterina[d] , Garcia, Andrés J. [e,f], Shea, Lonnie D.[b*]

[a] Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Rd / E310, Evanston, IL 60208, USA

[b] Department of Biomedical Engineering, University of Michigan, 1119 Carl A. Gerstacker Building, 2200 Bonisteel Boulevard, Ann Arbor, MI 48109-2099

[c] Interdisciplinary Biological Sciences Program, Northwestern University, Evanston, IL 60208

[d] Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL 60611

[e] Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA

[f] Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, USA

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*** Corresponding author.**

**** Co-first authors**

**Peter Daniel Rios, PhD, peterdrios@gmail.com, 757-329-5106

**Michael Skoumal, PhD, michael.j.skoumal@gmail.com,

Jeffrey Liu, PhD, jeffreyliu6@gmail.com,

Richard Youngblood, MS, youngblr@umich.edu,

Ekaterina, Kniazeva, PhD, ekniazeva@gmail.com,

Andrés Garcia, andres.garcia@me.gatech.edu

*Lonnie D. Shea, PhD, ldshea@umich.edu, 734-764-7149

Abstract

Islet transplantation is a promising therapeutic option for Type 1 diabetes mellitus, yet the current delivery into the hepatic portal vasculature is limited by poor engraftment. Biomaterials have been employed as a means to promote engraftment and function at extrahepatic sites, with strategies being categorized as encapsulation or microporous scaffolds that can either isolate or integrate islets with the host tissue, respectively. While these approaches are typically studied separately using distinct material platforms, herein, we developed non-degradable polyethylene glycol (PEG)-based hydrogels for islet encapsulation or as microporous scaffolds for islet seeding in order to compare the initial engraftment and function of islets in syngeneic diabetic mice.

Normoglycemia was restored with transplantation of islets within either encapsulating or microporous hydrogels containing 700 islet equivalents (IEQ), with transplantation on microporous hydrogels producing lower blood glucose levels at earlier times. A glucose challenge test at one month post-transplant indicated that encapsulated islets had a delay in glucose-stimulated insulin secretion, whereas microporous hydrogels restored normoglycemia in times consistent with native pancreata. Encapsulated islets remained isolated from the host tissue, whereas the microporous scaffolds allowed for re-vascularization of the islets post-transplant. Finally, we compared the inflammatory response post-transplantation for the two systems, and noted that microporous hydrogels had a substantially increased presence of neutrophils. Collectively, these findings suggest that both encapsulation and microporous PEG scaffold designs allow for stable engraftment of syngeneic islets and the ability to restore normoglycemia, yet the architecture influences islet function and responsiveness following transplantation.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that impacts 5-10% of diabetic patients and destroys pancreatic beta cells, rendering patients unable to regulate blood glucose levels (Shapiro et al., 2016). Despite use of exogenous insulin and the development of insulin pumps, tight control of normal blood glucose levels and secondary complications remain a concern. Alternatively, islet transplantation into the hepatic portal vein has been used to restore endogenous insulin production and aid in maintenance of normoglycemia and prevention of severe hypoglycemic events (Gibly et al., 2011a; Hering et al., 2016). However, this procedure is not widely employed

clinically due to poor survival and engraftment of transplanted islets in the hepatic vasculature. Upon injection into the hepatic portal vein, transplanted islets are subject to the instant blood inflammatory response (IBMIR), which can contribute to the loss of more than 60% of the islets within days post-transplant (Gibly et al., 2011a). IBMIR promotes a pro-inflammatory environment, which then leads to activation of adaptive immunity and additional injury to islets. Challenges associated with delivery of islets into the liver have motivated the development of extrahepatic sites conducive to islet engraftment to prevent IBMIR-mediated destruction of islets and improve clinical outcomes (Berman et al., 2016).

Biomaterial-based strategies have been employed as a means to provide a controlled environment for transplantation of islets into extrahepatic sites. A variety of scaffold materials have been used to localize islets in extrahepatic locations, including the kidney capsule and omentum, and restore normoglycemia in diabetic rodent models (Blomeier et al., 2006; Gibly et al., 2011b; Pedraza et al., 2013; Phelps et al., 2013; Rios et al., 2016; Weaver et al., 2017). The design of these scaffolds has implications for islet engraftment. Two of the more common scaffold designs used for housing islets involve either encapsulating or microporous biomaterials. Encapsulating materials protect islets from direct contact with host immune cells, yet permit efflux of insulin and exchange of metabolites. Micro- and macro-encapsulation approaches have been attempted in large-animal models and clinically, with mass transport being a challenge (Buder et al., 2013; Song and Roy, 2015; Yang and Yoon, 2015), along with long-term graft function despite refinements in specific immunosuppressive drugs and islet dosage used for transplantation (Brennan et al., 2016; Desai and Shea, 2016; Ryan et al., 2005; Yang and

