560g Differentiation Characteristics of Bone Marrow Derived Mesenchymal Stem Cells on Immobilized Glycosaminoglycans

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Proteoglycans in the extracellular matrix play an important role in determining the cell behavior such as proliferation, migration and differentiation and the glycosaminoglycan (GAG) side chains of proteoglycans are the primary determinants of their function. Therefore we hypothesized that the mesenchymal stem cell (MSC) proliferation and differentiation could be manipulated by adjusting the nature and composition of the extracellular matrix polysaccharide environment in vitro. In this study we explored the proliferation and differentiation responses of marrow-derived MSCs cultured on surface-grafted GAGs.

In order to obtain a variety of GAG surfaces for MSC culture, and differentiation, immobilization surfaces were formed in tissue culture plates by casting chitosan (poly-beta-1,4-N-acetylglucosamine) membranes. Various GAGs were then immobilized on the membranes by covalently linking the GAG carboxyl groups to the free amine groups on chitosan using EDC as the crosslinking reagent. Six different GAGs (heparin, heparan sulfate, dermatan sulfate and chondroitin sulfateA, chondroitin sulfate C and hyaluronan) were immobilized on chitosan membranes at GAG to chitosan mass ratios ranging from 0.04 to 1.0. Cell growth kinetics were quantified by cell counts and by measurement of metabolic activity using the MTT assay.

The results show a progression in cell response as a function of both GAG type and surface density, with heparin providing the most favorable environment for cell attachment and proliferation. In contrast, MSC growth was lowest on hyaluronic acid. For all GAGs, the proliferation rate increased with increasing GAG surface density. An ELISA-based evaluation of the relative levels of serum protein binding to the GAG surfaces indicated clear, positive correlations between vitronectin and fibronectin binding and the MSC growth responses. Adipogenic differentiation of MSCs on GAG-chitosan surfaces was also studied. Differentiation of the cells was assessed by Oil Red O staining and measurement of intracellular triglyceride concentration. It was found that a higher number of cells displayed cytoplasmic lipid accumulation on hyaluronan surfaces as a result of differentiation when compared to other GAG surfaces. This result, coupled with the lower proliferation rates and serum protein binding exhibited on hyaluronan surfaces, suggests a negative regulatory role for bound serum proteins with regard to MSC differentiation on immobilized GAG surfaces. However, differential GAG-growth factor binding or direct GAG-receptor interactions may still be significant in this system. Studies on the effects of GAG type and density on the osteogenic and chondrogenic differentiation of MSCs are underway and results will be reported.