

PEG-based hydrogels as an in vitro encapsulation platform for testing controlled β -cell microenvironments

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Abstract

An in vitro encapsulation platform for systematically testing the effects of microenvironmental parameters on encapsulated islets was developed. The base encapsulation matrix was a biocompatible hydrogel formed via the photoinitiated polymerization of dimethacrylated poly(ethylene glycol) (PEGDM). The resulting inert encapsulation matrix affords control over the biochemical and biophysical cellular microenvironment and the introduction of systematic changes to this environment. The compatibility of the PEG-based encapsulation platform with pancreatic β -cells was first established using a murine β -cell line, MIN6. When cell–cell contacts were introduced via aggregation of MIN6 β -cells prior to encapsulation, MIN6 β -cells remained viable within the PEG hydrogel platform throughout 3 weeks of in vitro culture. Proliferating cells were observed within encapsulated MIN6 aggregates qualitatively with bromodeoxyuridine staining and quantitatively by measuring the DNA content of encapsulation samples with time. MIN6 β -cells were encapsulated in hydrogels formed from three PEGDM macromers of varying molecular weights ($\bar{M}_n = 4000, 8000, 10,000$ g/mol), and the resulting differences in hydrogel crosslinking density, which influences transport properties, did not affect encapsulated β -cell survival. Encapsulated MIN6 β -cells transplanted into diabetic mice returned blood glucose levels to normal levels, indicating in vivo function. Finally, the compatibility of the PEG encapsulation system with freshly isolated islets was confirmed.

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1. Introduction

A widely researched method for protecting islets for transplantation involves encapsulation within a mechanical barrier that permits the diffusion of cell-secreted insulin and small molecules required for islet survival, but prevents contact between encapsulated cells and host cells and passage of large immune system molecules such as antibodies. Many potential encapsulation systems have been explored, employing both natural and synthetic polymer barriers.

Specific examples include an ionically crosslinked alginate-polylysine encapsulation system [1], a covalently crosslinked poly(ethylene glycol) barrier [2], agarose beads [3], a copolymer of polyacrylonitrile and poly(vinyl chloride) (PAN–PVC) [4], a poly(*N*-isopropylacrylamide-co-acrylic acid) polymer gel [5], and a membrane formed via copolymerization of hydrophilic poly(*N,N*-dimethylacrylamide) and hydrophobic polyisobutylene stars [6]. Discussions of these systems and many others are found in several excellent literature reviews [7–11].

Despite various advantages and successes of specific barrier systems, no single material or encapsulation method exists that provides an ideal system for long-term islet survivability and immunoprotection. Common problems include insufficient biocompatibility with either the encapsulated islets or with the surrounding host tissue,

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both resulting in limited islet survival [7], and the inability to protect encapsulated islets from all avenues of immune rejection indefinitely [12]. Important considerations in the design of an encapsulation system include material biocompatibility, permeability, and long-term stability. The ideal encapsulation material must not only meet the physical barrier requirements listed above, but also not elicit an immune response, maintain physical integrity indefinitely, and promote long-term survival of encapsulated islets.

Our aim is to develop an encapsulation platform for systematically testing in vitro the effects of biophysical and biochemical microenvironmental parameters on encapsulated islets. A poly(ethylene glycol) (PEG)-based hydrogel was selected as the base matrix for developing such a platform. A chemically crosslinked network of dimethacrylated PEG (PEGDM) provides a highly biocompatible cell niche and is crosslinked via covalent bonds for maximum long-term stability. These PEG hydrogels have tunable structural properties, such as crosslinking density for controlled diffusion, and allow for changes in such structural properties without altering the chemistry of the network. The resistance of PEG to protein adsorption creates an environment that does not present any non-specific cell–matrix interactions, thus allowing the controlled introduction of specific interactions to encapsulated cells. Finally, PEG has previously been used to improve the biocompatibility [13,14] and mechanical integrity [15] of other islet encapsulation materials.

In this work, PEG hydrogels were formed via photoinitiated polymerization of dimethacrylated PEG chains, a technique previously shown to be compatible for the encapsulation of multiple cell types [16–19]. Structural properties, and subsequently transport properties, of the network can be controlled by changes in PEG molecular weight or the processing conditions, such as the percentage of PEG in the initial formulation. The PEG encapsulation system used in this work differs greatly from the PEG barrier previously reported by Cruise et al. [2]. Previous work focused on the interfacial photopolymerization of a thin PEG coating surrounding single islets via a photoinitiator localized to the surface of the islets by absorption and excited by an argon ion laser, while the presented approach employs bulk photopolymerization of a macroscopic hydrogel containing multiple islets via a photoinitiator in solution excited by a 365 nm ultraviolet lamp.

Results herein describe the initial screening of this PEG hydrogel encapsulation system using a murine immortalized pancreatic β -cell line, MIN6. MIN6 β -cells retain physiological characteristics of primary β -cells, including glucose-dependent insulin secretion, but offer the advantages of a readily available cell source conducive to extended in vitro culture. Using the MIN6 β -cell line as a model, we focused on cell survival as the initial test of our in vitro encapsulation platform. The compatibility of encapsulation in PEG hydrogels via photopolymerization with MIN6 β -cells and β -cell aggregates is first investigated,

and then the translation of this encapsulation platform from a model β -cell line to freshly isolated islets is demonstrated.