Yoon, 2015). Alternatively, microporous scaffolds can be used to seed islets and permit infiltration of host tissue and vasculature around transplanted cells, which reduces the challenges related to mass transport. In the context of islet transplantation, microporous poly(lactide-co-glycolide)(PLG) scaffolds have demonstrated efficacy *in vivo* in mouse and porcine models of diabetes (Blomeier et al., 2006; Gibly et al., 2011b; Graham et al., 2013; Hlavaty et al., 2014; Liu et al., 2016). However, microporous scaffolds with seeded cells are subject to infiltration by host immune cells, which can impact islet engraftment and function. Strategies to utilize encapsulation, in combination with a porous architecture for vascular growth near encapsulated islets, have been examined (Rios et al., 2016). A direct comparison of the relative efficacy for the encapsulation and microporous approaches has been challenging as the material platforms are often distinct for the two systems.

In this report, we investigated islet function and the host response as a function of the delivery platform, and have used a common material platform of non-degradable polyethylene glycol (PEG) hydrogels. An encapsulating hydrogel representative of macroencapsulation strategies was fabricated from PEG using a crosslinking chemistry that is compatible with cells. A microporous scaffold was created from PEG using a particulate leaching strategy to create pores that allow for cell infiltration and islet integration with the host (Blomeier et al., 2006; Desai and Shea, 2016; Rios et al., 2016). A syngeneic transplant model was employed to focus on engraftment and function as a biomaterial platform and avoid loss of graft function due to immune rejection. Islets were transplanted into an extrahepatic site (i.e. epididymal fat pad) of diabetic mice and function was followed during a 30-day period. Additionally, we characterized the innate

immune cell response using flow cytometry to identify the host cells populating the graft and their relative abundance for the two material platforms. The use of syngeneic islets transplanted on similar materials provides insight into how the architecture of the cell delivery system can influence islet engraftment and function.

Materials and Methods

Encapsulating and Microporous Hydrogel Fabrication

Encapsulated hydrogels were formed by mixing PEG-maleimide (4-arm, 20kDa MW, JenKem Technology USA, Plano, TX) and CGRGDS (CelTek Peptides, Franklin, TN) in HEPES Buffer (pH 7.2) to yield a final PEG concentration of 10% (wt/vol) and RGD concentration of 5 mM. The PEG-CGRGDS solution was allow to react via Michael-type addition for 5 minutes at room temperature and then stored on ice. Next, the functionalized PEG precursor was added to sedimented islets in an Eppendorf tube (in approximately 6 μL of HBSS 1X media (Corning, Corning, NY) supplemented with 10% fetal bovine serum (FBS)). The bottom of a disc-shaped PDMS mold (diameter = 5 mm, height = 1 mm) was covered with 3 µL of a YKNR non-degradable peptide crosslinker solution (GCYKNRGCYKNRCG, custom synthesis and purification by CelTek Peptides). The peptide contained tyrosine (Y) and asparagine (N) amino acids in the D-configuration to prevent cleavage from plasmin, which inhibits hydrogel degradation (Shikanov et al., 2011). The PEG precursor containing islets was added to the mold containing the YKNR solution and an additional 3 µL of YKNR was added on top for a final YKNR concentration of 9.6 mM. The hydrogel was incubated at 37°C for

30 minutes to allow the crosslinking reaction to reach completion. Final gel volume was approximately 25 μ L.

Microporous PEG hydrogels were fabricated by dissolving 20 kDa 4-arm PEG-maleimide (JenKem Technology USA) in HEPES buffer for a final concentration of 20% (wt/vol). The photoinitiatior, Irgacure 2959 (BASF, Florham Park, NJ) was dissolved in N-vinylpyrrolidone at a concentration of 600 mg/mL and added to the PEG precursor solution for a final concentration of 1 wt%. NaCl was added to the PEG precursor to make a saturated solution. Forty milligrams of NaCl particles (sieved to a diameter between 250 and 425 μm) were then added to a polydimethylsiloxane (PDMS) mold (diameter = 5 mm, height = 1 mm) and 10 μL the saturated PEG solution was added. After irradiation with UV light, salt was leached from the scaffolds in ultrapure water for two 10-minute washes. Final gel volume was approximately 25 μL.

