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A transient cell-shielding method for viable MSC delivery within hydrophobic scaffolds polymerized *in situ*

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ABSTRACT

Cell-based therapies have emerged as promising approaches for regenerative medicine. Hydrophobic poly(ester urethane)s offer the advantages of robust mechanical properties, cell attachment without the use of peptides, and controlled degradation by oxidative and hydrolytic mechanisms. However, the application of injectable hydrophobic polymers to cell delivery is limited by the challenges of protecting cells from reaction products and creating a macroporous architecture post-cure. We designed injectable carriers for cell delivery derived from reactive, hydrophobic polyisocyanate and polyester triol precursors. To overcome cell death caused by reaction products from *in situ* polymerization, we encapsulated bone marrow-derived stem cells (BMSCs) in fastdegrading, oxidized alginate beads prior to mixing with the hydrophobic precursors. Cells survived the polymerization at >70% viability, and rapid dissolution of oxidized alginate beads after the scaffold cured created interconnected macropores that facilitated cellular adhesion to the scaffold *in vitro*. Applying this injectable system to deliver BMSCs to rat excisional skin wounds showed that the scaffolds supported survival of transplanted cells and infiltration of host cells, which improved new tissue formation compared to both implanted, pre-formed scaffolds seeded with cells and acellular controls. Our design is the first to enable injectable delivery of settable, hydrophobic scaffolds where cell encapsulation provides a mechanism for both temporary cytoprotection during polymerization and rapid formation of macropores post-polymerization. This simple approach provides potential advantages for cell delivery relative to hydrogel technologies, which have weaker mechanical properties and require incorporation of peptides to achieve cell adhesion and degradability.

1. Introduction

Autologous and allogeneic cell-based therapies have emerged as promising approaches for regenerative medicine [1]. While direct injection of cells has limited therapeutic efficacy due to poor cell survivability [2–4], delivery of cells within a 3D matrix can improve integration with host tissue and promote healing [5]. Injectable and settable cell carriers could be advantageous as a minimally invasive surgical approach to rapid filling of complex defects followed by *in situ* curing to form a porous scaffold with suitable mechanical properties [6].

Lysine-derived poly(ester urethane)s (PURs) offer potential advantages as injectable carriers for local cell delivery, such as curing using non-cytotoxic catalysts [7] without the need for UV radiation [8], support of cell attachment without cell adhesion peptides [9,10], tunable hydrolytic and oxidative degradation to non-cytotoxic breakdown products [11,12], and adjustable mechanical properties ranging from those of soft tissue [13] to bone [9,14]. Furthermore, macropores can be generated within PUR scaffolds by CO_2 gas foaming via the reaction of isocyanate groups with water [15]. When using these materials as acellular scaffolds, the CO_2 and

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heat generated by the *in situ* reaction is well tolerated at the biomaterial–tissue interface [7,16] due to the relatively long length scales (>1 mm) between the material and surrounding cells (Fig. 1A). However, cells encapsulated within the reactive hydrophobic polymer experience steeper CO_2 and temperature gradients due to transport of reaction products over much smaller length scales (<100 μm, Fig. 1A). Furthermore, after the reaction is complete, hydrophobic polymers absorb negligible amounts of water and allow less diffusion of vital cell nutrients and wastes than swollen hydrogels. While hydrophobic biomaterials such as PUR provide a generalizable, biodegradable platform for tissue scaffolding, their use as an injectable carrier for cell delivery has not been achieved due to two primary challenges: (1) maintenance of cell viability during *in situ* polymerization, and (2) provision of an interconnected, macroporous structure to allow effective nutrient and waste exchange post-cure. Overcoming these key barriers was the goal of the current work in order to enable the use of injectable, settable, mechanically robust, and cell-adhesive PUR networks to fill tissue defects and to locally deliver and retain viable cells *in vivo*.

Achieving these goals will provide a new alternative to photopolymerizable systems that utilize cytocompatible initiators [17,18] and water-soluble macromers [19–21] to encapsulate cells in injectable hydrogels [8]. Polyethylene glycol (PEG)-based hydrogels have generated considerable interest for localized cell delivery since they can be administered by minimally-invasive injections, set within clinically relevant working times, exhibit tissue-like structure, and induce a minimal inflammatory response [1,22–24]. However, PEG hydrogels must be functionalized with an optimal combination of peptides that serve as integrin-binding sites for cell adhesion and peptide crosslinkers that are matrix metalloproteinase (MMP) substrates to enable cellular infiltration and cell-mediated hydrogel degradation [5,25].

Alternative settable carriers must protect cells from reaction products prior to cure and then set *in situ* to form an interconnected, macroporous scaffold that supports cell adhesion and growth. In this study, we designed injectable PUR scaffolds for concurrent incorporation of macropores and cells within PUR scaffolds (Fig. 1B). Through encapsulation within partially oxidized sodium alginate (o-Alg) beads, cells were protected from the PUR reaction prior to gelation. Hydrolytic degradation of the o-Alg beads within the first 1–2 days after gelation was anticipated to result in cell release and attachment to the scaffold. Thus, in contrast to the porogen co-encapsulation approach [26,27], the o-Alg beads functioned both as a temporary barrier to transport of reaction products as well as a porogen. We varied bead size, timing of bead addition, and bead loading within PUR scaffolds to investigate the effects of heat and CO_2 generation on cell survivability both prior to and after gelation *in vitro*. In a proof-of-concept experiment, the lead-candidate formulation that produced maximal cell survivability *in vitro* was injected into full-thickness excisional skin wounds in Sprague–Dawley rats to evaluate the potential of the injectable PUR cell carrier for wound repair and restoration.