2. Materials and methods

2.1. PEGDM synthesis and hydrogel characterization

PEGDM was synthesized by reacting linear PEG (Sigma, St. Louis, MO) ($\bar{M}_n = 4000, 8000, 10,000$ g/mol) with methacrylic anhydride (Sigma) at a molar ratio of 1:10 via microwave irradiation [20]. Percent methacrylation was determined using ^1H NMR, by comparing the area under the integrals for the vinyl resonances ($\delta = 5.7$ and 6.1 ppm) to that for the PEG backbone (methylene protons, $\delta = 4.4$ ppm). Percent methacrylation for all macromers used in this work was $\sim 95 \pm 3\%$.

Hydrogels were formed from a 10 wt.% solution of PEGDM in Hank's Balanced Salt Solution (HBSS, Gibco, Carlsbad, CA), with 0.025 wt.% of the photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Ciba-Geigy, Tarrytown, NY). This solution was filter-sterilized using a 0.2 μm syringe filter, and 40 μL aliquots were exposed to 365 nm ultraviolet light at an intensity of ~ 7 mW cm^{-2} for 10 min within a disk-shaped mold ~ 5 mm in diameter. Hydrogel samples were swollen in phosphate buffered saline (PBS, pH 7.4, Gibco) at 37 $^\circ\text{C}$, and weighed to obtain the swollen mass, M_s . Samples were then placed in deionized water to remove PBS salts, frozen, and lyophilized overnight, and the dry polymer mass, M_d , determined. The volumetric swelling ratio, Q , was calculated from the mass swelling ratio (M_s/M_d), using density conversion factors.

The hydrogel crosslinking density, ρ_x , was then estimated using the following modified Flory–Rehner equation [21]:

$$\rho_x = -\frac{1}{\bar{v}} \left(\frac{\ln(1 - v_p) + v_p + \chi v_p^2}{v_p^{1/3} - \frac{v_p^2}{2}} \right) \quad (1)$$

where \bar{v} is the specific volume of the solvent; v_p is the polymer volume fraction (Q^{-1}); and χ is the solvent–polymer interaction parameter. For PEG in water and PBS with v_p values from 0.04 to 0.2, χ was reported to be constant with a value of 0.426 [22].

2.2. MIN6 culture, aggregation, and encapsulation

The murine pancreatic β -cell line MIN6 was cultured in RPMI 1640 (Gibco) supplemented with 1% penicillin–streptomycin (Gibco), 0.5 $\mu\text{g}/\text{mL}$ fungizone (Gibco), and 10% fetal bovine serum (Gibco). Cells were plated in 75 cm^2 treated tissue culture flasks and incubated at 37 $^\circ\text{C}$ in humid conditions with 5% CO_2 .

For aggregate formation, MIN6 β -cells were suspended in culture medium in non-treated 12-well tissue culture plates at a density of $\sim 1 \times 10^6$ cells/mL and placed on an

orbital shaker at ~ 70 rpm for 24 h. Images of aggregates taken under light microscopy were used to measure the average cross-sectional area of cellular aggregates and characterize the associated size distribution. Scion Image software (Scion Corp., Frederick, MD) was used for these calculations. Aggregate formation was characterized to establish that aggregate size was reproducible and controllable. The standard conditions listed above produce cellular aggregates with cross-sectional areas ranging from 11,300 to 17,600 μm^2 (95% confidence), with a corresponding approximate average diameter of 120 ± 30 μm .

For encapsulation, MIN6 β -cells and β -cell aggregates were suspended in the PEGDM macromer solution and exposed to ultraviolet light as described above. MIN6 were encapsulated in PEG hydrogels under the same conditions as used for other cell types, with one exception [16–19]. Because cell survival has been shown to improve with decreasing photoinitiator concentrations, a lower concentration of photoinitiator was used, 0.025 wt.% compared to 0.05 wt.%, resulting in a decrease in the rate of radical generation during photoinitiation by a factor of two [16]. Each 40 μL hydrogel sample with a diameter of ~ 5 mm and thickness of ~ 2 mm contained $\sim 50,000$ MIN6 β -cells. Post-polymerization, encapsulation samples were immediately placed in culture medium in 6-well tissue culture plates. Samples were kept on an orbital shaker at ~ 40 rpm, and the culture medium was changed every 3 days.

Murine islets were obtained from the Diabetes and Endocrinology Research Center at the Barbara Davis Center for Childhood Diabetes (Denver, CO). Following digestion of pancreatic tissue with collagenase, islets were isolated by density gradient purification and hand picked under a dissection microscope. Islets were encapsulated and maintained in culture by the same methods used for MIN6 β -cells.