Islet Isolation and Transplantation

Islets were isolated from healthy 10-12 week old male and female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) following standard islet isolation procedures. Male C57BL/6J recipient mice were between 14-18 weeks of age. Four days prior to islet transplantation, recipient mice were injected with 220 mg/kg of streptozotocin (Sigma Aldrich, St. Louis, MO) to chemically induce irreversible diabetes. Nonfasting blood glucose levels were taken using a OneTouch Basic Glucose Monitor (Aviva, West Des Moines, IA) and only those mice with a measurement of 300 mg/dL or greater on consecutive days (day before and day of transplant) were used as recipients. Normoglycemia was denoted as <200 mg/dL in syngeneic studies. Hydrogel materials,

encapsulating or microporous, contained either 700 islet equivalent (IEQ). Encapsulating hydrogels were submersed in supplemented media for at least 5 minutes prior to transplantation. To load microporous hydrogels, islets were concentrated in 30 μL of supplemented media and applied to the top of a dehydrated hydrogel. Supplemented media contained CMRL 1066, 10% FBS, 1% penicillin-streptomycin, 25 mM of HEPES (Corning, Corning, NY), and 2 mM of L-Glutamine (Sigma Aldrich, St. Louis, MO). Each mouse received one gel into the fat pad transplantation site. All studies were approved by the Northwestern University Animal Care and Use Committee or the University of Michigan Unit for Laboratory Animal Medicine (ULAM).

Intraperitoneal Glucose Tolerance Test (IPGTT)

Intraperitoneal glucose tolerance tests (IPGTTs) were performed at 4 weeks post-transplantation to assess the ability of the islet grafts to respond to glucose challenges. A D-glucose solution (250 mg/mL sterile phosphate buffered saline (PBS) (-/-)) was created for injection. After a 3-hour fast period, 2 g/kg of D-glucose was injected intraperitoneally. Blood glucose levels were measured at baseline (before injection), 15, 30, 60, 90, 120, and 150 minutes after the glucose injection.

Immunohistochemistry

Scaffolds were harvested from euthanized mice and immediately snap frozen in isopentane at -20°C to preserve tissue architecture. Frozen scaffolds were embedded in Tissue-Tek O.C.T. (Fisher Scientific, Waltham, MA) supplemented with sucrose. Cryosections (14 μm) were prepared and stored at -20°C until staining. Representative sections were fixed in 4% PFA and blocked with 10% normal donkey serum and 0.1%

Triton-X in PBS before staining with primary antibodies. Sections were stained with guinea pig polyclonal anti-swine insulin (Jackson Labs, West Grove, PA, 1:250) and Hoechst for nuclear counterstaining (Invitrogen, Carlsbad, CA, 1:2000). Dylight donkey anti-guinea pig 488 (Jackson Labs, West Grove, PA, 1:400) was used as a secondary antibody for visualization.

Flow Cytometry

Mice were euthanized by cervical dislocation under isoflurane-induced anesthesia. Tissue was dissociated into a single cell suspension. Tissues were harvested immediately and stored in HBSS on ice. The resulting tissue homogenate was filtered through a 70 μm cell strainer and washed with MACS (PBS supplemented with 2mM EDTA and 0.5% BSA). For scaffold implants and adipose tissue, enzymatic digestion was used to create a single cell suspension. Tissues were weighed and placed into petri dish with 0.5 mL of 10 mg/mL Collagenase Type II (Sigma Aldrich, St. Louis, MO) and 2.5 mL of digestion buffer (HBSS with Calcium Chloride and Magnesium Chloride (Thermo Fisher, Waltham, MA) supplemented with 0.5% Bovine Serum Albumin). Tissue was finely shredded and transferred to a 15 mL conical tube. Dish was washed with 2 mL of digestion buffer and added to tissue homogenate to bring final concentration of collagenase to 1 mg/mL. Tissue was incubated in a 37° C water bath for 30 minutes with gentle shaking every 5 minutes. 100 μL of 0.5 M EDTA was added to each tube to a final concentration of 10 mM and incubated for an additional 5 minutes at 37° C. Tissue homogenate was strained through a 70 μm filter and washed with MACS. The resulting cell pellets were then incubated with 1mL ACK buffer on ice to lyse the red blood cells

and washed with MACS. In preparation for staining with Live/Dead fixable stain, cells were washed with PBS.

