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Injectable Polyethylene Glycol Hydrogel for Islet Encapsulation: an *in vitro* and *in vivo* Characterization

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Abstract

An injection of hydrogel-encapsulated islets that controls blood glucose levels over long term would provide a much needed alternative treatment for type 1 diabetes mellitus (T1DM). To this end, we tested the feasibility of using an injectable polyethylene glycol (PEG) hydrogel as a scaffold for islet encapsulation. Encapsulated islets cultured *in vitro* for 6 days showed excellent cell viability and released insulin with higher basal and stimulated insulin secretion than control islets. Host responses to PEG hydrogels were studied by injecting PEG hydrogels (no treatment and vehicle controls used) into the peritoneal cavities of B6D2F1 mice and monitoring alterations in body weight, food and water intake, and blood glucose levels. After 2 weeks, peritoneal cavity cells were harvested, followed by hydrogel retrieval, and extraction of spleens. Body weights, food and water intake, and blood glucose levels were unaltered in mice injected with hydrogels compared to no treatment and vehicle-injected control mice. Frozen sections of a hydrogel showed the presence of tissues and small number of immune cells surrounding the hydrogel but no cell infiltration into the hydrogel bulk. Spleen sizes were not significantly different under the experimental conditions. Peritoneal cavity cells were slightly higher in mice injected with hydrogels compared to control mice but no statistical difference between vehicle- and hydrogel-injected mice was noted. As an *in vivo* feasibility study, streptozotocin-induced diabetic mice were injected with vehicle or hydrogels containing 50 islets each into two sites, the peritoneal cavity and a subcutaneous site on the back. Transient control of blood glucose levels were observed in mice injected with hydrogels containing islets. In summary, we developed an injectable PEG hydrogel that supported islet function and survival *in vitro* and *in vivo* and elicited only a mild host response. Our work illustrates the feasibility of using injectable PEG hydrogels for islet encapsulation.

Keywords

diabetes; islet; polyethylene glycol; hydrogel; transplantation; encapsulation

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Introduction

Approximately 1.25 million Americans have type 1 diabetes mellitus (T1DM) (American Diabetes Association 2012 statistics). T1DM is a chronic, progressive autoimmune disease caused by selective destruction of insulin-producing β -cells within the pancreatic islets of Langerhans [1]. Insulin delivered through an insulin pump or daily injections is essential to sustain life for these patients. Despite careful monitoring, a subset of patients with complicated T1DM are at high risk of life-threatening hypoglycemia episodes. Islet transplantation, when successful, eliminates hypoglycemic episodes, slows or prevents the progression of complications, and improves patient quality of life [2]. However, major hurdles for this therapy include poor islet survival, side effects of life-long immunosuppressants and shortage of islets from donors [3]. To circumvent islet shortage and post-transplantation rejection, islet encapsulation within semi-permeable, biocompatible membrane as a strategy to mask islets from host immune cells has emerged.

A variety of encapsulation approaches have emerged, including macroencapsulation, microencapsulation, conformal coating and nanoencapsulation as well as multiple transplantation sites such as liver (via the portal vein), peritoneal cavity, kidney capsule, omental pouch, skeletal muscle, and subcutaneous sites [4]. Transient independence or reduction in insulin requirement to control blood glucose levels has been reported with different approaches. However, maintaining a long-term survival and function of encapsulated islets is still a major challenge to overcome. Strategies such as promotion of vascularization using growth factors [5], co-encapsulation of islets with mesenchymal stem cells [6], tethering extracellular matrix protein fragments or cytokine receptor fragments to the encapsulating polymer meshwork [7], and others have been explored.

Developing encapsulated islet transplantation as a routine treatment option for severe diabetes requires advances in the development of novel engineered scaffolds and devices that promote long-term islet survival and function. A simple safe minimally-invasive procedure will also enhance wide clinical applications of encapsulated islet transplantation. Subcutaneous sites as transplantation location and injectable scaffolds would meet these conditions. Subcutaneous sites, however, are known to be inadequate for transplantation due to lack of optimal vascular networks [8]. To circumvent this problem, strategies of pre-vascularization using devices such as Sernovas's Cell Pouch or TheraCyte [9], implantation of adipose tissue-derived stromal cells [10], and bioactive vascular endothelial growth factor (VEGF)-releasing polyethylene glycol (PEG) hydrogel [11] have been used.

