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# PEG-based hydrogels with tunable degradation characteristics to control delivery of marrow stromal cells for tendon overuse injuries

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ABSTRACT

Marrow stromal cells (MSCs) have been suggested as a means to improve healing in tendon overuse injuries (tendinopathy), but optimal delivery methods for these cells have yet to be determined. In this study novel degradable hydrogels based on oligo(poly(ethylene glycol) fumarate) (OPF) and acrylated poly(ethylene glycol)–dithiothreitol (Ac PEG–DTT) with tunable degradation times ranging from a few days to >1 month were synthesized as MSC carriers for tendon overuse injuries. The addition of higher amounts of OPF or higher dithiothreitol (DTT) concentrations resulted in enhanced fold swelling and degradation. Three formulations, including non-degrading, slower degrading (degraded in ~10 days) and faster degrading (degraded in ~5 days) hydrogels were selected for studies with MSCs in tendon tissue explants that had been treated with collagenase as a reproducible model of tendinopathy. Quantitative analysis of the resulting histology images indicated that cell delivery from the hydrogels was dependent on the degradation rate, with cells present in the tissue only after hydrogel dissolution. In addition, significantly more cells were found in the tendon after 14 days with the fast degrading (53 ± 19) vs. slow degrading (20 ± 6) hydrogels. Based on these results, OPF/Ac PEG–DTT hydrogels provide a versatile biomaterial platform to control cell delivery and thus better identify dosing regimens required for MSC-based therapies for tendinopathy.



## 1. Introduction

Tendinopathy is a common tendon disorder resulting from repetitive motion in the workplace or during athletic activity leading to significant pain and associated morbidity that can last for weeks to months [1,2]. Overused tendons undergo significant histological and biochemical changes, which include microtearing, thinning and disorganization of the collagen fibers [3], in-growth of blood vessels [4], morphological transformation of tendon fibroblasts from a spindle to a more rounded shape [5], abnormal regulation of matrix metalloproteinases and their inhibitors [6,7], and increased glycosaminoglycan content [8]. Over time, such alterations in the extracellular matrix organization lead to decreased mechanical properties [3,9], and may predispose the tissue to complete rupture [2]. Current treatment options, including prescribing general anti-inflammatory drugs and/or physical therapy regimes, only suppress the pain temporarily and do not address the underlying causes of the pathology [10].

Prior studies focusing on regeneration of other tendon/ligament injuries suggest that mesenchymal stem cells/marrow stromal cells (MSCs) could be a potent therapy for tendinopathy [11,12]. Use of autologous MSCs, which can subsequently differentiate to tendon/ligament fibroblasts [10], would eliminate any deleterious immune response to implanted cells. Moreover, MSCs have been shown to act in a paracrine manner on nearby cells, enhancing proliferation, migration and adhesion of tendon fibroblasts in a co-culture model [13]. The potential of MSCs to assist healing in tendinopathy is further supported by several recent pilot studies in equine models [14–16]. Compared with the controls, that were treated only with phosphate-buffered saline (PBS), the tendons that received an injection of MSCs in PBS exhibited significantly better histological characteristics, improved mechanical properties and reduced re-injury rates. However, like MSC-based therapies for repairing other tissues [17,18], a major challenge in using stem cells to treat tendinopathy is understanding when to introduce cells to achieve optimal healing outcomes, as well as ensuring that adequate viable cells are delivered and engraft in the damaged areas.

To address these key issues, our laboratory has developed a poly(ethylene glycol) (PEG)-based hydrogel system with a range of degradation rates to control the timing of MSC delivery to

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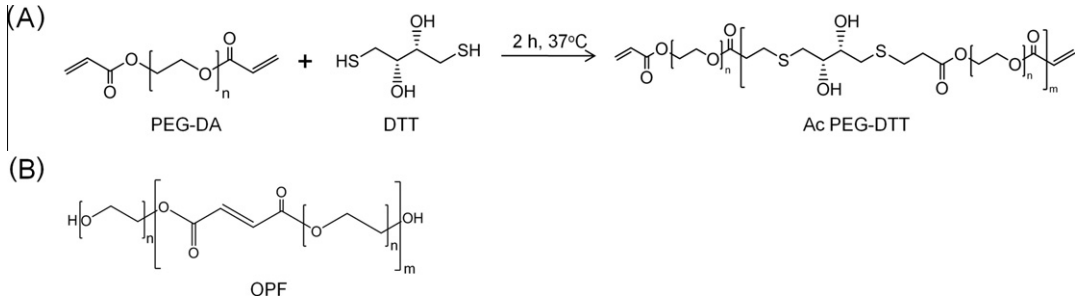


**Fig. 1.** Polymers used in these experiments. (A) Synthesis of Ac PEG–DTT; (B) chemical structure of OPF.

tendinopathic tendons. These hydrogels contain varying amounts of oligo(poly(ethylene glycol) fumarate) (OPF) and acrylated poly(ethylene glycol)–dithiothreitol (Ac PEG–DTT). OPF is a highly cytocompatible polymer that has been previously explored in our laboratory [19–21]. Ac PEG–DTT is synthesized by a Michael-type addition reaction between PEG diacrylate (PEG–DA) and dithiothreitol (DTT) (see Fig. 1A) and has also been demonstrated to be cytocompatible [22,23]. The introduction of DTT results in hydrolytic degradation of the resulting Ac PEG–DTT hydrogels due to the proximity of the thiol group and the ester linkage in the final polymer [22]. Therefore, by cross-linking OPF with different amounts of Ac PEG–DTT, hydrogels with tunable degradation times, ranging from a few days to >1 month, can be fabricated.