Live Dead Fixable Violet stain (Thermo Fisher Scientific, Waltham, MA) was used for removal of dead cells from analysis. The Foxp3/Transcription Factor Staining Buffer (Ebioscience) was used for cells requiring intracellular staining. The following antibodies (clone) were purchased for analysis from Biolegend (San Diego, CA) or Ebioscience (Waltham, MA): CD45 (30-F11), Ly6G (1A8), F4/80 (BM8), Siglec F, and CD11b. Isotype antibodies were used to establish gating. Samples were analyzed on the DAKO Cyan 5 ADP.

Results

Encapsulating and Microporous Hydrogel Fabrication

Hydrogels were formed using a 4-arm PEG maleimide, with distinct crosslinking strategies applied to create the encapsulating or microporous constructs. Encapsulating hydrogels were crosslinked in a PDMS mold, using a non-degradable, three-cysteine-containing crosslinking peptide (GCYKNRGCYKNRCG), which has previously been applied to safely encapsulate islets (Rios et al., 2016). After incubation in media, the dimensions of the encapsulating gel were approximately 6 mm in diameter and approximately 1.5 mm in height **(Figure 1A)**, adequate for implantation into the epididymal fat pad. Microporous hydrogels were fabricated by mixing salt particles in functionalized PEG precursor containing photoinitiator. Following UV photopolymerization and salt leaching, the gels had a microporous architecture **(Figure 1B-C).** The PEG wt% (wt/vol) of the microporous hydrogel was 20%, as lower concentrations resulted in collapsible gels with insufficient integrity for islet seeding. The

volume and dimensions of the microporous hydrogel were the same as the encapsulating hydrogels. Following swelling, microporous hydrogels were approximately 6.0 mm in diameter and 1.5 mm in height, a size suitable for transplantation into the epididymal fat pad. Furthermore, resultant hydrogels were sufficiently robust for surgical handling and implantation.

Syngeneic Islet Transplants in Encapsulating and Microporous Hydrogels

The engraftment and function of islets encapsulated in bulk hydrogels or seeded on microporous hydrogels was investigated by transplantation into the epididymal fat pad of streptozotocin-induced diabetic mice. Bulk, non-degradable encapsulating hydrogels with 700 islet equivalent (IEQ) reversed diabetes in recipient mice and achieved stable normoglycemia (i.e., <200 mg/dL) by day 17 **(Figure 2A)**. Salt-leached, microporous hydrogels achieved stable normoglycemia by day 15 post-transplant **(Figure 2B).** Interestingly, the blood glucose dynamics of the pre-engraftment period varied between the two designs. For the encapsulated islets, blood glucose levels remained consistently elevated above 300 mg/dL and were as high as 400mg/dL between day 2 and 10 before gradually declining and achieving normoglycemia by day 17. In contrast, the microporous hydrogels attained near normoglycemic blood glucose levels for the first 6 days after transplant, transiently rose to a maximum of 300 mg/dL between days 7 and 14, with re-establishment of normoglycemia by day 15. In both conditions, removal of the hydrogel from the mouse resulted in a return to hyperglycemia. Collectively, these syngeneic transplant studies indicated that non-degradable, encapsulating and microporous PEG hydrogels support islet function post-transplantation into the epididymal fat pad.

Graft Function

An intraperitoneal glucose tolerance test (IPGTT) was performed 30 days after islet transplantation to investigate glucose responsiveness of the two hydrogel types**.** The encapsulated islets had a delayed response to restoring normoglycemia in response to bolus glucose injection. The blood glucose levels of diabetic recipient mice with encapsulating hydrogels peaked at 30 minutes post-injection of glucose, versus control mice which peaked at 15 minutes post-injection. At the 60 minute time point, blood glucose levels had decreased toward normoglycemia in both groups. Normoglycemic blood glucose levels were re-established at 120 minutes and 90 minutes for the encapsulating gel (175±9 mg/dL) (n=5, ± SEM) and control group (187±4 mg/dL) (n=4, ± SEM), respectively **(Figure 3A)**. Area under the curve analysis confirmed statistical differences at 30, 60, and 90-minute time points ($p \leq 0.006$) between the encapsulating and control group **(Supplemental Figure 1).** Mice transplanted with islets on microporous gels had no significant difference in blood glucose levels for the glucose tolerance test relative to the control **(Figure 3B)**. The blood glucose levels of the microporous hydrogel and control groups peaked at 15 minutes post-injection of glucose. At 30 minutes, blood glucose levels for mice decreased toward normoglycemia in both groups. At 60 minutes, the microporous gel (153±14 mg/dL) and control group (150±18 mg/dL) both achieved normoglycemic levels and their blood glucose remained normoglycemic for the remainder of the time points. For control groups, the blood glucose for the control group relative to the encapsulating hydrogel peaked higher at 15 minutes post injection (352 mg/dL) compared to the control group for the microporous hydrogel (300 mg/dL). This

delay resulted in a statistically significant difference at the 60, 90, 120, and 150-minute time points between the two control groups.