Injectable hydrogel scaffolds for drug, peptide, and cell delivery have been used for various biomedical applications including treatment of diabetes. Glucose-dependent insulin release using glucose-responsive hydrogels has been recently reported [12], which could potentially reduce hypoglycemic episodes associated with insulin injections. Injectable insulin-lysozyme-loaded nanogel for basal insulin treatment [13], thermo-reversible injectable gel for exenatide delivery [14], and injectable PEG-bovine serum albumin (BSA)-Coumarin-GOx hydrogel for continuous glucose monitoring [15] are a few examples of potential innovative new advances in diabetes therapy. A number of papers were published in 1980s on injectable microencapsulated islet transplantation using alginate-polylysine-alginate

hydrogel [16–18]. The seemingly successful strategies, however, were not translated into clinical applications due to highly variable and inconsistent results from one laboratory to another [19]. Fibrosis of capsules [20], irregular pore size of the alginate hydrogels [21], and breakage of alginate crosslinks [22] are some of the postulated reasons of graft failure. Injectable hydrogel scaffolds composed of non-alginate material for islet transplantation has recently emerged as the subject of renewed research interest. An injectable synthetic saccharide-peptide (SP) hydrogel has been shown to promote islet viability and function both *in vitro* and *in vivo* [23]. Although the authors used syngeneic rather than allogeneic islets, and a surgical method rather than an injection for SP hydrogel-encapsulated islet transplantation in an omental pouch, this study provided evidence for proof of concept for using injectable hydrogel scaffolds for islet transplantation.

PEG-based hydrogels have been extensively used for islet encapsulation, because of their nanoporosity and, hence, immunoprotective properties, inertness, exceptional biocompatibility, and mechanical properties closely emulating that of soft tissues [24, 25]. However, while previous work has focused on either microencapsulating islets in the polymer [25] or in pre-formed hydrogel slabs [26], here we focus on minimally-invasive injectable scaffolds. While microencapsulation does not prevent injectability, the added benefit of an injectable scaffold is its ability to secure the islet location and be retrievable in the case of an adverse effect. Here, we describe the ability of injectable PEG hydrogels to support islet viability and function *in vitro* and *in vivo* as well as the minimal *in vivo* inflammatory response to the PEG material alone when injected in the peritoneal cavity. PEG hydrogels were formed via Michael-type addition of a multiarm PEG-VS and a PEG-based dithiol crosslinker. The non-immunogenicity of the material and its ability to support islet function demonstrate that an injectable PEG hydrogel could be a viable islet encapsulation strategy to treat T1DM.

Materials and Methods

Materials

Male Sprague-Dawley rats (8 wk old) and male B6D2F1 (the F1 hybrids of C57BL/6 and DBA/2, 4–6 wk old) were purchased from Harlan Sprague-Dawley. PEG-dithiol (PEG-diSH; MW 3.4 kDa) was obtained from Laysan Bio, Inc (Arab, AL) and 4-arm PEG-vinyl sulfone (PEG-VS; MW 10 kDa), obtained from Jenkem Technology (Plano, TX). Collagenase type XI, Hanks' balanced salt solution, 4',6'-diamidine-2'-phenylindole dihydrochloride (DAPI) and streptozotocin were obtained from Sigma (St. Louis, MO). Tissue culture medium, CMRL-1066, was obtained from Invitrogen (Carlsbad, CA). Rat insulin radioimmunoassay (RIA) kit was obtained from Millipore (St. Louis, MO). Bright Cryo-M-Bed was obtained from Hacker Instruments & Industries (Winnsboro, SC). Hematoxylin was obtained from Santa Cruz Biotechnology (Dallas, TX). Alexa fluor 488 anti-mouse Ly-6G/Ly-6C antibody, Alexa fluor 647 anti-mouse F4/80 antibody, and Alexa fluor 647 anti-mouse CD19 antibody were purchased from BioLegend (San Diego, CA). Contour Blood Glucose Test Strips were obtained from American Diabetes Wholesale (ADW) Diabetes (Pompano Beach, FL). All other chemicals were from commercially available sources.

