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Degradative properties and cytocompatibility of a mixed-mode hydrogel containing oligo[poly(ethylene glycol)fumarate] and poly(ethylene glycol)dithiol

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Abstract

Our laboratory is currently exploring synthetic oligo(poly(ethylene glycol)fumarate) (OPF)-based biomaterials as a means to deliver fibroblasts to promote regeneration of central/partial defects in tendons and ligaments. In order to further modulate the swelling and degradative characteristics of OPF-based hydrogels, OPF crosslinking via a radically initiated, mixed-mode reaction involving poly(ethylene glycol) (PEG)-diacrylate and PEG-dithiol was investigated. Results demonstrate that mixed-mode hydrogels containing OPF can be formed and that the presence of 20 wt.% PEG-dithiol increases swelling and decreases degradation time vs. 10 wt.% PEG-dithiol and non-thiol-containing hydrogels (20% thiol fold swelling 28.7 ± 0.8; 10% thiol fold swelling 11.6 ± 1.4; non-thiol 8.7 ± 0.2; 20% thiol-containing hydrogels degrade within 15 days *in vitro*). After encapsulation, tendon/ligament fibroblasts remained largely viable over 8 days of static culture. While the presence of PEG-dithiol did not significantly affect cellularity or collagen production within the constructs over this time period, image analysis revealed that the 20% PEG-dithiol gels did appear to promote cell clustering, with greater values for aggregate area observed by day 8. These experiments suggest that mixed-mode OPF-based hydrogels may provide an interesting alternative as a cell carrier for engineering a variety of soft orthopedic tissues, particularly for applications when it is important to encourage cell–cell contact.

Keywords: Hydrogel; Mixed-mode polymerization; Fibroblasts; Tendon/ligament tissue engineering

1. Introduction

A number of hydrogel systems have recently been explored as carriers for cell delivery in orthopedic tissue engineering, including both naturally-based and synthetic materials to promote regeneration of cartilage, bone, tendon/ligament and muscle [1–16]. Studies in this area have demonstrated that altering hydrogel properties, such as adhesivity [7,14,17], mechanical and swelling characteristics [3,4,6,13], and degradation time [1,2,8,11,12,18], can affect cell function within the carrier material, as well as construct engraftment with surrounding tissue [10,17].

Our laboratory is currently exploring synthetic oligo(poly(ethylene glycol)fumarate) (OPF)-based biomaterials as a means to deliver fibroblasts to promote regeneration of central/partial defects in the anterior cruciate ligament or patellar tendon, both of which demonstrate limited ability for self-repair and thus may require cell-based approaches for complete restoration of tissue architecture and function [19–22]. Previously, OPF crosslinked with poly(ethylene glycol diacrylate) (PEG-DA) has been shown to be cytocompatible *in vitro* [23,24] and produce a minimal inflammatory response *in vivo* [25], making it a useful starting material for tendon/ligament tissue engineering applications.

However, in order to further modulate the swelling and degradative characteristics of OPF-based hydrogels to better understand the effects of these properties on viability

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and function of tendon/ligament fibroblasts, we have investigated the addition of a third component, PEG-dithiol (PEG-diSH), to this hydrogel system. In this case, the hydrogel precursors are polymerized via the use of the photoinitiator Irgacure 2959 (D2959), which has been found to be non-toxic to cells and has been used in encapsulation of a variety of cell types, including osteoblasts, fibroblasts and chondrocytes [4,8,26]. Prior work has demonstrated that hydrogels or biofunctionalized polymers can be made through a radically initiated, mixed-mode reaction scheme involving thiol groups and acrylate end groups that produces a covalently crosslinked network [14,27–29]. Hydrogels fabricated through the thiol–acrylate mixed-mode reaction scheme degrade hydrolytically at physiological pH through cleavage of ester linkages [27] with the number of carbon atoms between the ester and sulfide groups affecting the rate of ester hydrolysis [27].

Therefore, the aims of this study were first to determine if such a reaction scheme could be utilized in the presence of OPF and, after fabrication, to characterize the swelling and degradative properties of mixed-mode hydrogels containing OPF, PEG-DA and PEG-diSH. Subsequently, the effect of PEG-diSH incorporation in the hydrogels on the viability, morphology, proliferation and collagen synthesis of encapsulated tendon/ligament fibroblasts was monitored over 8 days *in vitro*.

