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Injectable and inherently vascularizing semi-interpenetrating polymer network for delivering cells to the subcutaneous space

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ABSTRACT

Injectable hydrogels are suitable for local cell delivery to the subcutaneous space, but the lack of vasculature remains a limiting factor. Previously we demonstrated that biomaterials containing methacrylic acid promoted vascularization. Here we report the preparation of a semi-interpenetrating polymer network (SIPN), and its evaluation as an injectable carrier to deliver cells and generate blood vessels in a subcutaneous implantation site. The SIPN was prepared by reacting a blend of vinyl sulfone-terminated polyethylene glycol (PEG-VS) and sodium polymethacrylate (PMAA-Na) with dithiothreitol. The swelling of SIPN was sensitive to the PMAA-Na content but only small differences in gelation time, permeability and stiffness were noted. SIPN containing 20 mol% PMAA-Na generated a vascular network in the surrounding tissues, with 2–3 times as many vessels as was obtained with 10 mol% PMAA-Na or PEG alone. Perfusion studies showed that the generated vessels were perfused and connected to the host vasculature as early as seven days after transplantation. Islets embedded in SIPN were viable and responsive to glucose stimulation *in vitro*. In a proof of concept study in a streptozotocin-induced diabetic mouse model, a progressive return to normoglycemia was observed and the presence of insulin positive islets was confirmed when islets were embedded in SIPN prior to delivery. Our approach proposes a biomaterial-mediated strategy to deliver cells while enhancing vascularization.

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1. Introduction

Cell therapy [1,2] is an attractive approach to treat diseases in which whole organ transplantation is not required, obviating the need for major surgery. However, the inflammatory response, the limited retention of cells at the target site, and low survival rates post-injection are challenges that need to be addressed. In this regard, the immobilization of cells within hydrogels is considered an effective means of localizing cells at the delivery site while affording a favorable microenvironment that improves cell viability and facilitates engraftment [3].

The capacity to absorb a large amount of water and a microstructure that superficially mimics the natural extracellular matrix (ECM) found in tissues [4] make hydrogels attractive for cell delivery. In practice, however, implanting cells immobilized in prefabricated hydrogels often requires surgery. Consequently, significant focus has shifted to the design of hydrogels that form *in situ* upon injection of a liquid precursor solution [5,6]. These injectable hydrogels minimize patient discomfort and reduce infection risk, scar formation, and treatment cost.

The design criteria for injectable hydrogels are reviewed in detail elsewhere [7,8]. Most important among these criteria are the nature of the polymeric materials applied to form the 3D network and the cross-linking chemistry. Combining poly(ethylene glycol) (PEG) and Michael-type reaction [9] is a widely investigated approach for injectable hydrogel preparation [10] for cases where cell adhesion is not required. Besides being biocompatible, non-immunogenic, and protein repellant [11], PEG hydrogels exhibit highly tunable physical properties [12]. The Michael-type addition of thiols onto unsaturated vinyl sulfone enables the preparation of hydrogels under physiological and cytocompatible conditions. Further, the reaction is thiol-selective as amines react at least 1 order of magnitude slower than thiols [13].

Of the many transplantation sites being investigated [14], the

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subcutaneous space has favorable characteristics such as ease of access and the capacity to accommodate a relatively large volume of transplant, as well as potential for biopsy, imaging, and retrievability [15]. However, owing to the lack of a perfusable vascular network that supplies the cells with oxygen and nutrients, several attempts to subcutaneously deliver cells have failed [16,17]. We have previously demonstrated that biomaterials that contain methacrylic acid (MAA) have inherent vascular regenerative properties in the absence of exogenous factors, such as growth factors or cells. In the form of beads, these biomaterials have shown vascular regenerative effects in rat skin grafts [18], wounded diabetic [19], and non-diabetic mice [20,21]. Scaffolds made of MAA were also investigated [22], and higher microvessel densities were observed *in vivo*.

The objective of this study was to prepare an injectable hydrogel that promotes a perfusable vascular network in the subcutaneous space of mice and which is suitable for delivering cells to this transplantation site. As shown in Fig. 1, we reacted a blend of vinyl sulfone-terminated polyethylene glycol (PEG-VS) and sodium polymethacrylate (PMAA-Na) with a stoichiometric amount of dithiothreitol (DTT) to form a semi-interpenetrating polymer network (SIPN). The SIPN consisted of PMAA-Na physically entrapped within the chemically cross-linked PEG network. Three hydrogel formulations were prepared and investigated throughout this study, as described in Table 1. PP8020 is a SIPN composed of 80% mol ethylene glycol and 20% mol sodium methacrylate. PP9010 is a SIPN composed of 90% mol ethylene glycol and 10% mol sodium methacrylate. PEG is the 3D network made from PEG-VS and DTT in the absence of PMAA-Na. The vascular regenerative response was assessed upon subcutaneous injection in CD1 mice. PP8020 was used with rat pancreatic islets in a 7-day study to illustrate the potential of SIPN as cell delivery vehicle.