2.3. Encapsulated β -cell viability and proliferation

A membrane integrity assay, LIVE/DEAD[®], from Molecular Probes, Inc. (Eugene, OR) was used to evaluate cell viability. Cells were placed in the LIVE/DEAD[®] staining solution for 10 min at 37 °C. After staining, live cells fluoresce green via enzymatic conversion of calcein AM to calcein, and dead cells fluoresce red via labeled ethidium homodimer binding to exposed DNA. Two samples per condition per time point were stained and visualized using confocal laser scanning microscopy. Data from LIVE/DEAD[®] images was quantified by counting live and dead cells in multiple (≥ 3) images per sample. The cell counts from images of each sample were averaged and recorded with standard deviations.

Proliferating cells were identified through immunohistochemical staining with bromodeoxyuridine (BrdU). Both non-encapsulated and encapsulated MIN6 β -cells were incubated overnight with 10 $\mu\text{g}/\text{mL}$ BrdU in culture medium, followed by incubation in fresh culture medium

overnight ($n = 3$). Samples were then fixed in 4% paraformaldehyde and cryosectioned. Sections were stained with a BrdU antibody and a fluorescently labeled secondary antibody, and visualized by confocal laser scanning microscopy. Samples that had not been incubated with BrdU were used as a negative control. Proliferation was quantified using the PicoGreen[®] dsDNA Quantitation Reagent (Molecular Probes, Inc.) to measure the amount of DNA in non-encapsulated and encapsulated samples after 1 and 2 weeks of in vitro culture ($n = 3$). Statistical significance was determined using a two-tailed Student's *t*-test with a confidence level of 0.05.

2.4. Encapsulated MIN6 transplantation

MIN6 β -cells encapsulated in PEG hydrogels ($\bar{M}_n = 10,000$ g/mol) were transplanted into C57BL/6 recipients in which diabetes was induced using streptozotocin at a dose of 180 mg/kg body weight by retro-orbital sinus injection. Following injection, the mice were monitored daily by urine or blood tests. Overt diabetes was defined as a positive urine glucose ($>1\%$) and then a positive blood glucose test of 14 mM. After mice were anesthetized with avertin, a small incision was made in the abdominal wall, and the encapsulated cells were implanted into the peritoneal cavity. The dose of transplanted cells was increased by implanting more cell-laden hydrogels. Non-fasting blood glucose was then measured daily using an Exactech pen and strips, with the normal glucose concentration range defined as 3–9 mM. Blood glucose measurements are reported for individual mice. Mice were sacrificed at the end of the experiments, and the transplanted grafts were retrieved from the reopened peritoneal cavity and placed in culture media. The associated animal protocols were approved by the IACUC review board at the University of Colorado Health Sciences Center.

3. Results

MIN6 β -cells were encapsulated as a single cell suspension and remained viable immediately following polymerization and throughout 3 days of in vitro culture, as determined by LIVE/DEAD[®] staining. After this time, a rapid decrease in cell viability was observed. After 5 days of in vitro culture, only $30 \pm 2\%$ of encapsulated β -cells remained viable. The time delay between hydrogel formation and encapsulated cell death suggested that a condition within the hydrogel could be the cause of cell death instead of the photoencapsulation process. If cells near the exterior of the gel were consuming nutrients at a rate that restricted nutrient diffusion to the cells in the interior of the gel, then cell death should be localized to the interior of the hydrogel. However, cell death was observed uniformly throughout the hydrogels (data not shown).

The absence of cell–matrix interactions and cell–cell interactions in this encapsulation system was considered. Natural islet morphology and the propensity of MIN6

β -cells to form multi-cellular aggregates in in vitro culture both suggested the importance of cell–cell interactions. We hypothesized that restoring cell–cell interactions between encapsulated β -cells would improve encapsulated cell survival. To test this hypothesis, MIN6 aggregates, with an average diameter of $120 \pm 30 \mu\text{m}$, were formed by suspending β -cells in rotational culture for ~ 24 h. These aggregates were then encapsulated by the same methods used for encapsulating single β -cells. As aggregates, $96 \pm 2\%$ of encapsulated β -cells remained viable immediately after polymerization and throughout the first week in culture. The viability of aggregated MIN6 did not statistically differ from the viability of unencapsulated MIN6, $99 \pm 2\%$.

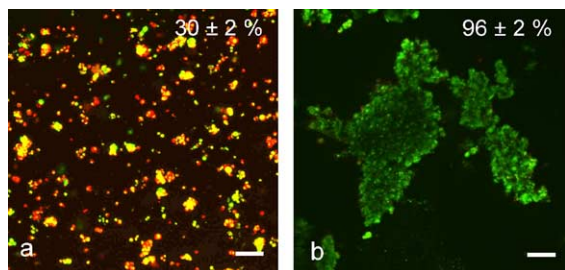


Fig. 1. Encapsulated MIN6 β -cells after 5 days in culture stained with LIVE/DEAD[®]: (a) single cell suspension, $30 \pm 2\%$ viable; (b) multi-cellular aggregates, $96 \pm 2\%$ viable. Live cells fluoresce green, and dead cells fluoresce red. Cell–cell interactions increase encapsulated MIN6 survival. Scale bar is $100 \mu\text{m}$.

