MMP-Degradable Carriers Promote Gene Upregulation & Production of Ligament ECM by Mesenchymal Stem Cells

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Introduction: Extracellular matrix (ECM) remodeling by fibroblasts is an important factor in tendon/ligament healing (Khatod, M. Daniel's Knee Injuries, 2003: 185-201). Matrix metalloproteinases (MMPs) are a family of enzymes secreted by mesenchymal stem cells (MSCs) and fibroblasts to facilitate ECM reorganization (Fox, SI. Human Physiology. 2002;7th ed.:127.). To establish parameters for design of future biomaterials for tendon/ligament tissue engineering, our laboratory incorporated MMP-degradable peptides in a model synthetic hydrogel system in an effort to understand the relationship between local degradation of the biomaterial carrier and gene and protein regulation by embedded cells. Specifically, this study sought to determine whether a biomaterial environment containing MMP-cleavable motifs would influence human MSCs to produce ECM-related gene expression profiles similar to human anterior cruciate ligament fibroblasts, as compared to the response of both cell types to a non-degradable carrier.

Methods: Oligo(poly(ethylene glycol) fumarate with a poly(ethylene glycol) (PEG) chain of molecular weight 3KDa was combined (1:1 wt/wt) with PEG-diacrylate (nominal M_n 3,400) to form non-degradable hydrogels (0%). A GGGLGPAGGK peptide conjugated on each end to an acylated PEG chain (3kDa) was used to form MMP-degrable hydrogels (100%). The GRGDS adhesive peptide (1 μmol/g hydrogel) was incorporated in all gels. Polymers were cross-linked (15mm dia discs) using N,N,N',N-tetramethylethylenediamine and ammonium persulfate thermal radical initiators (0.018M, 10min, 37°C) with hMSCs or fibroblasts (10 million cells/mL).

Bioasssays were conducted at d1, d7, d14, and d21. Media was examined for production of MMP-1 and MMP-13 (n=3). Real time RT-PCR was used to examine gene expression compared to GAPDH (n≥7) of major tendon/ligament matrix proteins (collagen I, collagen III, and tenascin-C), followed by immunohistochemisty to examine protein production. Significance was determined with a 2-way ANOVA followed by Tukey's Multiple Comparison Test ($p \leq 0.05$).

Results: While fibroblasts and hMSCs did not produce MMP-1 in an active form, modest levels of active MMP-13 were produced by both cell types (data not shown). Collagen I expression was upregulated (Fig. 1A) in fibroblasts compared to hMSCs in 0% and 100% hydrogels on d7 and d21. Collagen III expression was upregulated (Fig. 2B) in fibroblasts compared to hMSCs in 0% hydrogels on d7, d14, and d21 and in 100% hydrogels on d1 and d7. Tenascin-C expression was upregulated (Fig. 2C) in fibroblasts compared to hMSCs in 0% hydrogels on d7, d14, and d21 and in 100% hydrogels on d14. See Fig. 1 for additional comparisons.

IHC staining with hMSCs indicated the presence of collagen I, collagen III, and tenascin-C pericellularly in 100% MMP-cleavable hydrogels. 0% hydrogels did not demonstrate any staining for these proteins (not shown).

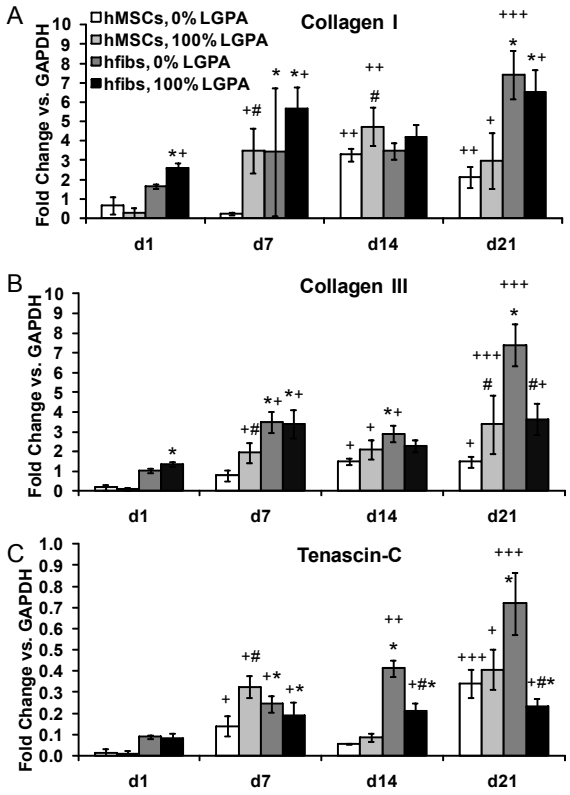

Fig 1. Gene expression of hMSCs and human fibroblasts (hfibs) in hydrogels (n≥7±SD). $p<0.05$ compared to hMSCs (*). $p<0.05$ compared to 0%(#). $p<0.05$ compared to d1(+); d1 and d7(++); or d1, d7, and d14(+++). Note different Y axis in C.

Discussion: Higher transcript levels were generally seen in fibroblasts compared to hMSCs, particularly in non-degradable gels. Therefore, the upregulation of collagen I and III expression seen in hMSCs in MMP-cleavable gels may indicate that an enzymatically degradable environment can promote hMSC differentiation toward a fibroblastic phenotype. This gene upregulation, combined with the IHC results, indicates that localized degradation of the hydrogel carrier by encapsulated MSCs may facilitate production of tendon/ligament ECM components and suggests that the amount of the ECM produced can be controlled by the degradability of the material. Previous studies conducted in our laboratory with non-degradable gels demonstrated MSC differentiation toward a fibroblast phenotype under tensile strain (Doroski, DM. Tissue Eng Part A. 2010;16:3457-66.). Further work will employ this MMP-sensitive carrier to better understand how local ECM deposition affects MSC responsiveness to tensile loading, thereby providing the basic parameters for next-generation biomaterial/bioreactor combinations to be used in production of tissue-engineered ligament grafts.

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