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ENGINEERED GAG-BASED COATINGS FOR MESENCHYMAL STEM CELLS

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The therapeutic potency of delivered mesenchymal stem cells (MSCs) in tissue engineering applications may be improved by priming cells toward a differentiated state prior to implantation. Mimicking native extracellular matrix (ECM) interactions, the electrostatic attraction between negatively-charged glycosaminoglycans (GAGs such as heparin and chondroitin sulfate) and positively-charged proteins may act to locally sequester factors present in the culture media (or secreted by nearby cells) to further enhance stem cell response to soluble factors prior to cell transplantation for musculoskeletal disorders.

In these experiments, we desulfated the GAG heparin and heparin (Hep) and desulfated (Hep-) heparin were biotinylated via HOBt/EDC chemistry. The dimethyl methylene blue (DMMB) assay indicated full removal of sulfate groups in the desulfated heparin. Human mesenchymal stem cells (MSCs) were then coated as single cells in suspension with sulfo-NHS-biotin, avidin and heparin (Hep) or desulfated heparin (Hep-). Coated cells were then aggregated using a centrifugation method. Results demonstrate that MSC aggregates can be coated without negatively affecting cell viability and anti-inflammatory properties (assayed through a monocyte co-culture study; Hep coating only). Positively-charged proteins bind preferentially to the Hep coated cells and an assay with a reporter cell line suggests that the positively-charged transforming growth factor- β 1 (a known chondrogenic factor) remains bioactive after sequestration and release from coated cells.

In follow-on studies, cells were cultured in serum-free media either with or without the addition of 10ng/mL fibroblast growth factor-2 (FGF-2) and (media changes every 3 days). DNA quantification revealed that heparin-coated MSC aggregates increased in cell number around ~1.5-fold at day 7, while all other groups either decreased in DNA amount (noncoated and noncoated+FGF) or maintained cell number over time (desulfated heparin-coated+FGF). Previous studies have not demonstrated an increase in DNA content of MSC aggregates over time; thus, this novel finding can represent a new culture platform for MSC aggregates. Since only sulfated heparin-coated samples showed increased DNA amount over time, this suggests that there may be interactions between the negatively-charged sulfate groups on the fully-sulfated heparin with the added growth factor.

Taken together, these results suggest that GAG-based coatings may be an exciting means to locally sequester soluble factors and enhance MSC response to soluble factors, and that, through chemical removal of the sulfate groups, the scaffolds' interactions with growth factors may be tuned to promote optimal MSC response for specific culture conditions.