2. Materials and methods

2.1. Hydrogel fabrication

2.1.1. Oligo(poly(ethylene glycol)fumarate) synthesis and characterization

2.1.1.1. Synthesis. OPF was synthesized as previously reported [30]. Briefly, poly(ethylene glycol) (PEG; $M_n = 10{,}000$ Da; Sigma–Aldrich, St. Louis, MO) was distilled and dissolved in dichloromethane (distilled before use) (Fisher Scientific, Waltham, MA) to produce a 40% (v/v) solution. Fumaryl chloride (FuCl; distilled before use, Sigma–Aldrich) and triethylamine (TEA; Sigma–Aldrich), in a molar ratio of 1 PEG:0.9 FuCl (2 TEA:1 FuCl), were added dropwise to the PEG solution and the reaction was held at approximately 0 °C under nitrogen. After the addition of FuCl and TEA, the OPF formulation was continuously stirred for 48–72 h at 25 °C under nitrogen to ensure reaction completion. At this time, the excess dichloromethane was evaporated and the Cl–TEA salt was removed. The OPF was recrystallized twice in ethyl acetate (Fisher) and washed three times in ethyl ether (Fisher). The resulting powder was vacuum dried at <5 mmHg and stored in a sealed container at −20 °C until further use.

2.1.1.2. Gel permeation chromatography (GPC) and nuclear magnetic resonance spectroscopy (NMR). After synthesis, the OPF was characterized via GPC and NMR. A gel permeation chromatography system (Prominence LC-20AD, CTO-20AC, SIL-20A, CBM-20A, DGU-20A; Shimadzu, Columbia, MD) equipped with a refractive index detector (RID-10A; Shimadzu) was used to determine the molecular weights of both the PEG used for synthesis and the resulting OPF polymer. The polymer samples were dissolved in chloroform, filtered (0.45 μm filter, Whatman, Florham Park, NJ) and injected into a column (50–100,000 Da range; Waters, Milford, MA) at a flow rate of 1 ml/min. Molecular weights were determined from elution time based on a calibration curve generated from PEG standards (seven standards ranging in molecular weights from 1400–73,500 Da; Waters). Samples were run in triplicate. In order to verify the addition of fumarate groups to OPF, samples were dissolved in $CDCl_3$ and ^{1}H-nuclear magnetic resonance (NMR) spectra were obtained with a Bruker Avance 400 MHz NMR system (Bruker Analytik, Billerica, MA).

2.1.2. Hydrogel construct fabrication

Hydrogel constructs were fabricated from OPF, PEG-diacrylate (PEG-DA; $M_n = 3400$ Da; Laysan Bio) and PEG-dithiol (PEG-diSH; $M_n = 3400$ Da; Laysan Bio) in ratios by weight (see Table 1). The ultraviolet (UV) photoinitiator Irgacure 2959 (D2959; Ciba, Basel, Switzerland) was dissolved in *N*-vinyl pyrrolidone (NVP; Sigma–Aldrich) at a concentration of 0.05% D2959 (wt./total wt.) in 10% NVP (wt./polymer wt.). The polymers were dissolved in phosphate-buffered saline (PBS; pH 7.4) and the photoinitiator was introduced to the system. The polymer solution was placed in 6 mm diameter by 1 mm thick Teflon molds (~30 μl) and polymerized under UV light (365 nm, 18 mW/cm^{-2}; UVP, Upland, CA) to create hydrogels (see Table 1 for polymerization times).

2.1.3. Swelling characteristics

Hydrogels were fabricated as stated above (see Table 1 for specific formulations) and allowed to swell in PBS for 24 h, at which point the wet weights were recorded. The constructs were then lyophilized for 24 h and the dry weights were recorded and used to calculate the fold swelling of the hydrogels (wet wt./dry wt.).

2.1.4. Sol fraction calculation

For sol fraction calculations, hydrogels were fabricated (see Table 1 for specific formulations) and weighed after fabrication (W_{d0}), then swelled in PBS for 24 h. After swelling, the gels were lyophilized for 24 h and their dry weights were recorded (W_{da}). Taking into account that the amount of polymer in the samples immediately after fabrication was 25 wt.% (75% initial water), sol fractions were calculated as:

$$\text{Sol fraction} = \frac{0.25W_{d0} - W_{da}}{0.25W_{d0}}$$

2.1.5. Degradative properties

Hydrogel constructs were fabricated (see Table 1 for specific formulations) and allowed to swell in PBS for

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