Animals

Male Sprague-Dawley rats and male B6D2F1 mice were maintained in our animal facility under controlled conditions (temperature 68–73°F and 12 h light-dark cycle). The rats (3 per cage) and the mice (4 per cage) were fed with a commercial lean chow diet (EI-Mel, St. Louis, MO) and water *ad libitum*. All animal maintenance and treatment protocols complied with the Guide for Care and Use of Laboratory Animals as adopted by the National Institute of Health and approved by the SIUE Institutional Animal Care and Use Committee (IACUC).

Rat islet isolation and culture

Islets were isolated from male Sprague-Dawley rats (250–270 g) by collagenase digestion as described previously [27]. Briefly, pancreases were inflated with Hank's balanced salt solution (HBSS) containing 0.5 mg/ml collagenase, isolated, minced, and digested for 5 min at 37°C while shaking. The digested cell suspension was filtered through a 70 µm cell strainer to remove acinar cells. Islets were hand-picked under a stereomicroscope and cultured in cCMRL-1066 culture medium (CMRL-1066 medium supplemented with 2 mM L-glutamine, 10% heat inactivated fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin) in a CO₂ incubator.

Encapsulation of rat islets in PEG hydrogels

Islets (50 in 200 µl of cCMRL) were centrifuged for 20 sec at 10,000 rpm. After medium was removed, 25 µl of TEA, 15 µl of PEG-VS (20% w/v stock solution), and 10 µl of PEG-diSH (20% w/v stock solution) were added in the order listed while mixing islets with the solution by gentle pipetting. About 90 sec prior to completion of gelation, the mixture of islets and hydrogel material was transferred to Petri dish containing 2 ml of cCMRL using a 200 µl pipet tip. The shape of islets-laden hydrogels is malleable depending on the injection site (thin sheet, rod, or sphere).

Encapsulated islet survival and insulin secretion

Control and encapsulated islets were cultured up to 6 d at 5% CO₂ in cCMRL-1066 medium containing 10 mM glucose. Brightfield images of islets cultured for 4 d were acquired using a 10X objective in a Leica DMI inverted fluorescent microscope (Leica Microsystems Inc., Buffalo Grove, IL).

To assess islet function, control and encapsulated islets were pre-incubated in cCMRL-1066 containing 5.6 mM glucose for 30 min. Islets were washed once with 2 ml of cCMRL-1066 medium and incubated for 1 h in cCMRL-1066 containing 5.6 mM (basal) or 20 mM (stimulated) glucose to determine both basal and glucose-stimulated insulin secretion. Supernatants were assayed for insulin content by RIA following manufacturer's instructions.

Injection of hydrogels into the peritoneal cavity and determining its effect on mice

A mixture of 50 µl TEA (pH=8.0), 30 µl PEG-VS (20% w/v stock solution, pH 8.0), and 20 µl PEG-diSH (20% w/v stock solution, pH 8.0) was loaded in 0.5 ml syringe. About 90 sec prior to completion of gelation (~6 min at pH=8.0), the mixture of hydrogel material was

injected into the peritoneal cavity of a mouse using a 27G needle. No treatment and TEA (100 µl)-injected mice were included as controls. Body weights and food/water intake were determined every other day for 2 wk. Blood glucose levels were measured before and after the 2 wk monitoring period.

Frozen sectioning, Hematoxylin staining, and immunohistochemistry

After retrieval, each hydrogel was cut into 8 pieces and placed in a microfuge tube containing 800 µl of Bright Cryo-M-Bed freezing medium. Hydrogels in microfuge tubes were snap-frozen in liquid nitrogen, and stored at -70°C until use. Frozen hydrogel sections of 10 µm thickness were cut using a Vibratome (St. Louis, MO) and every 10th section was transferred to a coverslip. Hydrogel sections were placed in Wheaton staining jars filled with de-ionized (DI) water and left to soak for 5 min. The hydrogel sections were stained with Hematoxylin solution for 1 min, rinsed with water, and mounted on microscope slides. Images of hydrogel sections were captured using a 5X objective in a Leica DMI inverted fluorescent microscope. For immunohistochemistry, hydrogel sections were washed, blocked, and immunostained with Alexa fluor 647 anti-mouse F4/80 antibody for murine macrophages and DAPI for nuclear staining. Fluorescent images were obtained using a 40X objective in an Olympus FluoView confocal microscope (Olympus Corporation, Waltham, Massachusetts).

