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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 ($\overline{M}_n$ = 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.

Keywords: Islet encapsulation; Poly(ethylene glycol) hydrogels; β-Cell viability

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) ($\overline{M}_n = 4000$, 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 ^{1}H 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 °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]:

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

where $\bar{v}$ is the specific volume of the solvent; υ_p is the polymer volume fraction (Q^{-1}); and χ is the solvent–polymer interaction parameter. For PEG in water and PBS with υ_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 μg/mL fungizone (Gibco), and 10% fetal bovine serum (Gibco). Cells were plated in 75 cm^2 treated tissue culture flasks and incubated at 37 °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

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