Histological Analysis of Hydrogel Explants

Transplanted hydrogels remained intact and were well-secured in the epididymal fat pad upon removal **(Figure 4A)**. Insulin-positive islets were identified within histological sections for both the encapsulating (**Figure 4B**) and microporous hydrogels at day 30 post-transplant **(Figure 4C)**. Islets encapsulated within the hydrogel or seeded onto the microporous hydrogel maintained their morphology, function, and engraft in the fat pad transplant site. The encapsulated islets were isolated from the host tissue, whereas islets within the microporous gel were integrated with the host tissue, as expected.

Innate Immune Cell Infiltration in Encapsulating and Microporous Hydrogels

Given the differences in initial blood glucose levels and the interaction with host tissue, we investigated the innate immune response during the initial stages of engraftment changes with respect to hydrogel architecture. At day 7 post-implantation, the microporous PEG hydrogel had a large population of neutrophils (Figure 5). Approximately 56% of recovered leukocytes from microporous gels expressed a CD11b+ Ly6G+ F4/80- phenotype consistent with neutrophils compared to only 24% of cells isolated along with the encapsulated islet graft. We also investigated eosinophils and macrophage percentages, as both cell types can be enriched in adipose tissue. Though not statistically significant, we also observed a trend towards decreased percentages of eosinophils (6% vs 16%) and macrophages (9% vs 17%) in the encapsulating relative to the microporous hydrogels.

4.5 Discussion

In this report, we formulated both encapsulating and microporous hydrogels from PEG in order to compare islet engraftment and function for these two platforms. PEG is soluble with water and can be formed into a hydrogel in cytocompatible conditions,

allowing for both prefabricated and *in situ* gelation strategies (Weaver et al., 2017; Zhu, 2010). Both hydrogels were formed as non-degradable on the time frame of the study. The cross-linking peptides employed to crosslink the encapsulating hydrogel are not recognizable by endogenous proteases to produce the non-degradable hydrogel. Islet encapsulation using this strategy has previously been shown to support high viability (Rios et al., 2016). We also developed a non-degradable PEG microporous hydrogel by incorporating salt as a leachable porogen. These PEG and salt mixture was cross-linked with UV and extensively washed to remove the porogen. The porogen had a diameter in the range of 250 to 425 μm, which creates pores sufficiently large for the islets to be seeded. The seeding of islets into microporous scaffolds occurs with high efficiency and cell viability (Blomeier et al., 2006; Gibly et al., 2011b). While the same backbone is used for the encapsulating and microporous hydrogel, the different crosslinking strategies may result in distinct crosslink densities and network structure. Crosslinking density can impact the behavior of cells and delivery from gels and is thus a consideration for cell-laden hydrogel platforms. The encapsulating hydrogel was formed via Michael-type addition (a form of step growth polymerization) in which fabrication can be performed under ambient conditions without use of free radical initiators. This crosslinking approach ultimately results in less defects in the hydrogel network and better control of the crosslinking density compared to photopolymerization (a form of chain polymerization) used for the microporous hydrogel (Lin and Anseth, 2009). In the context of islet encapsulation, previous studies have demonstrated altering crosslinking density did not impact encapsulated islet survival or insulin secretion *in vitro*, yet did impact the amount of free volume available for diffusion (Weber et al., 2009). This result

has direct implications for delivery of insulin and the time frames for restoration of normoglycemia after a glucose load post-transplantation. A high PEG concentration of 20% was used to fabricate the microporous hydrogel to improve mechanical robustness for potential clinical use, which may alter crosslinking density and thus substrate stiffness (DeForest and Anseth, 2012). However, isolated islets have been shown to survive and function on or within a wide range of polymeric biomaterial substrates with varying stiffness both *in vitro* and *in vivo* in numerous studies (Apeldoorn, 2015; Buitinga et al., 2013; Foster and García, 2017; Graham et al., 2013; Pedraza et al., 2013; Phelps et al., 2013; Smink et al., 2017).