Determination of spleen size

After retrieval, spleens were dabbed on paper towel to remove moist, weighed, and photographed. Spleen size (% body weight (wt) = (wt of spleen/wt of mouse) * 100) was calculated to normalize across mice of different body sizes.

Determination of the number and types of peritoneal cavity cells

After CO₂ asphyxiation, the abdomen of each mouse was wetted with 70% alcohol to sterilize the area, followed by removal of the outer skin with fur and a midline incision (~1"). While holding abdominal skin with forceps, 3 ml of 1X PBS was injected into the peritoneal cavity, the mouse was gently rocked, and the peritoneal fluid slowly withdrawn. The collected peritoneal fluid was dispensed into a 15 ml conical tube on ice. The same procedure was repeated once more. The pooled peritoneal fluid was centrifuged at 1,500 rpm and cell pellets were re-suspended in 1 ml 1X PBS. Samples (20 µl) were removed, mixed with 4% Trypan Blue solution (20 µl), and the number of cells was determined using a hemacytometer. To determine the types of peritoneal cavity cells, cells (100 µl suspension) were mixed with 2 µl of fluorescently labeled antibodies and DAPI for nuclear staining and incubated for 20 min. Samples (20 µl) were loaded onto a microscopic slide, covered with a glass coverslip, and sealed with a nail polish. Fluorescent images were obtained using a 40X objective in an Olympus FluoView confocal microscope.

Induction of diabetes by streptozotocin injection

Mice were fasted for 4 h prior to streptozotocin injection. Mice were anesthetized by placing them in a gas anesthetizing chamber filled with isoflurane, followed by an intraperitoneal injection with 50 mg/kg streptozotocin (7.5 mg/ml in Na Citrate Buffer, pH=4.5). Each

mouse was given one injection for 5 consecutive days. Mice were supplied with 10% sucrose water during the 5 d of injection period to avoid sudden hypoglycemia post-injection. Mice were tested for hyperglycemia (blood glucose levels > 360 mg/dl) at 2 wk post-injection.

Injection of encapsulated islets into streptozotocin-induced diabetic mice

Blood glucose levels were measured intermittently for ~30 h prior to injection of hydrogels containing 50 islets each into two different sites, the peritoneal cavity and a subcutaneous site on the back. For preparation of islet-encapsulated hydrogels, 50 islets were suspended in 50 μ l of TEA (pH=8.0), followed by addition of 30 μ l of PEG-VS (20% w/v stock solution, pH 8.0) and 20 μ l of PEG-diSH (20% w/v stock solution, pH 8.0), and loaded into a 0.5 ml syringe. About 90 sec prior to completion of gelation (~6 min at pH=8.0), the mixture of hydrogel material was injected into the peritoneal cavity of a mouse anesthetized with isoflurane using a 22G needle. The second hydrogel containing 50 islets was injected into the subcutaneous site on the back of the same mouse under anesthesia through a nose cone. Post-injection, blood glucose levels were measured intermittently for ~90 h.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Single factor analysis of variance (ANOVA) was used to determine statistical significance for multiple parameters followed by Tukey's post-hoc test. Two-tailed Student's t-test, paired or unpaired, was used to determine statistical significance between two parameters. Significant differences are indicated by * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001. More details on statistical tests are given in the figure legends. GraphPad Prism software was used for all statistical tests.

