Heparin Cell Coating Effects on Cell Number During Mesenchymal Stem Cell Aggregate Culture with FGF-2

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Statement of Purpose: Mesenchymal stem cells (MSCs) are known to differentiate down multiple lineages as well as secrete trophic factors, thus making them a promising source in cell-based therapies for treatment of numerous pathologies and injuries. While MSCs can be formed into 3D aggregates for therapeutic use, it has been seen that aggregates decrease almost 50% in size over the course of 21 days in culture[1]. This compaction may be an indication or cytoskeleton and extracellular matrix rearrangement or of cell loss, which can lead to reduction in the aggregates' capacity to promote regeneration. We have previously seen that coating cells with a heparin derivative can maintain cell number during *in vitro* culture of MSC aggregates without addition of soluble growth factors (previous SFB 2015 abstract). To further characterize this coating, the objective of this study was to examine the effects of heparin-based coatings on cell number in aggregates when cultured in the presence fibroblast growth factor-2 (FGF-2). FGF-2 is often administered to MSCs during expansion due to its ability to maintain self-renewal properties and promote proliferation.[2] We hypothesize that the negative charge of heparin on cell surfaces can interact with the positively charged FGF-2, thus potentiating the effects of the growth factor and increasing MSC number during *in vitro* aggregate culture.

Methods: Human MSCs were coated as single cells in suspension with sulfo-NHS-biotin (4mM), avidin (0.5mg/mL) and biotinylated-heparin (Hep) or biotinylated-desulfated heparin (Hep-) (5mg/mL). Each layer was incubated for 30 minutes in rotary suspension. Once coated, 2000-cell aggregates were formed by forced aggregation of coated or noncoated cells in Pluronic-treated96-well V-bottom plates and cultured in serum-free MSC maintenance media either with or without the addition of 10ng/mL FGF-2 (media changes every 3 days). On days of sample collection, aggregates were fixed in 10% formalin and processed for sectioning. Samples were sectioned at 10µm and stained with hematoxylin and eosin or with an antibody to α-smooth muscle actin (α-SMA). Additionally, samples were collected for DNA quantification via the CyQUANT assay (n=4). Statistical significance was determined by a two-way ANOVA with Tukey's post hoc test ($p<0.05$).

Results: We have previously shown that fluorescently tagged heparin was observed on cell surfaces for at least 14 days in culture using layer-by-layer deposition[3] and that the heparin coating alone is a key component in cell number maintenance during MSC aggregate culture. In this study, hematoxylin and eosin staining revealed that heparin- and desulfated heparin-coated aggregates cultured with FGF-2 exhibited rounded cell morphology within the aggregate (white arrows), while this was not observed in noncoated aggregates (Fig 1A). Further immunohistochemical staining demonstrated that there appeared to be brighter staining of α-SMA around the border of the heparin- and desulfated heparin-coated aggregates when compared to both noncoated groups (Fig 1B). DNA quantification revealed cell number in heparin-coated MSC aggregates increased ~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 (Hep- + FGF) (Fig 1C).

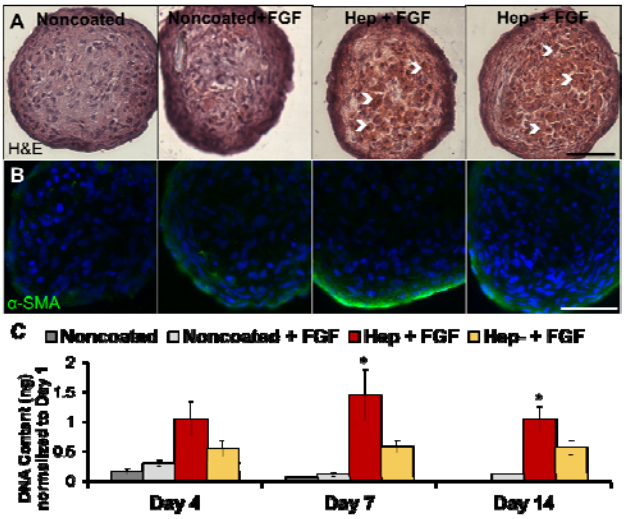

Figure 1. A) H&E staining of noncoated, noncoated +FGF, heparin-coated+FGF and desulfated heparin-coated+FGF aggregates at day 14, white arrows indicate rounded morphology, scale bar=100µm. B) α-SMA staining of aggregates at day 7, scale bar=100µm. C) DNA quantification of aggregates. * indicates significant difference from all other samples on the same day, $p<0.05$. n=4.

Conclusions: We have observed that, while both types of heparin coatings demonstrate morphological differences of MSCs within aggregates and had increased α-SMA staining on the aggregate periphery, only the sulfated heparin coating resulted in an increase in cell number over time when cultured in the presence of the proliferative growth factor FGF-2. Previous studies have not demonstrated an increase in DNA content of MSC aggregates 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. Interestingly, this increase in cell number occurred even in the presence of increased α-SMA deposition at the border, which may represent a tight cell periphery that would act as diffusional barrier of nutrient and growth factor exchange. While future studies are needed to better understand the mechanism by which this glycosaminoglycan coating affects cell number, early findings indicate that this coating can be used as a new technique to enhance the effects of soluble growth factors in culture of MSC aggregates. As such, these results may present a novel culture platform for expansion of MSC aggregates in serum-free conditions for future cell therapies.

References: [1]Sart S. Tissue Engineering B 2014 20(5). [2]Tsutsumi S. Biochem and Biophys Res Comm. 2001 288:413-419. [3]Lei J. Biomaterials Science 2014 2: 666.