Evaluation of ECM components in Biodegradable Hydrogels for Chondrogenic Differentiation of Mesenchymal Stem Cells

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Statement of Purpose: Numerous works have demonstrated that the incorporation of biopolymers, such as chondroitin sulfate and hyaluronic acid into hydrogel scaffolds, enhances chondrogensis [1-2]. Chondroitin sulfate (CS) and hyaluronic acid (HA) are natural components of cartilage extracellular matrix (ECM), thus their incorporation into scaffolds helps mimic the physiological environment of native cartilage. In tissue engineering, an essential property of scaffolds is their ability to degrade. The hydrogel scaffold serves as a temporary replacement matrix for the damaged tissue until the encapsulated cells have established their own ECM. To accomplish this we will incorporate an MMP-sensitive peptide that will degrade by cell specific collagenase. In previous studies, only one component, either CS, HA or an MMP-sensitive peptide is incorporated into the hydrogel network. No prior work explores the effects of combining these components together within a single polymer network. We propose to explore the fabrication of a multi-faceted scaffold by combining CS, HA and MMP into a PEG-based hydrogel and study their effects on MSC differentiation into chondrocytes. Our ultimate goal is to combine this multi-faceted scaffold for chondrogenesis with a scaffold that is conducive to osteogenesis to create a multilayer scaffold for the differentiation of MSCs into both bone and cartilage.

Methods: Chondroitin sulfate and hyaluronic acid was modified in order to be incorporated into the PEG-based hydrogel network. Chondroitin Sulfate A (CS) and hyaluronic acid was purchased from Sigma and acrylated using glycidyl methacrylate. The protocol was adopted from Elisseeff et al. (2003) and Schmidt et al. (2004), respectively. An MMP-sensitive (QPQGLAK) peptide was synthesized using an automatic peptide synthesizer (Protein Technologies, Inc. Symphony Quartet). The modification of the MMP-sensitive peptide was doneby 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 biopolymers such as CS and HA. The hydrogels were fabricated by dissolving the materials of each group in phosphate buffered saline (PBS) containing 0.05 wt% photoinitiator, Irgacure 2959 (Ciba Geigy Corp.) and polymerized using a long-wave ultraviolet lamp (Model B100AP, Blak-Ray) at an intensity of ~10 mW/cm^2 for 10 minutes. The hydrogel constructs were cultured in chondrogenic medium containing 1% penicillin-streptomycin, no FBS, and 10ng/uL TGF-β1 for six weeks. Hydrogels were removed from culture and fixed in 4% paraformaldehyde at 4°C for an overnight period. Fixed hydrogels were then dehydrated for paraffin embedding. Paraffin-embedded hydrogels were sliced in transverse sections at 10 µm using a rotary microtome. Immunohistochemistry was performed on sections to stain collagen II green to determine differentiation. Rabbit polyclonal antibodies to collagen II (ABCam) were used as primary antibodies. The slides were imaged using a confocal fluorescence microscope (Leica SP2 AOBS).

Results: The addition of MMP-sensitive peptide aids the differentiation of MSCs into chondrocytes as demonstrated in figure 1. All constructs containing the MMP-sensitive peptide shows an increase in expression of collagen II, compared to its opposing construct without the MMP-sensitive peptide. In addition, for all constructs, the MMP-sensitive peptide increases the degradation rate, as shown in figure 2. The exception are the PEG:HA and the PEG:HA:MMP hydrogels. Steric hindrance could account for the slower degradation of the PEG:HA:MMP hydrogels. The HA is a very high molecular weight molecule ranging from 1 to 2 million Daltons, which can sterically hinder the collagenase and inhibit its diffusion towards the cleavage site of the peptide.

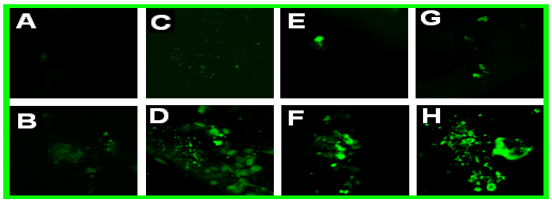

Figure 1. (A) PEGw/o cells, (B) PEG, (C) PEG:CS, (D) PEG:CS:MMP, (E) PEG:HA, (F) PEG:HA: MMP, (G) PEG:CS:HA and (H) PEG:CS:HA:MMP.

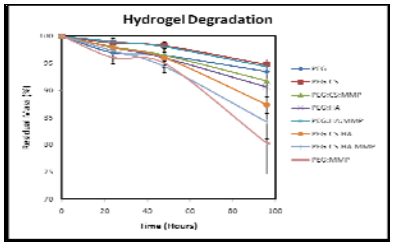

Figure 2. Degradation profiles of hydrogel constructs.

Conclusions: We have demonstrated that the combination of CS, HA and an MMP-sensitive peptide within a hydrogel aids in the induction of chondrogenic differentiation. The gene expression of collagen II will be quantified with qRT-PCR. Since the physical characteristics of the hydrogels also plays a role in chondrogenesis, further hydrogel characterization will be performed including swelling studies and mechanical testing studies. Since our ultimate goal is to induce hybrid differentiation of MSCs, we will investigate the effects of integrating the chondrogenic layer with an osteogenic layer within a single scaffold for the simultaneous differentiation MCSs into bone and cartilage.

References:

1. Bryant, S.J., Biomed Mater Res A, 2003. **64**(1): p. 70-9.
2. Varghese, S., et al., Matrix Biol, 2008. **27**(1): p. 12-21.