Fig. 1 compares the viability of MIN6 β -cells encapsulated as a single cell suspension and as multi-cellular aggregates after 5 days in culture. Due to the observed significant increase in encapsulated cell viability, MIN6 aggregates were used in all of the remaining experiments presented in this manuscript.

After observing aggregated cell viability during the first days of encapsulation, we investigated encapsulated β -cell viability with time. MIN6 multi-cellular aggregates were encapsulated, and cell viability was observed over 3 weeks of in vitro culture by LIVE/DEAD[®] staining. Fig. 2 displays images of cell-laden constructs after 1, 7, 14, and 21 days in culture, and at each time point, greater than 95% of encapsulated cells were viable.

Because MIN6 β -cells are an immortalized cell line, cell proliferation is one measure of normal cell function. We explored the ability of these cells to proliferate while encapsulated in PEG hydrogels. Proliferating cells within multi-cellular aggregates were identified by incorporation and subsequent staining of bromodeoxyuridine (BrdU). Fig. 3 compares non-encapsulated β -cell aggregates to encapsulated β -cell aggregates after 1 and 2 weeks of in vitro culture. Proliferating cells are observed spatially throughout the MIN6 aggregates in all samples. To quantify β -cell proliferation, the amount of DNA in non-encapsulated and encapsulated samples was measured after 1 and 2 weeks of in vitro culture. The graph in Fig. 4 displays an initial plateau in the amount of DNA measured between 1 and

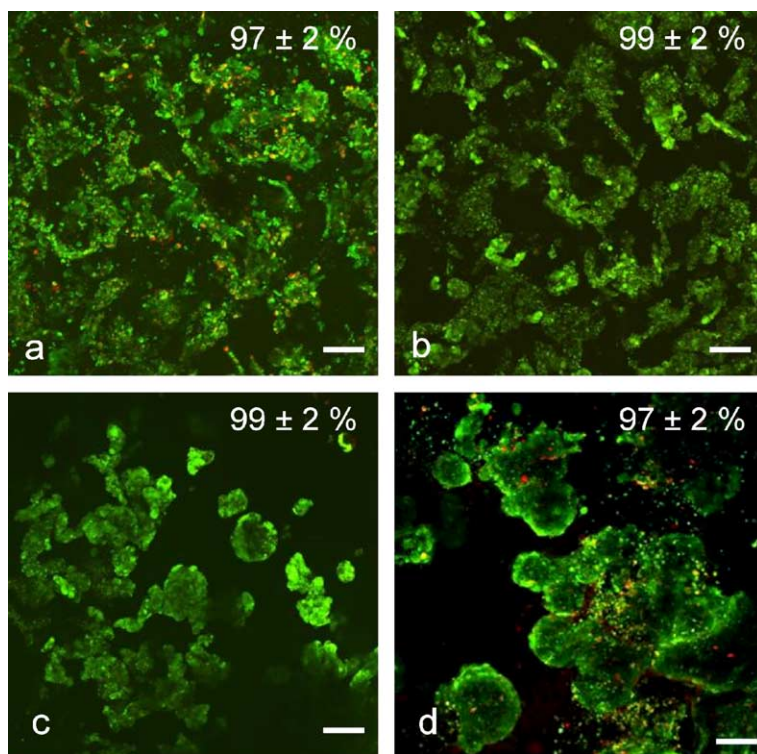


Fig. 2. Encapsulated MIN6 multi-cellular aggregates stained with LIVE/DEAD[®] after culture times of: (a) 1 day, $97 \pm 2\%$ viable; (b) 7 days, $99 \pm 2\%$ viable; (c) 14 days, $99 \pm 2\%$ viable; (d) 21 days, $97 \pm 2\%$ viable. Encapsulated MIN6 with cell–cell interactions remain viable throughout 3 weeks in culture. Scale bar is $100 \mu\text{m}$.

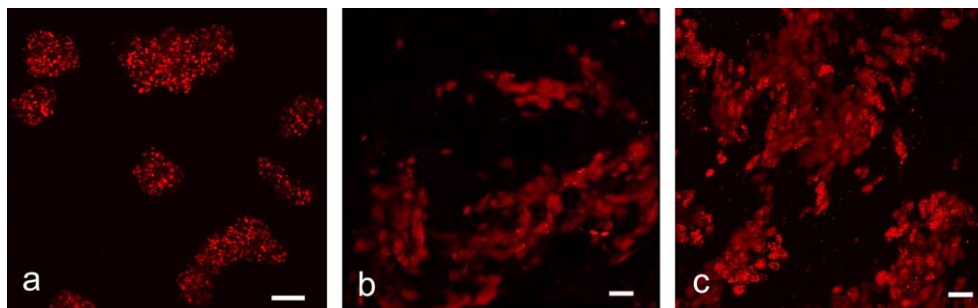


Fig. 3. BrdU staining of (a) unencapsulated MIN6 aggregates, (b) encapsulated MIN6 aggregates after 1 week in culture, (c) encapsulated MIN6 aggregates after 2 weeks in culture. Proliferating cells are identified in all samples. Scale bar on (a) is 100 μm . Scale bars on (b) and (c) are 20 μm . (For interpretation of the references in color in this figure, the reader is referred to the version of this article.)