The encapsulating and microporous hydrogels established stable normoglycemia by the third week after transplantation, though the microporous hydrogel had lower blood glucose levels at earlier times. These time scales are consistent with other studies which transplanted islets using degradable, encapsulating PEG hydrogels (Phelps et al., 2013; Weaver et al., 2017). Both material architectures ultimately restored blood glucose control using 700 IEQ. The encapsulating hydrogel had the highest blood glucose levels post transplantation, which has been proposed to be associated with the separation from the host tissue and the time for the cells to acclimate within the hydrogel (Pepper et al., 2013). Islets transplanted on microporous hydrogel produced lower blood glucose levels relative to the encapsulated platform, yet the blood glucose levels were elevated relative similar to our previous reports with microporous PLG scaffolds. The PLG scaffolds result in normoglycemia within a couple of days post-transplantation using a comparable quantity of syngeneic islets (Blomeier et al., 2006; Gibly et al., 2011b). Interestingly, the microporous PEG hydrogels had a modest increase in blood glucose between days 7 and

14 before returning to normoglycemic levels. This rise in blood glucose levels may be associated with our measurements that over 50% of CD45+ leukocytes recovered from the microporous hydrogel at day 7 are neutrophils. Previous work with PLG scaffolds have much lower percentages of neutrophils, and the persistence of neutrophils within the hydrogel is unusual given circulating neutrophils typically undergo spontaneous apoptosis within 5 days in the absence of extracellular stimuli. Neutrophils can cause damage to islets via release of reactive oxygen species or inflammatory cytokines that results in activation of antigen presenting cells (Gibly et al., 2011a).

Islets transplanted on microporous hydrogels offered greater responsiveness to blood glucose changes. At day 30 post transplantation, islets implanted on microporous hydrogels restored normoglycemia after bolus glucose delivery with kinetics similar to healthy mice with endogenous islets. The microporous platform has been reported to allow the ingrowth of host cells and the revascularization of transplanted islets (Blomeier et al., 2006; Gibly et al., 2011b), which can allow for rapid detection of blood glucose changes as well as the distribution of secreted insulin. In contrast, the encapsulated hydrogel showed a delayed recovery to normoglycemia. The encapsulated hydrogel platform creates distance between the vasculature and islets, thereby requiring time for the transport of glucose and insulin that likely contributes to the delay in responsiveness in the IPGTT.

The immune cell composition of the grafts at day 7 were distinct for the platform. The platforms were formed in a manner that would make them non-degradable, which are generally thought to initiate a foreign body response due to the inability of innate cells to phagocytose the material, leading to a “frustrated” phenotype that may lead to enhanced

secretion of pro-inflammatory cytokines(Lynn et al., 2010; Lynn et al., 2011). A neutrophil-based response was observed with the biomaterials implanted in the intraperitoneal space, consistent with previous reports that indicate that PEG has increased neutrophil accumulation (Jhunjhunwala et al., 2015; Jiang et al., 2007). The increased neutrophil recruitment with the microporous hydrogels was anticipated based on the increased surface area within the hydrogel, though a contribution of the crosslink density and network structure may also contribute. High porosity and hydrophilicity have been suggested as material properties influencing leukocyte adhesion that would diminish innate cell responses; however, the non-degradable nature of the hydrogel could negate the structural aspects of the microporous scaffolds (Anderson et al., 2008; Selders et al., 2017). Interestingly, the encapsulated non-porous hydrogel did not recruit a similar percentage of neutrophils despite being composed of the same PEG backbone. This reduction in neutrophils with encapsulation could suggest that UV crosslinking of PEG may introduce a response that is not observed with YKNR crosslinking. Slight alterations to the biomaterial chemistry can induce substantial differences in the host response (Bratlie et al., 2012; Desai and Shea, 2016). Importantly, the presence of neutrophils was not sufficient to cause graft failure, as all recipients of microporous hydrogels seeded with islets recovered to stable normoglycemia. Despite neutrophils being increased, the population of macrophages was consistent between the platforms, with macrophages being a consistent component of a traditional foreign body response (Anderson et al., 2008; Blakney et al., 2012; Lynn and Bryant, 2011; McWhorter et al., 2015).

4.6 Conclusion

Herein, the transplantation of islets within encapsulating hydrogels or on microporous hydrogels, both of which were formed from PEG, was compared. Both transplantation platforms support function of the islets, yet the encapsulation system had initially greater blood glucose levels relative to the microporous hydrogels. Microporous hydrogels were able to support lower blood glucose levels at earlier times, and provided a more rapid restoration of normoglycemia following an intraperitoneal glucose tolerance tests, which likely results from a direct integration with host tissue. However, the microporous hydrogels had greater numbers of neutrophils associated with the graft relative to the encapsulating hydrogels. These results highlight the impact of the material selection and architecture (encapsulating relative to microporous) can have on the engraftment and function of transplanted cells.

