A Cartilage-Like PEG Hydrogel Directs Chondrogenesis of hMSCs Under Mechanical Stimulation

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Statement of Purpose: The specific goal of this study was to identify a suitable hydrogel environment that enhances chondrogenic differentiation of human mesenchymal stem cells (hMSCs). Our approach is to mimic key biological and mechanical factors that are found in native cartilage. Specifically, we investigated two factors: extracellular matrix (ECM) cues and mechanical stimulation. Chondroitin sulfate (ChS) is a negatively charged glycosaminoglycan and is a major component of the ECM of cartilage. The cell adhesion peptide RGD has previously been shown to enhance hMSC viability when incorporated in PEG hydrogels (Nuttleman CR. Matrix Biol. 2005;3:208.). Mechanical loading is well known to be critical in cartilage homeostasis (Lima EG. Osteoarthr Cart. 2007;15:1025.). We hypothesized that an environment similar to native cartilage comprised of negatively charged ChS, cell attachment sites and when coupled with dynamic mechanical compression provides the necessary cues to direct hMSC chondrogenesis.

Methods: Hydrogel were made from poly(ethylene glycol) dimethacrylate (PEGDM), chondroitin sulfate methacrylate (ChSMA), and Acryloyl-PEG-RGD. HMSCs (Texas A&M Cell Distribution Center) were photoencapsulated in hydrogels formed from 10wt% macromer consisting of 0-2wt% ChSMA and 0-1mM RGD and cultured in chondrogenic differentiation media (1ml/100ml media ITS+ Premix, 100 nM dexamethasone, 5 ng ml^{-1} TGF-β3, 50 mg ml^{-1} l-ascorbic acid 2-phosphate, 50 U ml^{-1} penicillin, 50 mg ml^{-1} streptomycin, and 20 mg ml^{-1} gentamicin in DMEM). A bioreactor system (Nicodemus GD. J Biomech. 2008;41:1528.) was utilized to apply unconfined dynamic compression to hydrogels under one of four regimes (5% or 10% amplitude strain, 0.3Hz or 1.0 Hz in a sinusoidal wave form, 1 hour/day). Hydrogels were cultured under free swelling conditions for one week followed by either free swelling culture or dynamic loading for an additional 1-2 weeks. Gene expression from qRT-PCR and immunohistochemistry were used to analyze differentiation, specifically for collagens I (marker for undifferentiated hMSCs), II (marker for chondrogenesis) and X (marker for hypertrophic chondrogenesis).

Results: Hydrogel formulations with varying concentrations of ChSMA (1or 2wt%) and PEG-RGD (0.1mM or 1mM) were investigated under free swelling conditions to identify hydrogel environments that enhanced chondrogenesis. Gene expression showed a down-regulation of collagen I from day 1 ($p<0.05$) and an up-regulation in collagen II from day 1 ($p<0.05$) for all constructs. Collagen X expression mirrored that of collagen II and was elevated in all constructs. The highest mean collagen II expression was observed with 1% ChSMA and 0.1 mM RGD, although, not statistically significant. Because collagen II is a definitive marker for chondrogenesis, collagen II protein deposition was also investigated, showing the greatest deposition for the 1% ChSMA and 0.1 mM RGD hydrogels (Fig 1).

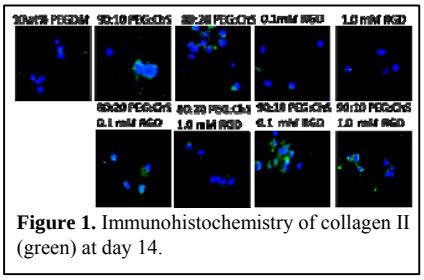

Figure 1. Immunohistochemistry of collagen II (green) at day 14.

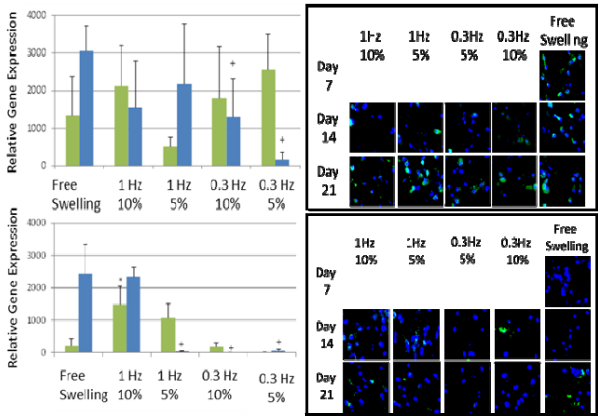

Figure 2 left. Normalized gene expression of Collagen II (top), and Collagen X (bottom). Green is day 7, blue is day 21. n=4. Error bars represent standard deviation. + indicates significant difference from free swelling ($p<0.05$). **Figure 2 right.** Immunohistochemistry of collagen (green) II (top), and X (bottom) at day 21. n=3.

This hydrogel formulation was selected and the effect of mechanical loading regime investigated (Fig 2). Collagen II gene expression was consistent with free swelling for the 1Hz loading, but down regulated for the 0.3 Hz loading by day 21 regardless of strain. Collagen X gene expression was significantly affected by mechanical loading. Hydrogels that were loaded at low strain or at low frequency exhibited the lowest expression of collagen X. By day 21, collagen II was detected in all conditions, while collagen X was only detected in free swelling and 1Hz 10% strain condition. Overall, the loading regime of 1 Hz at 5% strain enhances chondrogenic differentiation of hMSCs while minimizing hypertrophy.

Conclusions: This study demonstrates that creating a more biomimetic environment with biochemical and biophysical cues can enhance chondrogenesis, but is dependent on the amount of each cue. Most promising is that conditions were identified that supported chondrogenesis while inhibiting hypertrophy, where the latter appears to be largely a result of mechanical stimulation. Overall, this native-like environment provides the necessary cues to direct hMSC chondrogenesis.

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