Results and Discussions

Development of an injectable hydrogel scaffold may promote clinical applications of encapsulated islet transplantation, providing a minimally-invasive and efficient paradigm in islet transplantation. In particular, we chose an injectable PEG hydrogel because PEG is biocompatible, hydrophilic, immunoprotective, has tunable and reproducible physical and mechanical properties, and has been approved by the U.S. Food and Drug Administration for use in biomedical devices [24, 28]. Figure 1A shows that mixing multi-arm PEG containing reactive groups such as vinyl sulfone, acrylate, or maleimide with PEG-diSH or other dithiol crosslinkers and isolated islets generates islets encapsulated in a nanoporous PEG hydrogel. Figure 1B shows the chemical structures of 4-arm PEG-VS and the crosslinker PEG-diSH used in this study as well as a picture of the hydrogel injected in water, where a blue food dye was used for contrast (the hydrogel is completely transparent). Upon injection *in vitro* (as demonstrated in Figure 1B), the hydrogel assumed a slab geometry of ~11 mm in diameter and ~1 mm in height (height was calculated based on measured diameter, known volume and assuming a perfect cylinder) for a gel precursor solution volume of 100 μ l. Previous research on PEG hydrogels for islet encapsulation has shown >95% cell viability in PEG hydrogel slabs of 1 mm height even after 14 d of culture [29]; hence, the current hydrogel geometry alone was not expected to negatively impact islet viability due to reduced access of oxygen and nutrients from the media.

Figure 2A shows images of control (a) and encapsulated islets (b) cultured in cCMRL-1066 containing 10 mM glucose at 4 d post-encapsulation. The PEG hydrogel is not visible because it is transparent. Control islets appeared bigger than encapsulated islets because fibroblasts within islets adhered to the Petri dish, displaying an extended morphology, whereas islets contained in the three-dimensional (3D) hydrogels were unable to do so. Encapsulated islets maintained the same rounded morphology for 6 d post-encapsulation, the time period that we studied. Others have previously demonstrated that islets retained a round morphology up to 7 d post-encapsulation in silk hydrogels, where morphology was independent of the presence of adhesive extracellular matrix (ECM) proteins in the inert silk hydrogel [30]. The preservation of islet shape, morphology and physical integrity upon *in vitro* encapsulation is critical for proper metabolic function [31].

Figure 2B demonstrates that insulin secretion by encapsulated islets at both basal (5.6 mM glucose) and stimulated glucose (20 mM glucose) concentrations was significantly higher than that by control islets. During the 4 d of culture, the different microenvironments might have altered the expression levels/patterns of glucose transporters and insulin receptors in control and encapsulated islets. In a simple example, diffusion of glucose and insulin in and out of islets enclosed in the 3D hydrogel may take place all around the islet surface, whereas that in control islets could be impeded at the bottom surface where the islets were attached to the Petri dish. Note that an increased insulin secretion upon encapsulation in an inert hydrogel, specifically polyvinyl alcohol, has been noted by others [32]. Interestingly, increased basal and stimulated insulin secretion levels upon islet encapsulation in PEG hydrogels has been reported in response to PEG modification with the insulinotropic ligand GLP-1 [25]; here we used non-functionalized PEG. Importantly, encapsulated islets displayed glucose-stimulated insulin secretion suggesting that β -cells within the encapsulated islets were functional. Of note, the gelation times of PEG hydrogels were greatly affected by the pH of the reaction. For example, the gelation times of PEG-VS hydrogels at pH 7.4 and 8.0 were 22.2 ± 0.1 min and 5.7 ± 0.1 min, respectively. Figure 2B shows that the different gelation times, however, had no significant effect on β -cell function.

One of the primary objectives of an encapsulation strategy is immunoisolation to avoid immune rejection from the host [33]. However, the biomaterial used for the encapsulation scaffold itself could trigger inflammatory reactions and immune responses. Thus, we tested the biocompatibility of PEG hydrogels by injecting empty hydrogels into the peritoneal cavities of mice. The peritoneal cavity was selected as an optimal site for PEG hydrogel injection because this site is easily accessible, relatively safe, and can easily accommodate 100 μ l volumes of the PEG hydrogel precursor solution. Besides being one of the common sites for islet transplantation, recent studies have shown that alginate-encapsulated islet implants in the peritoneal cavity exhibited sustained function, which have led to a pilot study in a T1DM patient [34]. Interestingly, the authors noted an inflammatory reaction towards the material as well as fibrotic tissue formation [34], which further motivated us to test host responses towards the PEG material alone. Control, TEA, and hydrogel-injected mice (n=6) were monitored for 2 wk post-injection (Figure 3). Figures 3A, 3B, and 3C demonstrate that mice injected with empty PEG hydrogels showed no apparent adverse responses in terms of body weights, food/water intake, and blood glucose levels compared to control and TEA-

injected mice. The reason for the significant differences in blood glucose levels of control mice before and after 2 wk monitoring period is unknown. An intraperitoneal injection of a PEG hydrogel has not been previously attempted. However, the positive result was expected since PEG has been approved by the U.S. Food and Drug Administration for use in biomedical devices [35] and because the total mass of the PEG solution used was a fraction of the total body mass of the mice (~0.3%), hence should not interfere with mice daily activity.