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Figures

Figure 1. **Encapsulating and Microprous Hydrogels For Islet Transplantation.** (A) 10%(wt/vol) bulk PEG hydrogels were fabricated to encapsulate islets**.** (B, C) 20% (wt/vol) Microporous gel for islet seeding. Gels were stained with sirius red for visualization.

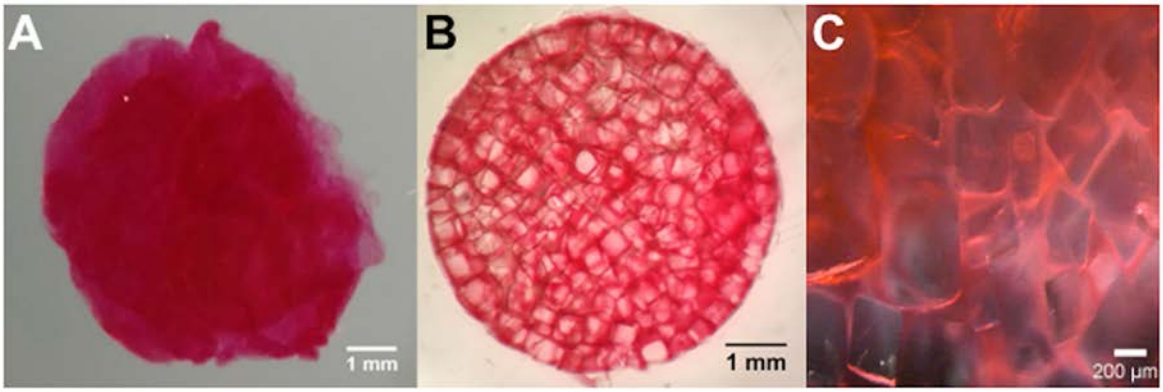


Figure 2. **Blood Glucose Monitoring Post-Transplant with Hydrogel Materials in Fat Pad Transplantation Site of Diabetic Mice.** (A) Bulk, non-degradable encapsulating hydrogels with 700 IEQ reversed diabetes in recipient mice, with consistent normoglycemia achieved by 3 weeks post-transplant (n=3, ± SEM)**.** (B) Salt-leached, microporous hydrogels seeded with 700 IEQ displayed consistent normoglycemia by 3 weeks post-transplant (n=5 pre-graft removal, n=4 post-graft removal, ± SEM). Recipient mice in both groups reverted to a diabetic state within 2-4 days following hydrogel removal (indicated with a black arrow).

