A Novel Degradable PEG-based Hydrogel to Improve Delivery of Progenitor Cells for Tendon Repair

Yongzhi Qiu, Larry Scott, Jr., Jeremy J. Lim, Johnna S. Temenoff
Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA

Introduction: Tendinopathy is a common tendon disorder resulting from repetitive motion in the workplace or during athletic activity. Recently, mesenchymal stem cells (MSCs) have been suggested as a means to restore extracellular matrix lost in tendinopathy. However, because tendinopathy does not often result in focal tissue defects, a major challenge is assuring that adequate cell numbers are delivered and remain localized in the damaged areas. Our laboratory is investigating a novel hydrogel system as a cell carrier for tendon repair. In this study, a novel degradable hydrogel based on oligo(poly(ethylene glycol) fumarate) (OPF) and acrylated poly(ethylene glycol)-dithiolthreitol (Ac PEG-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. For preliminary cell studies, a formulation with 3-5 day degradation time was chosen to examine cytocompatibility with bovine MSCs as well as quantify cell migration from degradable vs. nondegradable gels (containing no Ac PEG-DTT) into an *in vitro* model of tendinopathic tendon.

Methods: OPF [1] and PEG-dimethacrylate (PEG-DMA) [2] were synthesized as prevously reported. Four polymer formulations (Table 1) were used for the swelling/degradation studies. To synthesize Ac PEG-DTT, PEG-diacrylate and DTT were dissolved into phosphate-buffered saline (PBS) and incubated at 37°C for 2h [3]. OPF was then dissolved in PBS and mixed with Ac PEG-DTT and photoinitiator D2959 (0.05 wt%). The hydrogels were obtained by photopolymering the mixed solution in a Teflon mold via UV exposure for 15 minutes. The fold swelling was measured after hydrogels fully swelled in PBS overnight (fold swelling: equilibrium swelling wt/dry wt.) The degradation time of different formulations was observed by monitoring fold swelling over 28d in PBS. Statistical analysis on fold swelling was completed using ANOVA ($p<0.05$). In order to observe cell viability in degradable gels, bovine MSCs (10^7 cells/mL, n=3) were encapsulated into the gels of formulation 4. At day 3, the constructs were stained with LIVE/DEAD dye (Molecular Probes) and imaged with confocal microscopy. For the cell migration study, a defect (7.5 mm x 4 mm x 2.5 mm depth) was cut in bovine patellar tendons. To mimic tendinopathic tissue, the tendons were immersed into 0.4% collagenase type II (Invitrogen) for 4 hours followed by washing. Bovine MSCs labeled with CellTracker green (Molecular Probes) were encapsulated in these tendon defects ($3X10^5$ cells/defect, n=2) using gels of formulation 1 (non-degradable) or formulation 4 (degradable) and cultured for 14 days. After culture, the tendons were cryosectioned to provide cross-sections 10 μm thick. The slices were imaged directly with fluorescent microscopy (the labeled cells appeared green), followed by staining with hematoxylin and eosin (H&E) and re-imaging with light microscopy. The migrated bMSCs were counted from the H&E images of a full cross-section (merged in Adobe Photoshop) with the line drawn along the surface of the defect as a baseline. These cells were confirmed to be exogenous by comparing the H&E images with fluorescent images.

Results: Hydrogel with higher content of OPF or DTT demonstrated significantly greater swelling ratios (not shown), as well as faster degradation rates (Table 1).

Table 1: Formation and degradation time of hydrogels

Formulations	1	2	3	4
(OPF/PEG-wt%, mM is based on final volume)	50/50 OPF/ PEG-DMA	30/70 OPF/Ac PEG-DTT, 65mM DTT	50/50 OPF/Ac PEG-DTT, 65 mM DTT	50/50 OPF/Ac PEG-DTT, 75 mM DTT
Degradation time	>28 days	After 10days	After 5days	After 3days

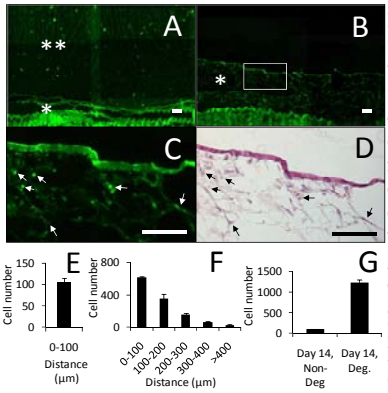

Figure 1. Cell migration from OPF hydrogels into tendon defects. Fluorescent images of bovine tendon defects with MSCs encapsulated in nondegradable (A) and degradable (B) gels and cultured for 2 weeks (* tendon; ** nondegradable gels). C) Fluorescent image of the boxed area in B (cells are marked by arrows). D) Image of the boxed area in B after H&E staining (corresponding cells from C marked by arrows). Number of engrafted cells delivered from non-degradable (E) and degradable gels (F) (n=2) at various distances from defect surface. G) Total number of engrafted cells from nondegradable and degradable hydrogels at day 14 (n=2). Scale bar = 50 um.

LIVE/DEAD staining showed that the encapsulated bMSCs were largely viable (green) before the gels degraded (not shown). The cell migration study indicated that degradable gels were able to deliver bMSCs to the defects, and this formulation demonstrated greatly enhanced migration and cellular engraftment in tendon tissue compared to nondegradable gels (10 X greater cell numbers were found in defects with degradable gels, see Fig 1).

Conclusions: A novel degradable PEG-based hydrogel with tunable degradation time was designed. This type of gel is cytocompatible and can be used to locally deliver and control timing of "release" of MSCs into damaged tendon, thus demonstrating great potential for use as a cell carrier to enhance repair of tendon injuries such as tendinopathy.

References: 1.Brink KS et al. *Acta Biomaterialia* 2009 (5) 570–579; 2.Lin-Gibson, S. et al. *Biomacromolecules* 2004 (5) 1280-1287; 3. Hudalla GA, *Biomacromolecules* 2008 (9) 842-849

Acknowledgements: Arthritis Foundation Investigator Award (JST)