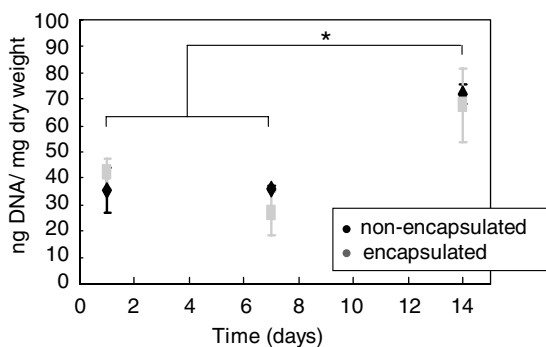


Fig. 4. Amount of DNA in samples of non-encapsulated and encapsulated β -cell aggregates with culture time (\star indicates $p < 0.05$). Proliferation within encapsulated MIN6 aggregates is not significantly different from that in non-encapsulated aggregates.

Table 1

Volumetric swelling ratio, Q , and crosslinking density, ρ_x , of PEG hydrogels formed from varying molecular weight PEGDM macromers

PEGDM molecular weight (g/mol)	Q	ρ_x (mol/L)
4000	13.1 ± 0.3	0.075 ± 0.004
8000	15.2 ± 0.2	0.056 ± 0.001
10,000	17.6 ± 0.04	0.042 ± 0.0002

7 days of culture followed by an increase in the amount of DNA, and therefore cell number, between 7 and 14 days of culture ($p < 0.05$) in both non-encapsulated and encapsulated samples. No statistical difference in proliferation was observed between non-encapsulated and encapsulated MIN6 aggregates.

The physical structure of the PEG hydrogel can be easily altered by changing the molecular weight of the starting PEG macromer. PEG chain length controls the crosslinking density, influencing the degree of hydrogel swelling and corresponding transport properties of the polymer network. To test the effects of such network properties on encapsulated cell viability, we encapsulated β -cell aggregates in hydrogels formed by three PEG molecular weights ($\bar{M}_n = 4000, 8000, 10,000$ g/mol). Table 1 reports the experimentally measured volumetric swelling ratio (Q)

and calculated crosslinking density (ρ_x) for hydrogels formed by each PEG molecular weight. Encapsulated cell viability was observed with time by LIVE/DEAD[®] staining. Fig. 5 displays images of viable cell aggregates encapsulated in hydrogels formed from each molecular weight PEG after 2 weeks in culture. No difference in viability was observed between the hydrogels formed from different molecule weight PEG chains.

The ultimate functional test of encapsulated islets or β -cells is insulin production. To determine whether encapsulated MIN6 cells could function in vivo, PEG hydrogels containing varying numbers of MIN6 cells were transplanted into the peritoneal cavity of C57BL/6 mice in which diabetes was induced using streptozotocin. As shown in Fig. 6, blood glucose concentrations in these mice decreased rapidly after transplantation, particularly at the higher cell doses, and eventually reached normal levels in all recipients. Grafts were found intact and not adhering to organs when mice were sacrificed 25–26 days after transplantation. In a second similar experiment after blood glucose levels reached normal levels, removal of the encapsulated grafts 21 days post-transplantation led to an immediate return to hyperglycemia in the recipient animal, indicated by the arrow in Fig. 6. Hyperglycemia was not reversed when non-encapsulated MIN6 were transplanted in a control experiment. The measured blood glucose concentration in this experiment was 30 ± 2 mM before and after transplantation.

After screening this PEG encapsulation system with MIN6 β -cells, preliminary testing of this platform was conducted with islets. Freshly isolated mouse islets were encapsulated in PEG hydrogels ($\bar{M}_n = 10,000$ g/mol) using the same conditions as for MIN6 β -cell aggregates. Viability was observed by LIVE/DEAD[®] staining throughout 3 weeks of in vitro culture. Images of stained islets shown in Fig. 7 display similar viability to encapsulated MIN6 aggregates.

4. Discussion

MIN6 β -cells were employed in the initial development of an in vitro islet encapsulation platform. The MIN6 cell

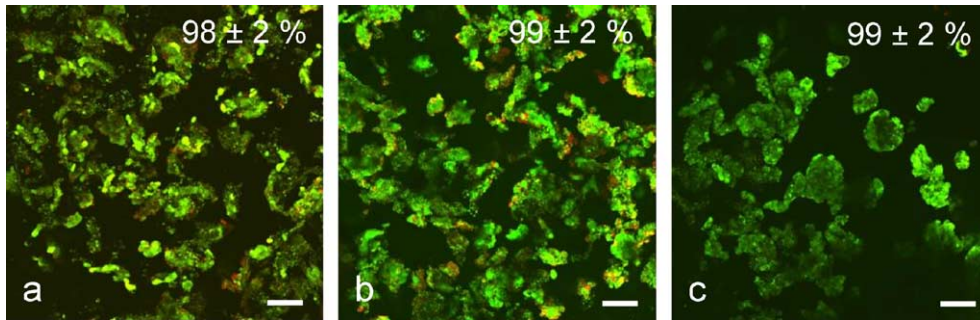


Fig. 5. Encapsulated MIN6 aggregates stained with LIVE/DEAD[®] after 2 weeks in culture within PEG hydrogels formed from PEGDM with an average molecular weight of: (a) 4000 g/mol, $98 \pm 2\%$ viable; (b) 8000 g/mol, $99 \pm 2\%$ viable; (c) 10,000 g/mol, $99 \pm 2\%$ viable. The resulting changes in PEG network properties do not alter encapsulated cell viability. Scale bars are 100 μm .

