Heparin Coating for Controlled Biomolecule Presentation to Mesenchymal Stem Cell Spheroids

Jennifer Lei[1], Louis McLane[2], Jennifer E. Curtis[2], Johnna S. Temenoff[3]
[1]School of Mechanical Engineering, Georgia Tech; [2]School of Physics, Georgia Tech; [3]Department of Biomedical Engineering, Georgia Tech/Emory University, Atlanta, GA

Statement of Purpose: Mesenchymal stem cells (MSCs) have been used as cell therapies to aid in regeneration of a variety of injured tissue. Moreover, MSCs aggregated into small spheroids have enhanced anti-inflammatory properties over single MSCs grown in monolayer[1]. However, MSC-based therapies may be rendered ineffective due to lack of control over cell fate post-injection. Therefore, we aim to develop a thin film coating for MSC spheroids to allow for simultaneous delivery of both cells and a soluble factor to direct cell differentiation. This system uses multilayer deposition of biotin and avidin to graft heparin onto cell surfaces[2]. Heparin is a negatively charged glycosaminoglycan that can sequester and release positively charged proteins[3]. The objectives of this study were to characterize the heparin coating and its effects on MSC viability and function, as well as the bioactivity of the growth factor (TGF-β1) subsequently sequestered on spheroid surfaces.

Methods: Biotin-heparin (heparin) and the model protein, histone (similar charge and MW as many growth factors), were fluorescently tagged for imaging. MSCs and mink lung epithelial cells (MLECs) were coated as single cells in suspension with sulfo-NHS-biotin (4mM), avidin (0.5mg/mL) and heparin (5µg/mL, 1mg/mL 5mg/mL). Each layer was incubated for 30 minutes with washing steps between layers. Once coated, 1000-cell MSC spheroids were formed using forced aggregation in AggreWells™. Imaging was performed using a Zeiss LSM 700 confocal microscope and analyzed in ImageJ. For particle exclusion assays, cells were seeded in monolayer, coated with heparin and incubated with 3µm polystyrene beads. Separation of beads from cell membrane surface was quantified as the pericellular matrix thickness. To examine protein bioactivity, MLECs were used as a biological reporter of TGF-β1 activity. MLECs are transfected with a luciferase reporter gene that is expressed in response to TGF-β1. MLECs were coated and loaded with 0pg/mL, 3000pg/mL or 3µg/mL TGF-β1 and formed into 500-cell spheroids. Control aggregates were loaded with protein without heparin coating. After 24 hours, aggregates and supernatant was collected. Cells were assayed for luminescence using ONE-Glo™ Luciferase assay buffer and supernatant was suspended over MLECs in a 96-well plate for 24 hours until luminescence was measured. Statistical significance was determined by one- or two-way ANOVA with Tukey's post hoc test ($p \leq 0.05$).

Results: MSCs were successfully coated using the multilayer technique to graft heparin onto the surface without affecting cell viability (data not shown). Results of the particle exclusion assay (data not shown) demonstrated that the heparin coating prevented pericellular matrix accumulation over 1 day, suggesting that this is a viable method to control loading and surface presentation of growth factors in the given timescale. A higher initial coating concentration resulted in more heparin on cell surfaces (Figure 1A). Over 14 days, fluorescent quantification showed a 40% loss of heparin for all three groups (Figure 1B). These results indicate that coatings can be tuned by using different initial concentrations. MLECs were used to study the bioactivity of sequestered and released TGF-β1. Without heparin, there was minimal protein localized to cell surfaces, however, when heparin was present the model protein histone is observed on cell surfaces (Figure 2A&B). Coated MLECs had significantly higher luminescence reported compared to noncoated samples at each concentration (Figure 2C). Supernatants taken from coated aggregates with TGF-β1 loading of 3000pg/mL and 3µg/mL elicited a luminescence response significantly higher than their noncoated counterparts (Figure 2D). These results suggest that a protein loaded onto these coatings remains bioactive after sequestration and release.

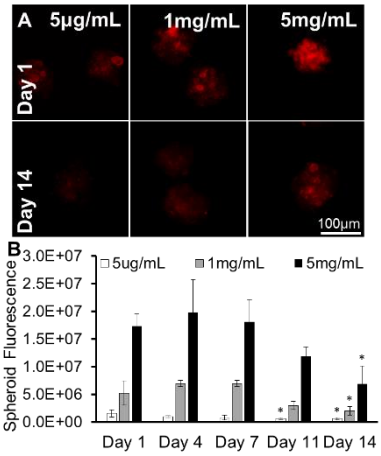

Figure 1. A) Flattened images of AF633-tagged heparin coating at 5µg/mL, 1mg/mL and 5mg/mL coating concentrations over 14 days. B) Quantification of fluorescence from tagged heparin over 14 days. *statistically different than day 1 levels at same concentration; $p<0.05$; n=10-12.

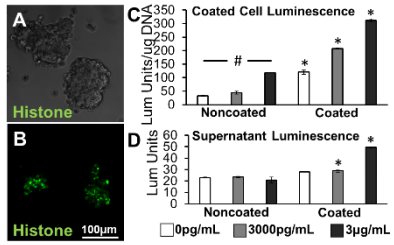

Figure 2. A) No FITC-tagged histone observed on control MLEC aggregates B) FITC-tagged histone loaded on heparin-coated aggregates C) Luminescence response of MLEC aggregates after TGF-β1 loading. D) Luminescence response resulting from released supernatant from MLEC aggregates. * statistically different from noncoated at same concentration; # statistically different from all other concentrations samples within same coating treatment; $p \leq 0.05$; n=4.

Conclusions: Through these studies, we have developed a multilayer coating system to graft heparin and facilitate protein sequestration onto MSC spheroid surfaces without loss in cell viability and while maintaining growth factor activity over 24 hours. In the future, this simple and efficient method of presenting growth factors to stem cell aggregates may have significant implications in enabling local signaling between transplanted cells and surrounding tissue post-injection.

References: [1]Bartosh TJ. PNAS. 2010 107: 13724; [2]Wilson JT. Acta Biomatl. 2010 6:1895; [3]Capila I. Angewandte Chemie. 2002 41: 390.