The development of such materials provides a platform to allow the investigation of questions regarding how the rate and amount of MSC delivery to tendinopathic lesions affects cell engraftment and, eventually, in vivo healing in a rat model of tendinopathy. As a first step in exploring these issues, in these experiments we determined the effect of different hydrogel formulations on swelling ratio and degradation time. Based on the degradation time, we selected three formulations, including those that were non-degradable on the timescale of the study (50 OPF/50 PEG–DA), as well as slower degrading (30 OPF/70 Ac PEG–DTT/65 mM) and faster degrading (50 OPF/50 Ac PEG–DTT/65 mM) hydrogels, and examined their cytocompatibility with rat marrow stromal cells (rMSCs). Furthermore, to test the hypothesis that localization of rMSCs in the tendon defects and subsequent delivery and infiltration of MSCs in the surrounding tissue could be controlled by the degradation rate of the biomaterial carrier, cell penetration from each of the three hydrogel formulations was quantified over 14 days in an in vitro model of tendinopathic tendon.

## 2. Materials and methods

### 2.1. Polymer synthesis

OPF ($M_n$ = 16,950 ± 130 Da, polydisperisity index [PI] = 4.9 ± 1.0) and PEG–DA ($M_n$ = 3760 ± 20 Da, PI = 1.1 ± 0.0) were synthesized, purified and stored as previously described [24,25]. PEG ($M_n$ = 10,000 and 3400 Da), fumaryl chloride, triethylamine, and acryloyl chloride were purchased from Sigma–Aldrich (St Louis, MO, USA). Dichloromethane, ethyl acetate, anhydrous potassium carbonate and ethyl ether were obtained from Fisher Scientific (Waltham, MA, USA).

### 2.2. Hydrogel fabrication

The ultraviolet (UV) photo-initiator Irgacure 2959 (D2959, Ciba, Basel, Switzerland) was dissolved in PBS at a concentration of 0.7 wt.% D2959 as a stock solution. To make solutions for degradable hydrogels, DTT (Sigma–Aldrich) and PEG–DA were dissolved in PBS and reacted at 37 °C for 2 h to synthesize Ac PEG–DTT. OPF was then added immediately to this solution and D2959 was included at a final concentration of 0.05 wt.%. The formulations for different hydrogels are listed in Table 1 (DTT refers to the concentration of DTT added to the reaction to synthesize the Ac PEG–DTT polymer). Hydrogel constructs were fabricated from OPF and PEG–DA or Ac PEG–DTT in ratios by weight (see Table 1) in 75 wt.% PBS. The polymer was placed between two glass slides and polymerized under UV light (365 nm, 10.5 mW $cm^{-2}$) (UVP, Upland, CA, USA) for 15 min. The polymerized hydrogel was cut with a size 10 cork borer to form samples (~13.8 mm diameter × ~1 mm thick) for further studies.

**Table 1**
Hydrogel formulations for the swelling and degradation study.

| Formulation | OPF (wt.%) | PEG–DA or Ac PEG–DTT (wt.%) | DTT (mM) |
|---|---|---|---|
| 50 OPF/50 PEG–DA | 50 | 50 | 0 |
| 50 OPF/50 Ac PEG–DTT/65 mM | 50 | 50 | 65 |
| 30 OPF/70 Ac PEG–DTT/60 mM | 30 | 70 | 60 |
| 30 OPF/70 Ac PEG–DTT/65 mM | 30 | 70 | 65 |
| 30 OPF/70 Ac PEG–DTT/70 mM | 30 | 70 | 70 |

### 2.3. Swelling and degradation study

Hydrogels fabricated as described above were allowed to swell overnight in PBS at 37 °C on a shaker table. The gels were blotted dry using weigh paper and their wet weights were recorded. These gels were then lyophilized for 24 h and the dry masses were recorded and used to calculate the fold swelling of the hydrogels (wet wt/dry wt). To characterize the degradation over time the fold swelling of hydrogels was determined on days 1, 5, 10, 17 and 24.

### 2.4. Cell harvest and isolation

Lewis rats were euthanized as per previous methods [26] using a chamber filled with $CO_2$. The femurs and tibiae were then dissected out. To expose the bone marrow, the distal ends of the tibiae and proximal ends of the femurs were cut off using rongeurs and holes were drilled in the bones using an 18G needle. The marrow was then flushed from the cavities with Dulbecco's modified Eagle's medium (DMEM) (VWR, West Chester, PA, USA). Collected marrow was dissociated with a transfer pipette and plated in tissue culture flasks. MSCs were allowed to proliferate until confluent in medium containing low glucose DMEM, 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 1% antibiotic/antimycotic (AA) (VWR), and then frozen and stored in liquid $N_2$ until further use. This was approved and performed according to guidelines of the Institutional Animal Care and Use Committee of Georgia Institute of Technology.