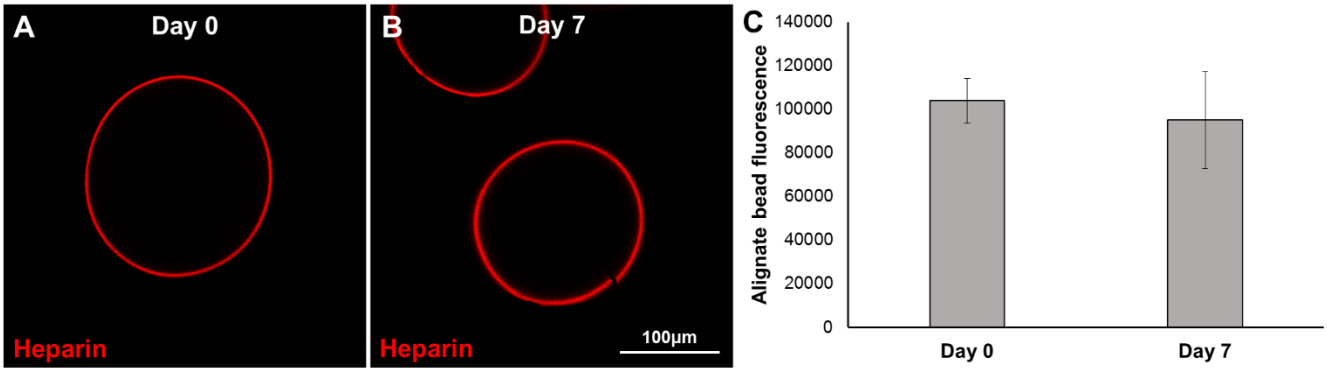
Supplemental Figure 1

Methods: Alginate beads were used as an acellular control for MSC spheroids due to their ease of formation and spherical shape. Alginate beads were made using the Nisco electrostatic bead generator, in which 3% alginate formed ~200 µm droplets in 100 mM calcium chloride. Beads were coated with 0.1% poly-L-lysine, followed by 4 mM EZ-Link Sulfo-NHS-LC-Biotin in PBS, followed by 0.5 mg/mL avidin in PBS, and, lastly, 5 mg/mL biotin-conjugated heparin in PBS. Each layer was incubated for 1 hour with washing steps between layers. The volume of 0.1 mL beads was incubated at 37ºC in MSC maintenance media (containing 10% FBS) replaced every other day. The heparin coating was imaged under the same imaging parameters (laser intensity, gain, and exposure time) via confocal microscopy immediately after coating (day 0) and at day 7. Fluorescent quantification was performed in ImageJ.

Results: After alginate beads were formed and coated, confocal imaging was used to image the heparin layer on the surface of the alginate beads. Immediately after coating, heparin was seen around the bead surfaces. After 7 days in MSC maintenance media, beads were imaged and heparin was still observed on the surface of beads with no statistical difference in fluorescence from day 0.

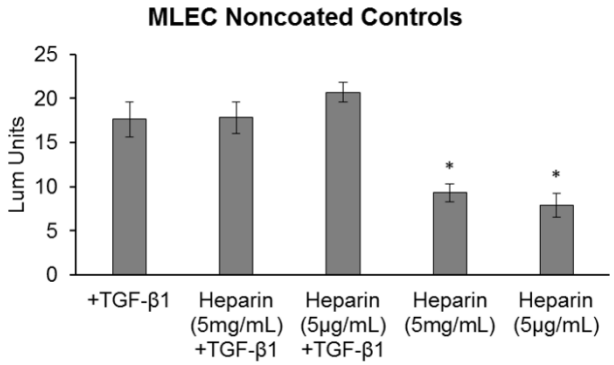


Supplemental Figure 1. Alginate bead heparin coating in media. A) Heparin seen in red on alginate bead surfaces immediately after coating procedure (day 0). B) Heparin on alginate bead surfaces after being cultured in MSC media for 7 days at 37ºC. C) Quantification of fluorescence on alginate bead surfaces over 7 days, $p < 0.05$, n=4.

Supplemental Figure 2.

Methods: Noncoated MLECs were cultured with soluble heparin (5 mg/mL or 5 μg/mL) and exposed to TGF-β1 (3 ng/mL) for 30 minutes to mimic the coating procedure used in this study, but using a non-modified heparin that should not attach to cell surfaces. Following the same procedure as outlined for experiments shown in Figure 4, cells were then formed into 500-cell aggregates. Subsequently, the aggregrates were cultured for 24 hours in media without heparin or with soluble heparin at 5 μg/mL or 5 mg/mL. After 24 hours in culture, cells were lysed with ONE-Glo™ Luciferase Assay buffer and luminescence was measured using a plate reader.

Results: It was observed that soluble heparin at both concentrations without any TGF-β1 stimuli resulted in significantly lower luminescence response when compared to the control group of 3 ng/mL TGF-β1 exposure. With TGF-β1 exposure, there was no difference observed in cells cultured with soluble heparin at either concentrationcompared to the control group. The resulting observations reveal that the luminescence response seen in Figure 4E is not a result of soluble heparin in solution.

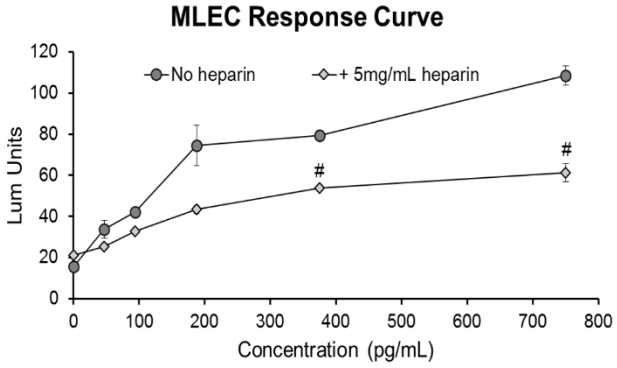


Supplemental Figure 2. MLEC aggregates without TGF-β1 exposure had significantly less luminescence response compared to control with TGF-β1 exposure. * Statistically different than TGF-β1 only control, $p < 0.05$, n=5.

Supplemental Figure 3.

Methods: The MLEC response curve was made by plating MLECs at 50,000 cells/cm^2 in 96-well plates and culturing them in media containing TGF-β1 ranging from 0 pg/mL to 750 pg/mL with or without 5 mg/mL heparin. This concentration was chosen based on the coating concentration of 5 mg/mL, representing the maximum amount of heparin that could be released into the supernatant from the coated surface. After 24 hours in culture, cells were lysed with ONE-Glo™ Luciferase Assay buffer and luminescence was measured using a plate reader.

Results: Higher luminescence levels were observed with higher concentrations of TGF-β1. Additionally, when heparin was is present in the media (5 mg/mL), the luminescence response was significantly lower at higher TGF-β1 concentrations (375 pg/mL and 750 pg/mL) when compared to samples without heparin in solution. These results confirm that the luminescence values reported in Figure 4F are not attributable to soluble heparin released from the cell coatings, even if all of heparin on the coatings was released within the 24 hour assay period.



Supplemental Figure 3. Luminescence response from noncoated plated MLECs when exposed to increasing TGF-β1 concentrations for 24 hours. # Statistically different than sample at same TGF-β1 concentration with no heparin, $p < 0.05$, n=3.