2. Materials and methods

2.1. Preparation of stock solutions

All solutions were prepared in endotoxin-free 3-(*N*-morpholino) propanesulfonic acid (MOPS, 10 mM, pH ~ 7.4, Teknova, Hollister, CA). A stock solution of 165 mg/mL of PEG-VS (20 kDa, JenKem Technology USA Inc. Plano, Tx) was prepared, sterile filtered (0.2 μm Millex-GP syringe filter unit) and stored at 4 °C until further use. Poly(methacrylic acid) (PMAA; 100 kDa; Polysciences, Warrington, PA) was first converted to sodium polymethacrylate (PMAA-Na) by incubation with NaOH (Sigma-Aldrich, Oakville) followed by dialysis against distilled water (MWCO 8 kDa) and lyophilization. Two PMAA-Na stock solutions were prepared at 105 mg/mL and 45 mg/mL, respectively. The solutions were sterile filtered and stored at 4 °C until use. Dithiothreitol (DTT, Sigma) was dissolved in MOPS at 26 mg/mL. Since DTT is air-sensitive, the solution was always freshly constituted, sterile filtered, and used shortly afterwards.

2.2. Preparation of hydrogels

PEG only (without PMAA-Na) and two formulations of SIPN differing in the PMAA-Na molar feed were prepared. The SIPNs were designated as PP8020 and PP9010, based on the molar ratio of ethylene glycol to sodium methacrylate in the formulation (Table 1). PEG were prepared by mixing 50 μL of PEG-VS stock solution and 50 μL of MOPS. The mixture was homogenized in a rotating shaker for 1 h. After the addition of 10 μL DTT, the mixture was vortexed for 10 s and allowed to gel at 37 °C. Similarly, PP8020 was prepared by mixing 50 μL of PEG-VS, 50 μL of 105 mg/mL PMAA-Na, and 10 μL DTT. PP9010 was prepared from 50 μL of PEG-VS, 50 μL of 45 mg/mL PMAA-Na, and 10 μL DTT.

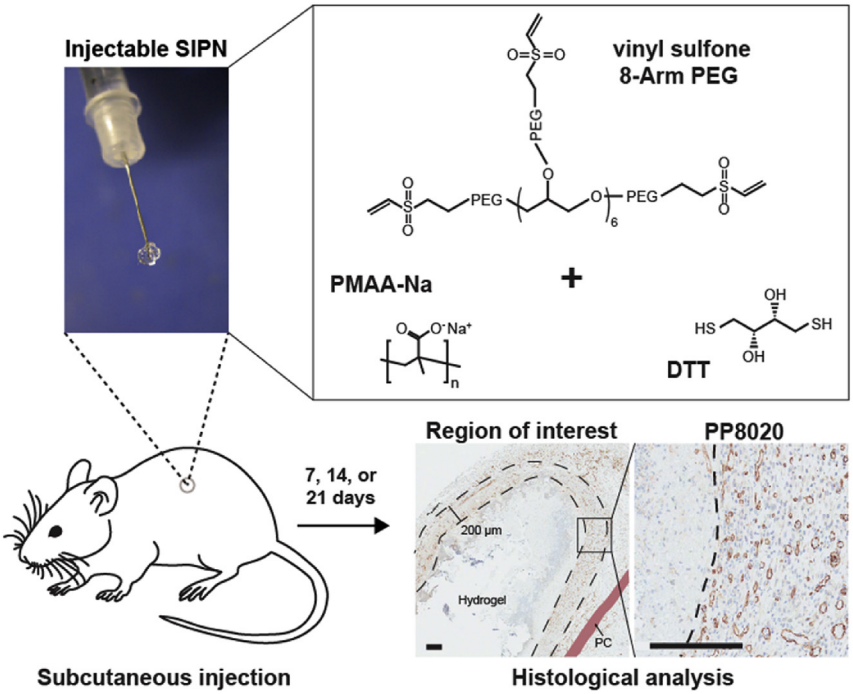

Fig. 1. SIPN made by blending PEG-VS, PMAA-Na, and DTT were prepared and subcutaneously injected into the dorsal flank of CD1 mice. Only PP8020 promoted the formation of blood vessels in the subcutaneous space below the panniculus carnosus (PC). Day 7 histological sections stained with CD31 are shown. $CD31^{+}$ vessels within the region of interest (ROI, dashed line) were counted. Scale = 200 μm.

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