Designing Cell-Mediated Degrading PEG Hydrogels for Personalizing Cartilage Tissue Engineering

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Statement of Purpose: A successful cartilage tissue engineering therapy will need to employ cartilage cells from patients of varying ages. However, donor age dramatically impacts the anabolic and catabolic metabolism of cartilage cells.[1] Therefore, scaffold (e.g., hydrogel) designs must adapt and account for these differences. Cell-mediated degradation is a promising strategy to exploit differences in catabolism, yet this degradation mode is complex, as enzyme secretion and network structure affect hydrogel degradation kinetics and behavior. Depending on the relative reactivity and diffusivity of the enzyme, hydrogel degradation can occur in bulk or localized around the cells. Recently, we showed through computational simulations that localized degradation improves macroscopic tissue evolution over bulk degradation[2]. Therefore, the goal of this study was twofold: (a) to develop an acellular model system where collagenase-secreting microparticles simulate enzyme-secreting cells as a means to characterize hydrogel degradation in the absence of matrix deposition, and (b) to investigate a cartilage-specific enzyme-degradable hydrogel in regenerating cartilage from different aged donors.

Methods: Collagenase was loaded into poly(lactic co-glycolic acid) microparticles by a double emulsion technique and its release measured (NanoOrange®). PEG hydrogels were formed from 10% w/w 8-arm PEG norbornene (10 or 20 kDa) and crosslinker (PEG-dithiol (1 kDa) or bis-cysteine peptides) and crosslinked in the presence of photoinitiator and UV light[3]. Bovine chondrocytes were isolated from 1-2 year old steers and 6 month old calves (referred to as adult and juvenile, respectively) and encapsulated at 20-50 million cells/mL in thiol-ene PEG hydrogels. ADAMTS-4 content (Anaspec SensoLyte™) and compressive modulus of cell-laden gels were assessed. Significance was assessed by one-way ANOVA (n=3) with Fisher's LSD post-hoc test, $p < 0.05$. Data are presented as mean (standard deviation).

Results: Hydrogels crosslinked with a collagenase-specific peptide (CVPLSLYSGC) degraded by either bulk or surface degradation when exposed to varying concentrations of collagenase (Fig 1a). Collagenase-loaded microparticles were fabricated and their release profiles characterized (Fig 1b), demonstrating the ability to model variation in enzyme production by cells of different aged donors. To design a cartilage-specific enzyme degradable hydrogel, ADAMTS-4 secreted by adult and juvenile cells was quantified in non-degrading hydrogels and varied with age (Fig 1c). Therefore, an ADAMTS-4 sensitive hydrogel was designed with the peptide crosslinker, CRDTEGEARGSVIDRC, which is based on the ADAMTS-4 cleavage site located in the aggrecan core protein. After 3 weeks of culture in ADAMTS-4 degradable hydrogels with or without the catabolic stimulator lipopolysaccharide (LPS), degradation was confirmed by the decrease in compressive modulus (Fig 1d).

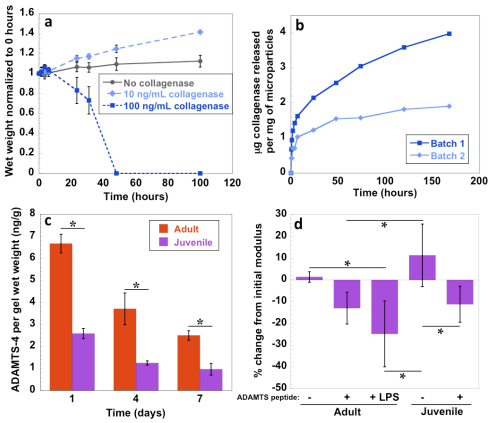

Figure 1. (a) Normalized wet weight of collagenase-degraded hydrogels. (b) Collagenase release from microparticles. (c) ADAMTS-4 produced by adult and juvenile cells in non-degrading hydrogels. (d) Week 3 compressive modulus normalized to day 1 for cell-laden non-degrading or ADAMTS-4 degradable hydrogels with or without 10 ng/mL LPS supplementation. * indicates significant difference ($p < 0.05$).

Conclusions: We demonstrated a collagenase microparticle system that models variable enzyme secretion from cells, without the added complication of matrix production. We also showed that altering enzyme concentration led to predominantly bulk or surface (i.e., localized) degradation. We are currently using this system to probe how variations in concentration and release of enzyme and hydrogel structure impact the degradation mode. By encapsulating cells from juvenile and adult donors, which secrete different amounts of ADAMTS-4 enzyme, into ADAMTS-4 degradable hydrogels, we can determine how differences in enzyme production affect both gel degradation and new matrix deposition. In the ADAMTS-4 degradable hydrogels, degradation was confirmed by a drop in compressive modulus when compared to non-degradable hydrogels, and this drop was more dramatic when catabolically stimulated with LPS, which did not adversely affect cell viability at 3 weeks (images not shown). Studies are continuing through 12 weeks where cartilage neotissue deposition will be characterized to determine how age, enzyme production, and degradation affect matrix deposition. Together with the microparticle system experiments, our goal is to determine the impact of degradation mode (bulk versus localized) on neotissue development and to use this information to direct patient-tailored design of degradable hydrogels for cartilage tissue engineering.

References: 1) Skaalure SC. Biomed Mater. 2012;7 doi:10.1088/1748-6041/7/2/024111. 2) Dhote V. Biomech Model Mechanobiol 2013; doi:10.1007/s10237-013-0493-0. 3) Fairbanks BD. Adv Mater. 2009;21:5005-5010.

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