After 2 wk of *in vivo* monitoring, PEG hydrogels were retrieved. Figure 4A panel (a) shows that the shape of a PEG hydrogel injected into the peritoneal cavity conformed to the space available at the injection site. The hydrogel is circled with a light blue dotted line. Figures 4A panel (b) and (c) show that the shapes of the hydrogels retrieved from other mice were also irregular with tissues and blood vessels attached to the surface. Figure 4B demonstrates a representative frozen section of a hydrogel stained with Hematoxylin, followed by capturing an image using a 5X objective in a Leica DMI inverted fluorescent microscope. The hydrogel mesh network is visible in the middle of the image with irregular shapes of tissues (indicated by white arrows) and host cells (indicated by short black arrows) present along the hydrogel section. The inset shows 3-fold magnification of the designated area. As the image shown in Figure 4A panel b indicated, the hydrogel was surrounded by tissues present at the injection site or recruited host immune cells, but the inside of the hydrogel was intact and relatively clear of host cells (Figure 4B). This was expected since PEG is nanoporous and should not allow cell infiltration in the hydrogel bulk [36]. However, some cells and peritoneal fluid and tissues could have been embedded in the hydrogel periphery during the injection: the hydrogel precursor solution was injected while a viscous liquid and then fully solidified *in situ*. To demonstrate the plausibility of this hypothesis, we also injected PEG hydrogels subcutaneously either directly (Supplemental Figure (SF) 1a, b) or by first creating an air pouch (injection of 1 ml air, SF 1c, d). When injected directly, the hydrogels showed the presence of tissues and blood vessels (SF 1b), but when injected in a pre-formed air pouch, the hydrogels appeared mostly clear of blood vessels (SF 1d). This data supported our hypothesis that the process of *in situ* gelation upon injection, rather than cell infiltration into the hydrogel bulk could be responsible for the presence of tissues and blood vessels in the hydrogel periphery.

Innate immune reaction occurs immediately after exposure and is typically initiated by cellular mediators such as macrophages, neutrophils, and natural killer cells [37]. To further study if the PEG hydrogel alone (no islets) was attracting some of the host immune cells, frozen hydrogel sections were immunostained with Alexa fluor 647 anti-mouse F4/80 antibody for murine macrophages (red) and DAPI (blue) for nuclear staining. Figure 4C shows images of hydrogel sections with cells staining positive for macrophages, suggesting that some of the cells surrounding the hydrogel (Figure 4B) were host immune cells. However, the immune reaction caused by the PEG hydrogel was relatively mild as evident by no significant differences in the sizes of retrieved spleens between the control, TEA- or hydrogel-injected mice (Figures 5A and 5B). Immune responses against various pathogens or disease states are associated with several-fold enlarged spleens (splenomegaly) [38, 39]. Figure 5A shows the images of spleens obtained from control, TEA-, or PEG hydrogel-

injected mice. The spleen size (normalized by body weight) showed no statistical difference under the experimental conditions, indicating no adverse effects from the injectable PEG hydrogel.

