Hyaluronic Acid Mediation of Mesenchymal Stem Cell Behavior in Photocrosslinkable Hydrogels

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Statement of Purpose: Mesenchymal stem cells (MSCs) are pluripotent progenitor cells with the ability to differentiate towards osteoblastic, chondrogenic, or adipogenic lineages under the appropriate conditions. Easily isolated and expanded *in vitro*, MSCs prove to be a promising cell source for cartilage tissue engineering. MSCs have also been shown to interact with hyaluronic acid (HA) through CD44, CD54, or CD168 receptors. CD44 is the major receptor of HA and it exhibits a wide range of biological functions including cell adhesion, matrix assembly, and cell signaling[1]. Recently, we have been investigating photocrosslinkable HA gels as cell carriers for the regeneration of cartilage[2]. In this study, human MSCs encapsulated in photocrosslinkable HA and polyethylene glycol (PEG) hydrogels are cultured *in vitro* to investigate the effects of MSC/hydrogel interactions on encapsulated cell behavior.

Methods: Human MSCs (Cambrex) were cultured in 2D on glass coverslips and fixed in accustain (Sigma) for immunofluorescence staining of CD44. Briefly, the cells were blocked with 5%FBS, stained with primary antibody anti-CD44 clone A3D8, incubated with secondary antibody anti-mouse IgG (whole molecule) $F(ab')_2$ fragment-FITC, and counterstained with DAPI (2μg/ml).

For *in vitro* culture, MSCs (20 million cells/mL) were photoencapsulated in hydrogels solutions of 2 wt%, 64kDa methacrylated HA or 10 wt% PEG 4600 diacrylate, containing 0.05 wt% Irgacure 2959. Hydrogels were then cultured in chondrogenic media for 14 days. Cell pellets (750,000 cells per pellet) served as additional controls. Gene expression analysis (n=4) for GAPDH (housekeeping gene), type I and type II collagen, and aggrecan was performed after 3, 7, and 14 days of culture using real-time PCR. For histological analysis, hydrogel sections were stained with hematoxylin and eosin to observe the morphology and distribution of encapsulated cells. In addition, presence of chondroitin sulfate was detected using the Vectastain ABC and DAB Substrate for peroxidase kits (Vector Labs) with mouse monoclonal anti-chondroitin sulfate primary antibody (Sigma) and biotinylated anti-mouse IgM, μ chain specific secondary antibody (Vector Labs).

Results / Discussion: Due to its resistance to protein adsorption, relative inert interactions with human MSCs, and extensive investigation for cartilage regeneration[3], a PEG diacrylate hydrogel system was chosen as a basis of comparison to examine the effects of cellular interaction between MSCs and the HA hydrogel. Immunofluorescence staining of CD44 (Figure 1) demonstrates that this HA cell surface receptor is uniformly expressed in human MSCs and thus, provides a means for interaction with the HA hydrogels.

Relative gene expression shows a general increase in type II collagen and aggrecan and a decrease in type I collagen with culture time for both HA and PEG hydrogels in chondrogenic media. A similar trend is also observed when HA hydrogels are compared with their PEG counterparts (Figure 1). MSCs in HA hydrogels show a 155.0-fold and 8.6-fold increase in type II collagen and aggrecan expression over PEG hydrogels at day 14. Similarly, MSCs in HA hydrogels show a 19.1-fold and 2.9-fold increase in type II collagen and aggrecan expression over cell pellets at day 14 (results not shown).

Hematoxylin and eosin staining indicated good cell viability and a uniform cell distribution throughout both HA and PEG hydrogels. Immunohistochemistry staining exhibited deposition of chondroitin sulfate by cells throughout the hydrogel after 14 days of culture, where deposition in HA hydrogels was greater and more uniform than in PEG hydrogels.

Conclusions: Relative gene expression and immunohistological analysis indicate that the interaction between MSCs and the hydrogel can influence extracellular matrix protein expression and production in a hydrogel network. MSCs in HA hydrogels exhibited significant up-regulation of type II collagen and aggrecan when compared to their PEG counterparts and exhibited increased production of chondroitin sulfate, indicative of greater cartilaginous extracellular matrix production. This work illustrates the importance of cell/biomaterial interactions in stem cell-based tissue engineering approaches.

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References: (1) Knudson CB. Birth Defects Res C Embryo Today. 2003;69(2):174-96. (2) Chung C. Tissue Eng. 2006;12(9):2665-73. (3) Elisseeff J. J. Biomed. Mater. Res. 2000;51(2):164-71.

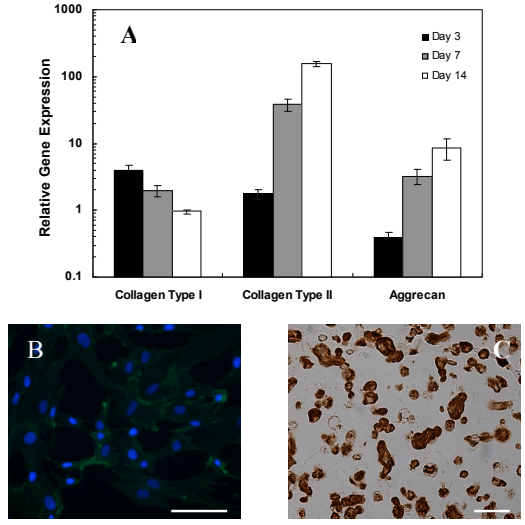

Figure 1: Relative gene expression of hMSCs in HA hydrogels calibrated with PEG hydrogels using GAPDH as a housekeeping gene (A). Immunofluorescence staining of CD44 (green) with nuclear staining (blue) on 2D cultured hMSCs (B). Chondroitin sulfate staining of the center of a HA hydrogel at Day 14 of *in vitro* culture (C). Scale bar = 100μm.