2. Materials and methods

2.1. Materials

The sodium salt of alginic acid (Alg, viscosity = 20–40 cPs) was supplied by Sigma Aldrich (St. Louis, MO). Acros Organics supplied calcium chloride and glycerol. αMEM and DMEM were supplied by GIBCO. Fetal bovine serum (FBS) was purchased from Thermo Scientific. Penicillin/streptomycin (P/S), trypsin EDTA and Amphotericin B were obtained from Corning Cellgro. Live/Dead kits for mammalian cells were supplied by Life Technologies. Glycolide and D,L-lactide were purchased from Polysciences (Warrington, PA). Lysine triisocyanate-poly(ethylene glycol) (LTI-PEG) prepolymer was supplied by Medtronic, Inc, and hexamethylene diisocyanate trimer (HDIt) was supplied by Bayer Material Science. Iron acetylacetonate (FeAA) was supplied by Sigma–Aldrich. ε-caprolactone was dried over anhydrous $MgSO_4$, and all other materials were used as received.

2.2. Cell culture

MC3T3 cells (ATCC) were cultured in a complete medium of αMEM with 10% FBS and 1% P/S. Primary rat bone marrow mesenchymal stem cells (BMSCs) were maintained in DMEM with 10% FBS, 1% P/S, and 0.1% Amphotericin B (Sigma). BMSCs were generated from pooled bone marrow from 4 male Sprague–Dawley rats. Rat femora and tibiae were removed after sacrificing and bone marrow flushed with BMSC culture medium. After centrifuging, cell pellets were suspended in BMSC medium and plated in T75 tissue culture flasks. Three days after seeding, floating

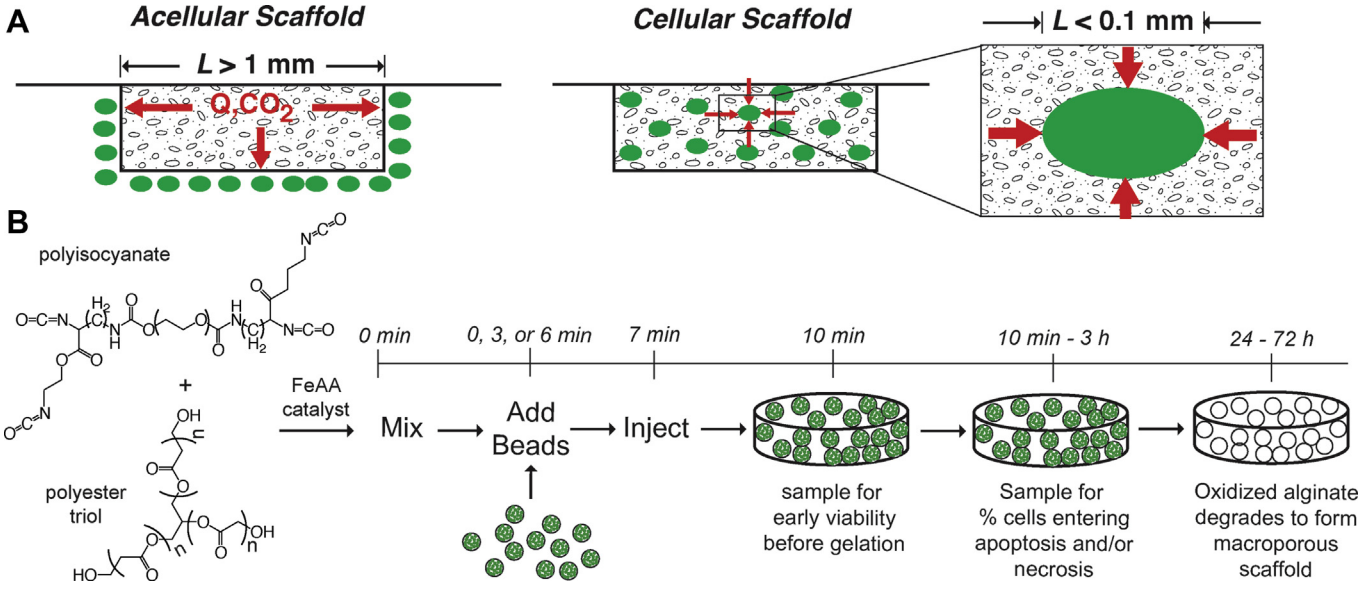

Fig. 1. Design of injectable, settable carriers for cell delivery. (A) For an acellular scaffold, the length scale of diffusion of reaction products is comparable to the size of the tissue defect. However, in a cellular scaffold, reaction products diffuse radially toward the encapsulated cell over a much shorter length scale (comparable to the size of the cell). (B) Schematic illustrating the design concept in which an NCO-functional prepolymer reacts with a polyester polyol in the presence of an iron acetylacetonoate (FeAA) catalyst to form a polyurethane network. Encapsulation of cells in oxidized alginate beads (green) provides temporary protection from the chemical reaction and is followed by hydrolytic degradation of the oxidized alginate to form interconnected macropores that are enhanced by the NCO-water reaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)