Regulation of Osteogenic and Chondrogenic Differentiation of MSCs in Extracellular Matrix-Hydrogels

Nathaniel S. Hwang [a], Shyni Varghese[a], Hanwei Li[a], Adam Canver, and Jennifer Elisseeff[a*]
[a] Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218

Statement of Purpose: Extracellular microenvironment regulates the differentiation of mesenchymal stem cells (MSCs). To define whether extracellular matrix (ECM) plays a functional role in regulation of osteogenic and chondrogenic differentiation by MSCs, we have encapsulated MSCs in ECM-hydrogels containing exogenous type I collagen, type II collagen, or hyaluronic acids (HA). Enhanced chondrogenic differentiation of MSCs was evident in collagen matrix-hydrogels, while osteogenic differentiation, as measured by calcium accumulation, was strongly induced in HA matrix-hydrogel. Altogether, these results indicate that microenvironment created by exogenous ECM components can regulate the fate of MSCs differentiation.

Methods: Photoencapsulation: Goat MSCs were isolated and expanded as previously described [1]. Hydrogels with exogenous ECM components were prepared by mixing 50/50 volume of 20% (w/v) poly(ethylene glycol)-diacrylate (PEGDA) solution with either bovine type I or II collagen solutions (neutralized with 0.1N NaOH and brought up to a concentration of 2 mg/ml by adding PBS), or HA solution (5 mg/ml in PBS). MSCs were gently mixed with the polymer solution at a concentration of 2×10^7 cells/ml. The photoinitiator, Igracure 2959, was added to the cell-hydrogel solution and mixed thoroughly to make a final concentration of 0.05% (w/v). Photopolymerization of 100µl cell-polymer-photoinitiator suspension was conducted using 365-nm light (4.5 mW/cm^2) for 5 minutes. The constructs were then cultured at 37 ºC with 5% CO_2 in 2.5 ml chondrogenic or osteogenic differentiation medium as described previously [1]. **Actin Staining and Histology:** Hydrogel constructs were fixed in 4% paraformaldehyde and cut into 1 mm sections and then permeabilized for 30 minutes with 0.1% Triton X-100 in DPBS. Subsequently, the samples were incubated with TRITC-conjugated Phalloidin diluted 1:500 in 1% BSA/DPBS for an hour at room temperature (Chemicon). For histology, constructs were processed into 5-µm sections and stained with Safranin-O and Alizarin Red S. **Biochemical Assays**: Proteoglycan content was determined by chondroitin sulfate using dimethylmethylene blue spectrophotometric assay at A_{525}, as previously described [2]. Calcium content was measured following manufacturer's protocol (Sigma Diagnostics 587). **RNA Isolation and Real-Time PCR:** Total RNA was extracted with Trizol and reverse-transcribed into cDNA using SuperScript First-Strand Synthesis System (Invitrogen). Real Time-PCR reactions were performed using the SYBR Green PCR Mastermix.

Results/Discussion: In chondrogenic condition after 6 weeks *in vitro*, cartilaginous tissues formed in the all ECM-hydrogels (**A-D**). Individual cells surrounded by an ECM containing sulfated polysaccharides were evident, resembling native articular cartilage. Extent of matrix accumulation was enhanced in collagen based hydrogels while HA-hydrogel resulted in minimal amount of GAG accumulation (**E**). Real-time PCR for type II collagen and Sox-9 confirmed that collagen-hydrogels showed higher expressions while cells in HA-hydrogels showed relatively lower expression levels of cartilage markers (**F, G**). The extent of mineralization on different ECM-hydrogel scaffolds were examined by Alizarin Red S staining following 6 weeks of incubation in osteogenic medium (**H-K**). The calcium content and extent of mineralization was modulated by ECM-hydrogels, HA-hydrogel promoting significantly larger amount of mineral deposits (**L**). In contrast chondrogenic condition, collagen-hydrogels resulted in minimal accumulation of calcium. The actin cytoskeleton organization and cellular morphology was strongly dependent on which extracellular matrix components were added to the hydrogel, indicating that these exogenous factors significantly modulated ECM-cell interactions **(M-O).**

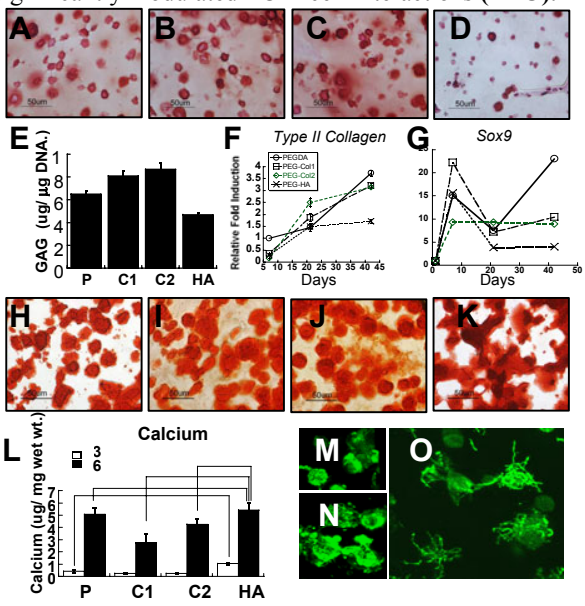

Figure 1: Safranin-O staining of cells in PEGDA (A), PEGDA-Collagen 1 (B), PEGDA-Collagen 2 (C), and PEGDA-HA (D). GAG content showed enhanced matrix accumulation in collagen-hydrogels (E). Real-time PCR of MSCs in ECM-hydrogels for *type II collagen* (F) and *Sox9* (G). Alizarin staining of cells in PEGDA (H), PEGDA-Collagen 1 (I), PEGDA-Collagen 2 (J), and PEGDA-HA (K) after 6 weeks in osteogenic medium. Calcium measurement of ECM-hydrogels after 3 and 6 weeks of incubation (L). Actin staining of MSCs in PEGDA (M), PEGDA-collagen 1(N), and PEGDA-HA (O) after 10 days in osteogenic medium.

Conclusions: Knowledge gained from these experiments can be utilized to exploit selective signals inducing differentiation scheme of mesenchymal stem cells.

References: [1] Williams, C.G. (2003) Tissue Eng 9, 679-88. [2] Farndale, R.W. (1986) Biochim Biophys Acta 883 173-7