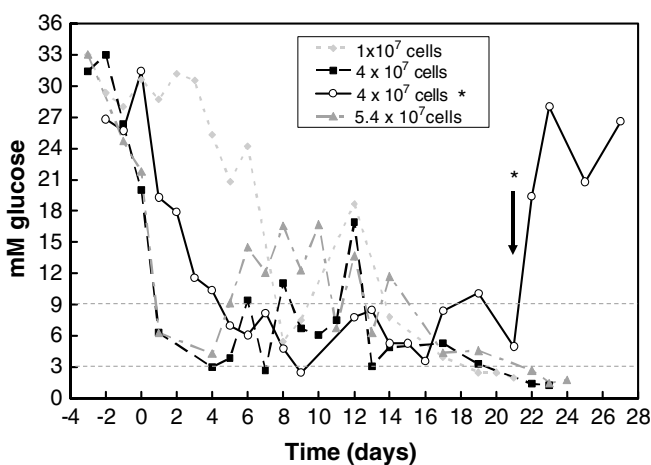


Fig. 6. Blood glucose levels of C57BL/6 mice before and after transplantation of encapsulated MIN6 β -cells into the peritoneal cavity. Transplantation occurred on day 0, and the 3–9 mM glucose range is considered normoglycemic. Three cell doses were used: (1) 1.0×10^7 cells, (2) 4×10^7 cells, and (3) 5.4×10^7 cells. Arrow indicates time of \star graft removal.

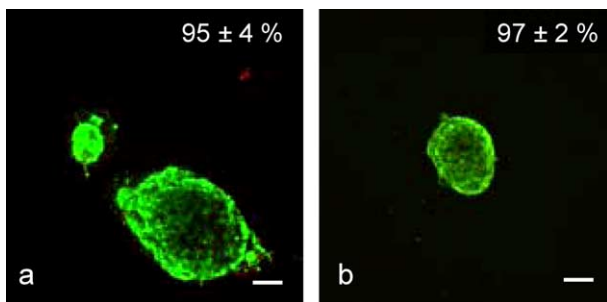


Fig. 7. Encapsulated whole islets stained with LIVE/DEAD[®] remained viable after in vitro culture time of (a) 3 days, $95 \pm 4\%$ viable, (b) 21 days, $97 \pm 2\%$ viable. Scale bars are 100 μm .

line is a valuable β -cell model for screening encapsulation conditions, while avoiding the complexities associated with the in vitro culture of primary islets. Cell survival was used to measure the compatibility of the proposed PEG-based encapsulation platform with MIN6 β -cells. Cell survival is our initial concern in establishing an in vitro testing

platform, because cell survival is a prerequisite for proper function of encapsulated cells. Additionally, function of viable cells may be influenced by environmental parameters beyond the base encapsulation scheme.

The photopolymerization conditions used to form the PEG hydrogels around suspended β -cells did not have a deleterious effect on β -cell survival. However, cell–cell contact is required for extended survival of MIN6 β -cells encapsulated within unmodified PEG hydrogels. The absence of any natural cell interactions within the PEG encapsulation platform allowed us to isolate the effects of cell–cell contact, and as a result, observe the importance of these contacts. This inert encapsulation system will allow us to explore systematically other controlled β -cell interactions that may further enhance cell survival and influence cell function, such as cell–extracellular matrix interactions. Bosco et al. [23] reported the importance of cell–matrix interactions for β -cell insulin secretion in vitro in a two-dimensional culture environment. The PEG hydrogel encapsulation system presented in this work will serve as a three-dimensional platform for investigating these interactions in a potentially clinically relevant encapsulation environment.

Observed proliferation within both non-encapsulated and encapsulated MIN6 β -cell aggregates formed by cell adhesion in rotational culture provides evidence that the three-dimensional culture environment within the PEG hydrogel does not disrupt normal MIN6 proliferation. Other cell types, such as anchorage dependent mesenchymal stem cells that are proliferative in non-encapsulated culture, do not proliferate when encapsulated in an unmodified PEG hydrogel [18]. A comparison of these results suggests that anchorage dependent cells may require interaction with the encapsulation material, while cell–cell contact may be sufficient to support MIN6 β -cells. Previous efforts have reported an arrest of MIN6 proliferation upon aggregation [24], inconsistent with the results presented in this work. This discrepancy may be the result of differing aggregate formation techniques, as well as differences in β -cell aggregate size. In the previous report, MIN6 aggregates with diameters of $220 \pm 40 \mu\text{m}$ were formed via self-assembly, compared to MIN6 aggregates

with diameters of $120 \pm 30 \mu\text{m}$ formed in rotational culture for these studies. Also, in the previous report, MIN6 proliferation within aggregates was assessed by measuring aggregate size and observing the expression of proliferative markers. While these techniques are indirectly related to cell proliferation, the DNA content of aggregate samples displayed in Fig. 4 is a more sensitive and direct measurement.

