Effect of acid and sulfate functional groups on chondrogenic differentiation of mesenchymal stem cells

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Introduction: Extracellular matrix (ECM) plays an important role in controlling several critical functions of the cell. Capturing important aspects of the ECM in the design of synthetic materials is essential to better control the differentiation of human mesenchymal stem cells *in vitro*. Benoit et al [1] has recently shown that small functional groups found abundantly in ECM of fat tissue and bone, alone have the ability to induce adipogenic and osteogenic differentiation in hMSCs. In this study, we explored the chondrogenic inducing ability of carboxylic acid and sulfate functional groups on hMSCs. The effect of materials functionalized with these functional groups was tested in a 2D microarray fashion, enabling rapid evaluation of triplicates of 36 different cell-material interactions simultaneously. Cells were cultured on 2D microarrays of the functionalized materials and chondrogenic differentiation was tracked by immunostaining for aggrecan and CD105. 2D screening results indicate that relatively high concentrations of both carboxylic acid and sulfate functional groups induce higher aggrecan expression in hMSCs.

Methods: Monovinyl monomers of methacrylic acid for the carboxylic acid functionality and ammonium sulfatoethyl methacrylate for the sulfate functionality were used to introduce pendant functionalities in poly(ethylene) glycol dimethacrylate (PEGDM). Nanoliters of solutions of desired concentrations of functional monomers and PEGDM were deposited onto pre-treated, silane functionalized glass slides. A triplicate of 6X6 array of materials was created on a single glass slide using a VersArray Chip writer Pro System (BioRad labs, Hercules, CA, USA). Microarrays were photopolymerized under UV light and dip coated with poly(HEMA) to prevent cell attachment to regions other than the hydrogel microspots.

hMSCs were isolated from bone marrow (Cambrex) and cultured in normal growth media. Cells at passage 3 were seeded onto the microarrays and immunostained with Cy3 labeled anti-aggrecan antibody and Cy3 labeled anti-CD105 antibody at days 2 and 9. Nucleus staining was also done (Cy5 labeled) to normalize the data on a per cell basis. Immunostained cell-microarrays were scanned for fluorescence of the stained markers using a microarray scanner (Agilent, Santa Clara, CA, USA). All antibodies were purchased from Abcam.

Results and Discussion: The dual channel (Cy3 and Cy5) fluorescent intensity data was read out from each of the microspots using the scanner. Values are reported as an average of three samples per composition per time point, relative to nucleus staining fluorescent intensity, normalized to the expression of cells on pure PEGDM gels (control). A 2D differentiation map was created from the obtained fluorescence intensity data (Figure 1). These maps are similar to topographical contour maps.

Aggrecan expression is known to increase from day 2 to 9, during chondrogenic differentiation of hMSCs. By day 9, we observed the highest increase in aggrecan expression for COOH and SO_4 concentrations both greater than 20mM. Lower concentrations of both functionalities did not perform better than controls. CD105 levels were maintained at low levels at day 9. We observed only a slight increase in the levels of CD105 in most of the regions in the map. This slight increase was attributed to proliferation of a small fraction of hMSCs. Also, the highest increase in CD105 was observed only in the regions where aggrecan expression was the lowest. Our results indicate that higher concentrations of COOH

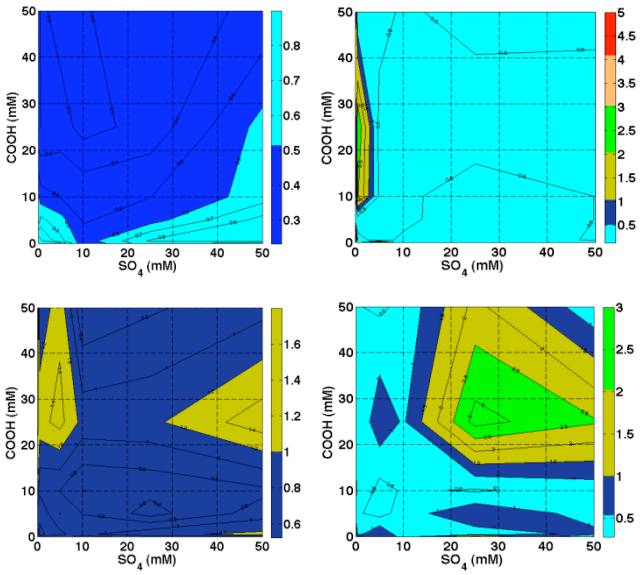

Figure 1: 2D differentiation maps plotting expression levels as a function of the gel composition. a,b- CD105 expression at day 2 and 9 respectively. c,d- Aggrecan expression at day 2 and 9 respectively.

and SO_4 may serve to induce chondrogenesis in hMSCs. The SO_4/COOH molar ratio in natural cartilage is roughly 1.3:1 [2]. The region in which we see the highest aggrecan expression includes this ratio. Current work is focused on further refining regions of interest and testing the translation of these 2D studies to 3D cell culture microenvironments.

Conclusions: A 2D microarray method was used to study the chondrogenic differenitiation inducing ability of acid and sulfate functional groups in hMSCs. Results indicate acid and sulfate functional groups alone have the ability to induce chondrogenesis in hMSCs and relatively high concentrations of the functional groups lead to increased chondrogenesis.

References:

1. (Benoit D.W. Nat. Mat. 2008;7:816-823)
2. (Asari A. J. Histochem. Cytochem. 1994:42;4:513-512)