Accelerating Cellular Spheroid Fusion Using Magnetic Forces to Fabricate a Vascular Tissue

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Statement of Purpose: A critical process in the fabrication of complex tissue structures with cellular spheroids is related to their fusion [1,2]. Tissue fusion is a self-assembly process in which two or more distinct cell populations, or tissues, make contact and coalesce to form a single cohesive structure. Though the process is not clearly understood, research has shown that factors mediating tissue fusion include cell migration, cell-cell interactions, and cell-matrix interactions [1]. Tissue fusion is driven by minimizing the overall system configurational energy, which results in smaller and more cohesive tissue aggregates. Conventional tissue assembly and fabrication methods include cell printing, cell sheet techniques, and patterned molds [1]. These methods spatially orient the cells into a desired position through passive contact, but do not promote active contact for accelerated fusion of the tissue. The objective of this work is to determine what factors are critical to accelerate the fusion of magnetic cellular spheroids and to understand their fusion mechanisms. The hypothesis driving this work is that magnetic forces, cell-cell interactions and cell-matrix interactions regulate the fusion of magnetic cellular spheroids. Here, magnetic forces were used to not only pattern and align magnetic cellular spheroids, but to accelerate the fusion of spheroids through active contact. Our preliminary results demonstrate that cell density and collagen content influence tissue fusion. We also demonstrate the critical importance of magnetic forces for promoting active contacts for accelerated fusion [3].

Methods: Spheroid fusion was analyzed by tracking the fusion of spheroids patterned into a ring. Ring magnets were commercially purchased (2 mm OD, 1 mm ID, 1 mm thick, Super Magnet Man) and 25 individual Janus Magnetic Cellular Spheroids (JMCSs) were patterned around the rings in a monolayer formation [3]. A range of cell densities (5,000-100,000 cells per spheroid) and ECM contents (0 mg/mL – 0.3 mg/mL collagen type I) were used for ring fabrication. Magnets were kept in place for 48 hours and then removed, followed by imaging with a Nikon AZ100 multizoom microscope at respective time points. Capillary tubes (500 µm diameter, CTechGlass, CT95-02) were also used to quantify the influence of magnetic forces on cellular spheroid fusion with varying cell densities and collagen. JMCSs were assembled, placed into capillary tubes full of cell culture media and exposed to magnetic forces (K&J Magnetics, Inc., B881). Measurements of the length of the tissue constructs were made over time and normalized to initial lengths. In another study, rat aortic fibroblast, smooth muscle cell and endothelial cell solutions were fluorescently labeled using a Vybrant® CFDA SE Cell Tracer Kit (Invitrogen) prior to spheroid fabrication. Spheroids were patterned into tissue sheets and left to fuse for 24 hours. Next, the tissue strips were wrapped around a silicon tube with a magnet in the lumen and left to fuse for 24 hours prior to analysis. To mimic the structure of a blood vessel, the endothelial cell tissue sheet was wrapped first, followed by the smooth muscle cell tissue sheet and then the fibroblast sheet.

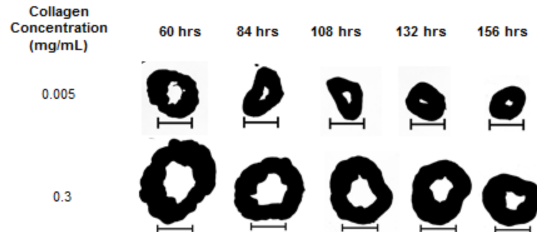

Figure 1: Fusion of tissue rings composed of magnetic cellular spheroids with varying collagen contents. Scale = 500 µm.

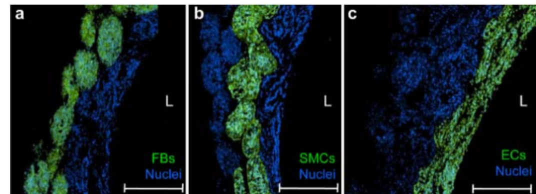

Figure 2: A fused trilayer tissue tube composed of (a) fibroblast, (b) smooth muscle cell and (c) endothelial cell magnetic cellular spheroids. Each individual cell type was fluorescently labeled and DAPI was used as a counterstain to visualize nuclei. Scale = 500 µm.

Results: The results of the ring fusion studies showed that lower cell densities and lower ECM contents allow for the most rapid and complete fusion over time. The fusion studies in capillary tubes demonstrated similar results, but further confirmed the need for magnetic forces to actively accelerate fusion of spheroids. Homogenous, cohesive tissue structures were formed from spheroids exposed to magnetic forces for 48 hours. In contrast, spheroids without magnetic forces experienced minimal fusion and would break apart from each other upon handling or removal from the capillary tubes. A trilayer tube that mimics the structure of native blood vessels was successfully fabricated using JMCSs. Each cell type was fluorescently labeled to demonstrate control over spatial orientation.

Conclusions: In conclusion, we have demonstrated that cell density, ECM content and magnetic forces are critical to the fusion of magnetic cellular spheroids. Studies showed that minimal fusion occurred between JMCSs without magnetic forces and spheroids without MNPs. Results suggest that magnetic forces can be used to actively promote enhanced contacts between cellular spheroids, ultimately leading to a more cohesive and homogenous structure. This fundamental understanding is expected to provide a strong theoretical and methodological foundation for the development of new tissue engineering technologies.

References:

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