Control of the PEG hydrogel structural properties is critical in balancing the need for rapid diffusion of cellular metabolites with the goal of protecting entrapped cells from immune challenge. In the initial study of the effects of hydrogel structural properties on encapsulated β -cell survival, differences in hydrogel crosslinking density produced by using different molecular weight PEG macromers did not influence aggregated β -cell viability. These results are important in demonstrating the ability to systematically vary the physical properties of the PEG network without negatively influencing encapsulated cell viability. This ability is critical to the applicability of the in vitro encapsulation platform for systematically testing the effects of biophysical and biochemical parameters within the cellular microenvironment. In the PEG networks tested, the diffusion of low molecular weight cell nutrients and waste products was not hindered sufficiently to affect encapsulated cell viability in in vitro culture. The influence of network properties, specifically the volumetric swelling ratio and the crosslinking density, on the diffusion coefficient of a solute diffusing through the hydrogel network, D_g , can be estimated using the following equation [25]:

$$\frac{D_g}{D_0} \cong \left(1 - \frac{r_s}{v_p^{-1/3} l C_n^{1/3} \left(\frac{3\bar{v}_p \rho_x}{M_r} \right)^{1/2}} \right) \exp \left(-Y \frac{v_p}{1 - v_p} \right) \quad (2)$$

where D_0 is the diffusion coefficient of the solute in solution; r_s is the solute radius; v_p is the equilibrium polymer volume fraction (Q^{-1}); l is the average bond length; C_n is the characteristic ratio of the polymer (3.8 for PEG); \bar{v}_p is the specific volume of the polymer; ρ_x is the crosslinking density; M_r is the molecular weight of the polymer repeat unit, and Y is the ratio of the critical volume required for translational movement of the solute to the average free volume per liquid molecule (usually assumed to be 1). When the radius of the solute is much smaller than the network mesh size (or crosslinking density), the diffusion coefficient within the hydrogel approaches that in solution. However, diffusion becomes sufficiently hindered as the gel crosslinking density increases and approaches the size of the solute. Of further importance is the time scale for diffusion, t_{diff} . The following equation relates t_{diff} to the diffusion coefficient of a solute within a given medium, D , and distance the solute must travel through that medium, L :

$$t_{\text{diff}} = \frac{L^2}{D} \quad (3)$$

The time for glucose induced insulin response of cells encapsulated in a PEG hydrogel can be approximated by determining the diffusion coefficients of each molecule within the hydrogel and the distance each molecule must diffuse through the network. The radii of glucose and insulin relative to the volumetric swelling ratios and crosslinking densities of the PEG hydrogels used to encapsulate MIN6 aggregates give rise to diffusion coefficients within the networks that range from 85% to 95% of the coefficients for diffusion in solution. Therefore, insulin response time is more readily influenced by changes in the distance each solute must diffuse through the network, a variable controlled by the initial geometry of the hydrogel.

The changes in network properties resulting from the use of varying molecular weight PEG macromers ($\bar{M}_n = 4000, 8000, 10,000 \text{ g/mol}$), do not greatly influence the diffusion of small molecules. However, in future studies related to the ability of these networks to protect encapsulated cells from immune rejection, small changes in network properties may have a greater effect of the diffusion of high molecular weight molecules such as antibodies. Altering the PEG network properties for controlled diffusion will be revisited when the subject of immune protection is addressed.

Results of preliminary transplantation experiments indicate that encapsulated MIN6 β -cells are able to secrete insulin at sufficient levels to maintain normal blood glucose control in diabetic mice. Because diabetes was chemically induced in the C57BL/6 mice used in these experiments, a diabetic autoimmune response against the transplanted β -cells was avoided. However, the inability of the transplanted non-encapsulated MIN6 to restore normal blood glucose concentrations suggests the limited survival of these tumor cells, as well as, the potential role of the PEG barrier in protecting encapsulated β -cells over the course of the transplantation experiments. The return of hyperglycemia in recipient mice following graft removal provides further evidence of the ability of encapsulated MIN6 to function in vivo. Together, these results demonstrate the ability of the PEG environment to permit encapsulated cell survival and function in the absence of an autoimmune challenge.

Finally, primary islets encapsulated in PEG hydrogels remained viable throughout 3 weeks of in vitro culture. These results suggest that the photopolymerization conditions used and the three-dimensional environment of the PEG hydrogel are conducive to islet survival, as well as MIN6 β -cell aggregate survival.

In summary, the observed viability of aggregated MIN6 β -cells and islets encapsulated within photopolymerizable PEG hydrogels, in combination with the inherent physical properties of these networks, supports the utility of these hydrogels for in vitro investigations of encapsulation parameters. Such experimentation will provide a better understanding of how material properties and other environmental factors, such as survival factors (e.g., growth factors or extracellular matrix molecules), affect encapsulated

cell viability and function and will contribute to the development of a clinically applicable encapsulation system.