The types and the number of peritoneal cavity cells were also determined. The peritoneal cells are in general composed of B cells (50–60%), macrophages (30%), T cells (5–10%), and other cell types [40]. Figure 6A shows that macrophages (shown in red in panel **a**), lymphocytes (shown in red in panel **b**), and a small number of polymorphonuclear neutrophils (shown in green in panel **a**) were readily visible. Figure 6B shows that the number of peritoneal cavity cells was slightly increased in mice injected with PEG hydrogels compared to control mice. Note that an untreated control mouse (negative control) and a Freund's complete adjuvant injected mouse (positive control) have ~5–10 million and ~130 million peritoneal cavity cells, respectively [40, 41]. The number of peritoneal cells upon TEA or PEG injection was ~10–15 million, which was similar to the untreated control mouse and much lower than a chemically-activated positive control mouse, indicating no adverse effect from the hydrogel. Further, there were no significant differences in the number of peritoneal cavity cells between the mice injected with TEA and PEG hydrogels, suggesting that the injection alone or the buffer only, but not PEG, might have contributed to the slightly elevated cell count. Note that the TEA buffer is used as a biocompatible base to initiate the Michael-type addition reaction that leads to hydrogel formation and could be substituted by another base if required.

As an *in vivo* feasibility study, streptozotocin-induced diabetic mice were injected with TEA (control) or PEG hydrogels containing 50 islets each. The islets-laden hydrogels were injected into two different sites, namely the peritoneal cavity and a subcutaneous site on the back (Encapsulated Islets). The blood glucose levels observed in the mice are shown in Figure 7. Transient reduction in blood glucose levels (from ~600 mg/dl to ~200 mg/dl) were observed ~2 d after implantation in mice injected with encapsulated islets in PEG hydrogels (closed triangle). An *et al.* [9] had reported that implantation of ~500 rat islets encapsulated in a tubular alginate device and implanted into the peritoneal cavity of a C57BL/6 mouse reversed diabetes 2 d after the implantation. Our experiment showed that implantation of ~100 islets encapsulated in a PEG hydrogel significantly reduced blood glucose levels ~2 d after implantation as well, but intermittently. While it is possible that ~100 islets were not sufficient to reverse the diabetic conditions of the mice, this experiment nevertheless demonstrated the preliminary feasibility of our approach. Survival of encapsulated islets in the peritoneal cavity or at a subcutaneous site was anticipated to be transient due to insufficient blood vessels that deliver oxygen and nutrients to the islets at these sites.

Conclusions

An encapsulated islet transplantation strategy, if successful, would overcome some of the hurdles of islet transplantation such as human islet shortage, damage of transplanted islets, side effects due to lifelong immunosuppressant regimen, and complications associated with current islet transplant procedures, especially involving islet infusion into the portal vein. For routine clinical applications of the strategy, simple, safe, and minimally-invasive procedure that provides optimal blood glucose regulation is needed. We envision injection of

encapsulated islets to peritoneal or pre-vascularized subcutaneous sites in intervals of 3–4 month, which will provide a cure for T1DM, that will alleviate hypoglycemic episodes and complications associated with diabetes. To that end, we developed an injectable PEG hydrogel that supported, albeit transiently, islet function and survival *in vitro* and *in vivo*, and illustrated the feasibility of using injectable PEG hydrogels for islet encapsulation. Importantly, there was a very minimal inflammatory response towards the hydrogel and no change in mice body weight or food and water intake upon hydrogel injection. Future work will focus on hydrogel modification and pre-vascularization of the transplantation site to prolong islet function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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The abbreviations used are

PEG VS	poly(ethylene)glycol vinyl sulfone
TEA	Triethanolamine
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
HBSS	Hank's balanced salt solution
cCMRL-1066	complete CMRL-1066
BSA	Bovine serum albumin
DAPI	4',6-damidino-2-phenylindole
RIA	radioimmunoassay
SIUE	Southern Illinois University Edwardsville

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Figure 1.

Encapsulation of rat islets in a PEG hydrogel. **(A)** Mixing multi-arm PEG with PEG-dithiol and isolated islets generates an islets-laden PEG hydrogel. **(B)** The structures of PEG-vinyl sulfone and PEG-dithiol and a picture of the injected hydrogel *in vitro*; blue food dye was used for color contrast.

Figure 2.