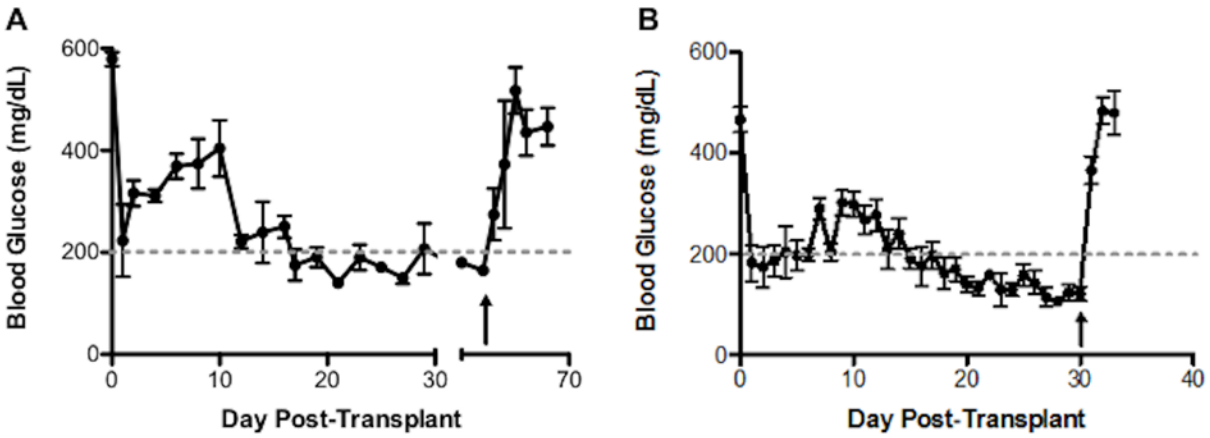


Figure 3. **Glucose Responsiveness of Encapsulating and Microporous Hydrogels 1 Month Post-Transplantation.** (A) Post-injection, normoglycemic blood glucose levels (<200 mg/dL) were achieved by the encapsulating (n=5, ± SEM) and control (n=9, ± SEM) groups at 120 and 90 minutes, respectively. (B) Post-injection, normoglycemic blood glucose levels were achieved by the microporous (n=5 pre-graft removal, n=4 post-graft removal, ± SEM) and control (n=4, ± SEM) groups at 60 minutes.

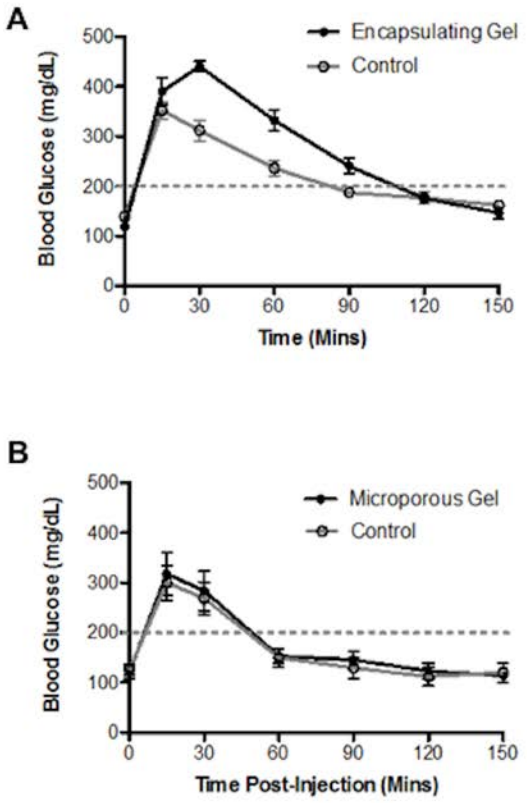
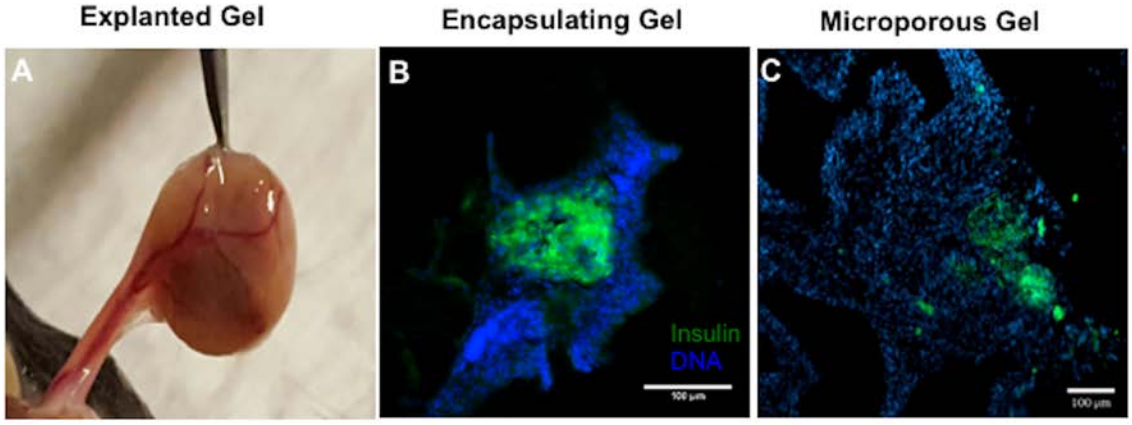


Figure 4. **Insulin-Positive Islets Identified in Explanted Hydrogels at Day 10 and 1 Month Post-Transplant. (**A) Explanted microporous hydrogel 1 month post-transplant. (B) Insulin-positive islets were identified encapsulating hydrogels removed at Day 32 (~1 month). (C) Insulin-positive islets were also identified in microporous hydrogels removed at Day 35 (Scale bar: 100 μm).



Increase size of scale bars

Figure 5. **Innate Immune Cell Populations in the PEG Hydrogel Environment**. Percentages of innate cells identified from hydrogels extracted from hydrogels 7 days after implantation. Graph depicts mean ± SEM. N = 4 hydrogels per condition *<0.05. Statistics determined by unpaired T test**.**