Modification of 3D Agarose Gels with Biomimetic Ligands: Influences on Mesenchymal Progenitor Cells

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Introduction: Interactions with the extracellular matrix provide critical signals for growth and differentiation of mesenchymal progenitor cells. The purpose of this study was to engineer 3D agarose gels presenting various biomimetic ligands and to investigate the effects of these specific ligands on the chondrogenic differentiation of bone marrow stromal cells (BMSCs).

Methods: The linear peptide, GRGDSPC, targeting multiple integrins, and a non-adhesive control, GRGESC, were synthesized by Bachem. The triple helical, collagen mimetic peptide, containing the GFOGER sequence and targeting the α2β1 integrin, was synthesized by the Emory University Microchemical Facility (Reyes, 2003) and labeled with biotin. A recombinant mono-biotinylated fragment of human fibronectin ($FnIII^{7-10}$), targeting the α5β1 integrin, was expressed in JM109 bacterial cells and affinity purified (Petrie, 2006). The synthetic and recombinant peptides were conjugated to 3% SeaPrep agarose using the heterobifunctional cross-linker, sulfo-SANPAH (Dodla, 2006). RGE, RGD, and GFOGER were reacted at 0.1mM, and the $FnIII^{7-10}$ was reacted at 10µM. The conjugation efficiency and resulting peptide density were measured by dot blotting agarase-digested gels and probing for biotin. Bovine BMSCs were isolated from the bone marrow of an immature calf and expanded in the presence of 10% FBS and 1ng/ml b-FGF. BMSCs were seeded into cylindrical agarose gels (4mmØ x 3mm) that were unmodified or conjugated with RGE, RGD, GFOGER, or $FnIII^{7-10}$. Agarose gels were cultured for 7 days in basal, serum-free medium or in chondrogenic medium (basal + 10ng/ml TGF-β1 + 100nM dexamethasone). After 7 days, the gels were examined for accumulation of sulfated glycosaminoglycans (sGAG), DNA content, cell viability, and cell morphology. The effect of RGD density on cell morphology was also examined after 24h in gels with input concentrations of 0, 0.1, and 1mM RGD.

Results: The GFOGER peptide was conjugated to the agarose at approximately 25% (±5%SD) efficiency, and the $FnIII^{7-10}$ was conjugated at approximately 33% (±7%SD) efficiency (not shown). After 7 days in culture, there were no visible differences in cell viability between any of the peptide treatments, but there was reduced viability for BMSCs cultured in the basal medium compared to the chondrogenic medium (not shown). BMSCs seeded in the unmodified, RGE, RGD, and GFOGER gels had primarily round morphologies (Fig1). However, BMSCs in gels with higher RGD densities (1mM input) formed cytoskeletal projections after 24h (Fig3). Cells in the $FnIII^{7-10}$ modified gels had more spread morphologies with large cytoskeletal projections and actin stress fibers (Fig1). BMSCs in the $FnIII^{7-10}$ gels were aggregated in multi-cell clusters, and projections could be seen extending between clusters. The chondrogenic medium stimulated sGAG accumulation in each of the agarose gel types, and there were no significant differences between the groups (Fig2A). The chondrogenic medium also increased the DNA content in the gels over basal medium, and the $FnIII^{7-10}$ modified gels cultured in chondrogenic medium had higher DNA contents than all other conditions (Fig2B).

Discussion: This study demonstrated that the biomimetic ligands, RGD, GFOGER, and $FnIII^{7-10}$, could be conjugated to agarose hydrogels for studying the effects of 3D, cell-matrix interactions on the differentiation of BMSCs. The striking changes in cell morphology for BMSCs in $FnIII^{7-10}$ indicate that the protein is accessible and can influence cell function in 3D. Although the low densities of RGD and GFOGER did not affect cell morphology, increasing the input RGD density to 1mM induced spreading, suggesting that higher densities may be required for the short peptides to be accessible to the cells. In these experiments, the $FnIII^{7-10}$ significantly increased the DNA content but did not influence the chondrogenic stimulation of sGAG accumulation. These results suggest that adhesion to $FnIII^{7-10}$ may regulate BMSC proliferation but not chondrogenic differentiation. Additional studies will further investigate the effects of $FnIII^{7-10}$ on chondrogenesis, as well as the density dependent effects of the other biomimetic ligands.

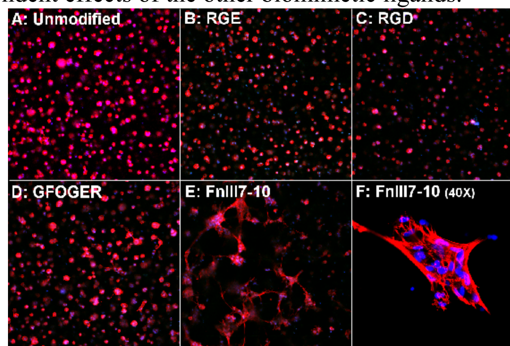

Figure 1: BMSCs in modified agarose gels after 7 days in chondrogenic medium. Red=f-actin. Blue =DNA.

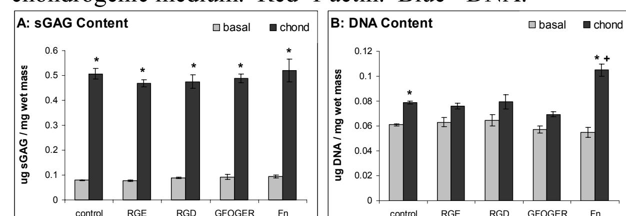

Figure 2: (A) sGAG accumulation and (B) DNA content within the agarose gels after 7 days in culture. n = 4/group, $*p<0.05$ with basal, $+p<0.05$ with control.

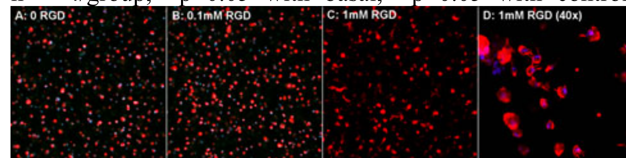

Figure 3: BMSCs in RGD-modified gels after 24h in chondrogenic medium. Red=f-actin. Blue=DNA.

References: [1]Reyes, *J Biomed Res A* 2003. [2]Petrie, *Biomaterials* 2006. [3]Dodla, *Biomaterials* 2006.