INTRODUCTION:
Tendinopathy is a common tendon disorder resulting from repetitive motion in the workplace or during athletic activity. Recently, mesenchymal stem/marrow stromal cells (MSCs) have been suggested as a means to restore extracellular matrix lost in tendinopathy. However, like MSC-based therapies for repairing other tissues, a major challenge in using stem cells to treat tendinopathy is assuring that adequate cell numbers are delivered and remain localized in the damaged areas. Our laboratory is investigating a novel injectable hydrogel system as a MSC carrier for treating tendinopathy. In this study, a novel degradable hydrogel based on oligo(poly(ethylene glycol) fumarate) (OPF) and acylated poly(ethylene glycol)-dithiobis(acrylate) (PEG-DA) and dithiothreitol (DTT), with tunable degradation times was designed to encapsulate MSCs and deliver them locally to enhance their engraftment in tendon tissue. In these experiments, we first explored the effect of different hydrogel formulations on swelling ratio and degradation time. Based on the degradation time, we selected 3 formulations, including those that were non-degradable on the time scale 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 engraftment of MSCs to the surrounding tissue could be controlled by the degradation rate of the biomaterial carrier, cell migration was quantified over 14 days from each of the 3 hydrogel formulations in an in vitro model of tendinopathic tendon.

METHODS:
OPF and PEG-diacrylate (PEG-DA) were synthesized as previously reported. To make solutions for degradable hydrogels, DTT (Sigma-Aldrich) and PEG-DA were dissolved in PBS and reacted at 37°C for 2 hours to synthesize Ac-PEG-DTT. OPF was then added immediately to this solution and photoinitiator was included at a final concentration of 0.05 wt%. The formulations for different hydrogels are listed in Table 1.

Hydrogel constructs were fabricated by placing the polymer solution made from OPF and PEG-DA or Ac-PEG-DTT between 2 glass slides and polymerizing under UV light (365 nm, 10.5 mW/cm²; UVP, Upland, CA) for 15 minutes. The fold swelling was measured after hydrogels fully swelled in PBS overnight (fold swelling: equilibrium swelling wt/dry wt; n=4). The degradation time of different formulations was observed by monitoring fold swelling over 24 days in PBS.

In order to observe cell viability in hydrogels, rMSCs (10⁶ cells/mL, n=3) were encapsulated into selected non-degrading, slower-degrading and faster degrading formulations. At day 3, the constructs were stained with LIVE/DEAD dye (Invitrogen) and imaged with confocal microscopy. For the cell migration study, a defect (7.5 mm × 4 mm × 2.5 mm depth) was cut in bovine patellar tendons (Animal Technologies). To mimic tendinopathic tissue, the tendons were immersed into 20 mL collagenase type II (1 unit/mL; Invitrogen) for 5 hours followed by washing. rMSCs labeled with CellTracker green (Invitrogen) were encapsulated in these tendon defects (4.5 × 10⁶ cells/defect; n=3 for each hydrogel type) using non-degrading, slower degrading and faster-degrading hydrogels and cultured for 14 days. At day 7 and 14, tendon segments were removed from culture and sectioned in 10 μm thick slices using a cryostat (HM560, Thermo Scientific). For each sample, 3 slices from the center portion of the defect, each site ~1 mm apart from each other, were imaged directly with fluorescent microscopy, followed by staining with hematoxylin and eosin (H&E, VWR) and re-imaging with light microscopy. The number of rMSCs migrated into each defect was counted from the H&E images of a full cross-section (merged Adobe Photoshop with a line drawn along the surface of the defect as a baseline and other lines created by shifting the baseline into the tissue at 100 μm intervals. These cells were confirmed to be exogenous by comparing the H&E images with fluorescent images.

The number of migrated cells was normalized to the linear distance of the line drawn along the surface of the defect.

All of the data from the cell migration study were square-root-transformed before statistical analysis due to the large variance among samples. Cell migration data from the faster-degrading hydrogels on day 7 and 14 were analyzed via two-way analysis of variance (ANOVA), and all other data, including hydrogel swelling, were analyzed via one-way ANOVA and Tukey’s multiple comparison test (p ≤ 0.05). Results are reported as mean ± standard deviation.

RESULTS:
Hydrogel with higher amounts of OPF or DTT demonstrated significantly greater swelling ratios (not shown), as well as faster degradation rates (Table 1). LIVE/DEAD staining showed that the encapsulated rMSCs were largely viable (green) before the gels degraded (not shown). The cell migration study indicated that no cells migrated outwards from non-degradable hydrogel after 14 days, but degradation was able to deliver rMSCs to the defects. Faster-degrading hydrogels resulted in significantly more total cell engraftment compared to slower-degrading hydrogels at day 14. Moreover, for faster-degrading hydrogels, significantly more cells were found in each layer at day 14 compared to day 7 (see Fig1).

DISCUSSION:
In this study, we successfully designed a novel injectable hydrogel carrier for MSCs based on OPF and Ac PEG-DTT. This hydrogel carrier is cytocompatible and the degradation rate can be adjusted by changing the ratio of the two polymers. An in vitro migration study using collagenase-treated bovine patellar tendons indicated that hydrogel-encapsulated MSCs were localized in tendon defects, and the migration and engraftment of MSCs in tendon tissue was controlled by hydrogel degradation time. Therefore, this type of hydrogel shows great potential as a cell carrier to better identify dosing regimens required for MSC-based therapies of tendon overuse injuries.

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