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References

- [1] Fan MY, Lum ZP, Fu XW, Levesque L, Tai IT, Sun AM. Reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets. *Diabetes* 1990;39(4):519–22.
- [2] Cruise GM, Hegre OD, Lamberti FV, Hager SR, Hill R, Scharp DS, et al. In vitro and in vivo performance of porcine islets encapsulated in interfacially photopolymerized poly(ethylene glycol) diacrylate membranes. *Cell Transplant* 1999;8(3):293–306.
- [3] Jain K, Asina S, Yang H, Blount ED, Smith BH, Diehl CH, et al. Glucose control and long-term survival in biobreeding/worcester rats after intraperitoneal implantation of hydrophilic macrobeads containing porcine islets without immunosuppression. *Transplantation* 1999;68(11):1693–700.
- [4] Lanza RP, Chick WL. Transplantation of pancreatic islets. *Ann NY Acad Sci* 1997;831(1):323–31.
- [5] Vernon BL, Kim SW, Bae YH. Insulin release from islets of Langerhans entrapped in a poly(*N*-isopropylacrylamide-*co*-acrylic acid) polymer gel. *J Biomater Sci, Polym Ed* 1999;10:183–98.
- [6] Isayeva IS, Kasibhatla BT, Rosenthal KS, Kennedy JP. Characterization and performance of membranes designed for macroencapsulation/implantation of pancreatic islet cells. *Biomaterials* 2003;24:3483–91.
- [7] DeVos P, Hamel AF, Tatarkiewicz K. Considerations for successful transplantation of encapsulated pancreatic islets. *Diabetologia* 2002;45:159–73.
- [8] Opara EC, Kendall WF. Immunoisolation techniques for islet cell transplantation. *Expert Opin Biol Therapy* 2002;2(5):503–11.
- [9] Uludag H, De Vos P, Tresco PA. Technology of mammalian cell encapsulation. *Adv Drug Deliv Rev* 2000;42:29–64.
- [10] Li RH. Materials for immunoisolated cell transplantation. *Adv Drug Deliv Rev* 1998;33:87–109.
- [11] Risbud MV, Bhonde RR. Islet immunoisolation: experience with biopolymers. *J Biomater Sci, Polym Ed* 2001;12(11):1243–52.
- [12] Gray DWR. An overview of the immune system with specific reference to membrane encapsulation and islet transplantation. *Ann NY Acad Sci* 2001;944:226–39.
- [13] Sawhney AS, Pathak CP, Hubbell JA. Interfacial photopolymerization of poly(ethylene glycol)-based hydrogels upon alginate-poly(L-lysine) microcapsules for enhanced biocompatibility. *Biomaterials* 1993;14:1008–16.
- [14] Chandy T, Mooradian DL, Rao GH. Evaluation of modified alginate–chitosan–polyethylene glycol microcapsules for cell encapsulation. *Artificial Organs* 1999;23(10):894–903.
- [15] Desai NP, Sojomihardjo A, Yao Z, Ron N, Soon-Shiong P. Interpenetrating polymer networks of alginate and polyethylene glycol for encapsulation of islets of Langerhans. *J Microencapsul* 2000;17(6):677–90.
- [16] Bryant SJ, Nuttelman CR, Anseth KS. Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro. *J Biomater Sci, Polym Ed* 2000;11(5):439–57.
- [17] Bryant SJ, Anseth KS. Controlling the spatial distribution of ECM components in degradable PEG hydrogels for tissue engineering cartilage. *J Biomed Mater Res* 2003;64A(1):70–9.
- [18] Nuttelman CR, Tripodi MC, Anseth KS. In vitro osteogenic differentiation of human mesenchymal stem cells photoencapsulated in PEG hydrogels. *J Biomed Mater Res* 2004;68A:773–82.
- [19] Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. *Biomaterials* 2002;23(22):4315–23.
- [20] Lin-Gibson S et al. Synthesis and characterization of PEG dimethacrylates and their hydrogels. *Biomacromolecules* 2004;5:1280–7.
- [21] Flory PJ, Rehner R. Statistical mechanics of crosslinked polymer networks. I. Rubberlike elasticity. *J Chem Phys* 1943;11:512.
- [22] Merrill EW, Dennison KA, Sung C. Partitioning and diffusion of solutes in hydrogels of poly(ethylene oxide). *Biomaterials* 1993;14:1117–26.
- [23] Bosco D, Meda P, Halban P, Rouiller D. Importance of cell–matrix interactions in rat islet β -cell secretion in vitro: role of $\alpha 6\beta 1$ integrin. *Diabetes* 2000;49:233–43.
- [24] Kinoshita N, Echigo Y, Shinohara S, Gu Y, Miyazaki J, Inoue K, et al. Regulation of cell proliferation using tissue engineering in MIN6 cells. *Cell Transplant* 2001;10:473–7.
- [25] Lustig SR, Peppas NA. Solute diffusion in swollen membranes. 9. Scaling laws for solute diffusion in gels. *J Appl Polym Sci* 1988;36:735–47.