Encapsulated islet survival and insulin secretion. **(A)** Control and encapsulated islets (PEG-VS hydrogel at pH=8.0) were cultured for 4 d in cMRL-1066 medium containing 10 mM glucose. After a 4 d incubation, brightfield images of islets were acquired using a 10X objective in a Leica DMI inverted fluorescent microscope. **(B)** Control and encapsulated islets (PEG-VS hydrogels formed at pH 7.4 or 8.0) were pre-incubated in cMRL-1066 containing 5.6 mM glucose for 30 min, washed, and incubated for additional 1 h in cMRL-1066 containing 5.6 mM or 20 mM glucose. Supernatants were assayed for insulin content by RIA. Data show the average of triplicates of 6 independent experiments. Values are presented as the means $\pm$ SEM. For Figure 2B, two-way ANOVA and Tukey's multiple comparison test was performed and significant differences are indicated by asterisks (n=6; **p<0.01, ***p<0.001, ****p<0.0001).

Figure 3.

Effect of PEG hydrogels on mice. To assess whether PEG hydrogel injection caused adverse effects on mice, mice were monitored for 2 wk post-injection. No treatment (Control) and TEA (100 μ l)-injected mice were included as controls. Body weights (A), food intake (B), and water (C) intake were determined every other day for 2 wk. Blood glucose levels (D) were measured before and after the 2 wk monitoring period. Data are the means $\pm$ SEM (n=6 for each group). Two-way ANOVA for repeated measure was performed and no statistical significance among the three different conditions was observed (A–C). Two-way ANOVA and Tukey's multiple comparison test was performed and significant differences are indicated as * p <0.05, ** p <0.01 (n=3) (D).

Figure 4.

Frozen sectioning, Hematoxylin staining, and immunohistochemistry of hydrogels after retrieval from mice. **(A)** Two wk post-injection hydrogels were retrieved from the peritoneal cavity. A hydrogel with an irregular shape conforming to the space available at the injection site (a) of a mouse is shown. Images of two retrieved hydrogels (b) and (c) show tissues and blood vessels attached on the surface. **(B)** Images of Hematoxylin-stained hydrogel sections were captured using a 5X objective in a Leica DMI inverted fluorescent microscope. The inset shows 3-fold magnification of the designated area. **(C)** For immunohistochemistry, hydrogel sections were immunostained with Alexa fluor 647 anti-mouse F4/80 antibody for murine macrophages and DAPI for nuclear staining. Fluorescent images were obtained using a 40X objective in an Olympus FluoView confocal microscope.

Figure 5.

Determination of spleen size. (A) After retrieval, spleens were weighed and photographed.

(B) Spleen size (% body weight) was calculated to normalize across mice of different body sizes. Data are the means $\pm$ SEM (n=6 for each group). One-way ANOVA test showed no statistical significance (n=3).

Figure 6.

Determination of the types and the number of peritoneal cavity cells in response to PEG or buffer injection. **(A)** To determine the types of peritoneal cavity cells, cells (100 μ l) isolated from TEA-injected mouse were stained with appropriate antibodies (macrophages, PMN, or lymphocytes) and DAPI for 20 min. Fluorescent images were obtained using a 40X objective in an Olympus FluoView confocal microscope. **(B)** The pooled peritoneal fluid was centrifuged and cell pellets were re-suspended in 1 ml 1X PBS. Samples (20 μ l) were removed, mixed with 4% Trypan Blue solution (20 μ l), and the number of cells was determined using a hemacytometer. Data are the means $\pm$ SEM (n=6 for each group). For Figure 6B, one-way ANOVA test was performed and statistical significance is reported as **p<0.01 (n=3).

Figure 7.

Injection of hydrogels containing islets reduced blood glucose levels. Blood glucose levels were measured intermittently for ~30 h prior to injection of TEA (Control) or hydrogels containing 50 islets each into two different sites, namely the peritoneal cavity and a subcutaneous site on the back (Encapsulated Islets) of diabetic mice. About 90 sec prior to completion of gelation (~6 min at pH=8.0), the hydrogel precursor solution was injected into the peritoneal cavity, followed by another injection at a subcutaneous site on the back of a mouse anesthetized with isoflurane using a 22G needle. Post-injection blood glucose levels were measured intermittently for additional ~90 h. The black arrow indicates the time when implantation of encapsulated islets or TEA injection was performed. Data are the means $\pm$ SEM (n=2 for each group). Unpaired student t- test was performed and significant differences between control and diabetic conditions are indicated by asterisks (*p<0.05, **p<0.01).