ECM-mimicking Hydrogels for Efficient Differentiation of Bone Marrow Stromal Cells into Various Zones of Articular Cartilage

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Statement of Purpose: Our primary hypothesis is that biomaterials play an important role in the chondrogenic differentiation of MSCs, especially in determining the phenotype of the resulting cartilage-like tissue. We believe that different combinations of synthetic (e.g. PEG) and natural biopolymers (e.g. extracellular matrix (ECM) components) as well as cell-induced matrix degradability would provide unique cues to the differentiating MSCs and could direct them to zone-specific articular chondrocytes. Our goal is to identify unique hydrogel compositions that specifically generate either superficial, middle, deep or calcified zones of articular cartilage from MSCs and to create multi-layered scaffold structures incorporating these biomaterial compositions such that a single stem/progenitor cell population gives rise to zonally-organized articular cartilage-like tissue. Here we demonstrate that such zone-specific differentiation of mouse MSCs is feasible by designing specific matrix material compositions.

Methods: Chondroitin sulfate (CS) and hyaluronic acid (HA) was acrylated using glycidyl methacrylate. The protocol was adopted from Varghese et al. [1] and Leach et al. [2], respectively. An MMP-sensitive (QPQGLAK) peptide was synthesized using an automatic peptide synthesizer (Protein Technologies, Inc.). The MMP-sensitive peptide was modified by adding acryl groups to amine group of the N terminal and to the amine group on the lysine. Hydrogel scaffolds were fabricated using poly(ethylene glycol) dimethacrylate (PEGDA), MMP peptide, and the modified CS and HA, by dissolving the materials of each group in PBS containing 0.05 wt% photoinitiator, Irgacure 2959 and polymerized using a long-wave ultraviolet lamp (Blak-Ray) at an intensity of ~10 mW/cm^2 for 10 minutes. Various MSC-hydrogel constructs were cultured in a chondrogenic medium containing 1% penicillin-streptomycin, no FBS, and 10ng/uL TGF-β1 for 2 and 4 weeks. Scaffolds were homogenized and RNA isolation was performed following previously published protocol. Quantitative real-time PCR for Collagen II and Collagen X was performed using an ABI Prism® 7900 Real Time thermal cycler. In addition extensive immuno-histochemistry was performed on paraffin-sections to determine distribution of collagen II and X in each tissue construct. Further, GAG content of the differentiated tissues were determined using colorimetric assays.

Results: Cartilage synthesis was determined by gene expression of collagen II and X within the hydrogel constructs at 2 and 4 weeks. The collagen II expression increases between week 2 and week 4 in all the hydrogel matrices except for the CS, PEG:CS and PEG:CS:MMP hydrogels as shown in **Fig. 1A**. Collagen X expression is very low for all the hydrogel matrices at 2 weeks with the exception of CS as shown in **Fig. 1B**, indicating that CS plays a role in inducing a hypertrophic phenotype. At all time points, the addition of CS and MMP into the PEG hydrogels increased collagen II expression indicating that CS and MMP encourages chondrogenesis. In all hydrogel groups GAG production increased from 2 to 4 weeks. However, the presence of HA within the hydrogel matrix induced maximal GAG production (data not shown).

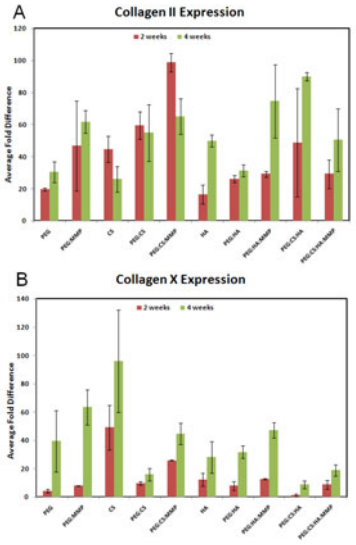

Figure 1. Gene expression of (A) collagen II and (B) Collagen X.

Conclusions: Our results indicate that the various hydrogel compositions induce chondrogenesis that can be correlated to each of the different zones of articular cartilage. As shown in **Figure 2**, the PEG:CS:MMP hydrogel composition had the highest collagen II expression as well as the lowest GAG production at both 2 and 4 weeks, which correlates to the superficial zone. The PEG:CS composition had mid-range expressions of collagen II and GAG, which is similar to that of the middle zone. PEG:HA hydrogels correlated to the deep zone of articular cartilage because of its high GAG content and low expression of collage II. The CS hydrogel composition induces hypertrophic chondrocytes with very high expression of collagen X, which correlates to the calcified cartilage zone.

	Collagen X		Collagen II		GAG Content	
Hydrogel Groups	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
PEG:CS:MMP	26	45	99	65	53	98
PEG:CS	10	16	59	55	99	137
PEG:HA	8	32	26	31	176	236
CS	49	96	45	26	89	255

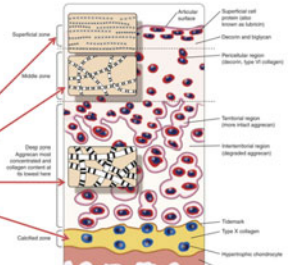

Figure 2. Correlation of cartilage generated in various hydrogel compositions to the specific zones of articular cartilage.

Our current studies are focusing on creating a single multi-layered structure with these zone specific biomaterials for the simultaneous differentiation of BMSCs into structurally organized articular cartilage.

References:

1. Varghese, S., et al., Matrix Biol, 2008. **27**(1): p. 12-21.
2. Leach,et al., Biotechnol Bioeng, 2003. **82**(5): p. 578-89.