Three-Dimensional Morphological Quantification to Understand Vasculogenesis

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Statement of Purpose: Our research focuses on the differentiation of human mesenchymal stem cells (hMSCs) embedded in PEGylated fibrin gels as a three-dimensional (3D) environment for the quantitative study of angio- and vasculogenesis. We recently developed a novel 3D quantification method to better characterize the branching morphology of our vascular networks[1]. Here, we aim to examine the relationship between biomechanical properties of our gels (rheology), the secretory profiles of the hMSCs in these gels (vascular endothelial growth factor, VEGF, production), and subsequent morphological development (confocal Z-stacks). We also used soluble Concanavalin A, a lectin known to increase extracellular matrix degradation and VEGF production, to explore these perturbations as well.

Methods: Gels were made based on our previously reported method[2]. Briefly, hMSCs were seeded onto collagen-coated Cytodex beads, embedded in fibrin or PEGylated fibrin gels and cultured for seven days. Samples for 3D analysis were stained with Calcein AM fluorescent dye and confocal Z-stacks were obtained. Supernatants were collected for secreted VEGF quantification via ELISA and normalized to cell viability data from an MTT assay. Rheological gel samples were prepared in a 40mm diameter mold for the plate-plate configuration and strain sweeps were measured from 0.1-2.5% at 15rad/s.

Results: Throughout our studies, we observed a significant increase in cell proliferation in fibrin gels compared to PEGylated (data not shown), with a similar but lesser trend across decreasing fibrinogen concentration. Both rheological and normalized VEGF expression data revealed no significant difference between PEGylated fibrin and fibrin groups. Instead, significance was universally observed between low and high concentration gels.

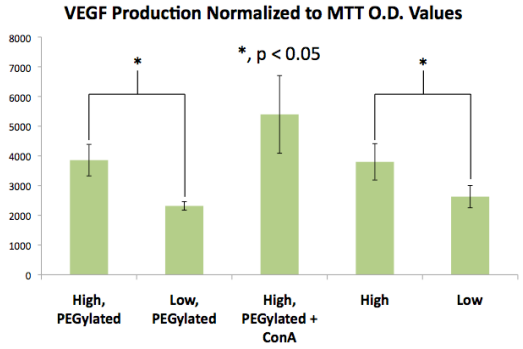

Figure 1. *Y-axis values are a ratio of VEGF (pg) to adjusted O.D. values obtained ($OD_{570nm} - OD_{650nm}$). Data labels: High, 10mg/mL Fgn; Low, 5mg/mL Fgn.*

Gel Formulation	G' (Pa)	G'' (Pa)
High, PEGylated	116.22 ± 8.02	4.75 ± 2.39
Low, PEGylated	45.98 ± 4.93	4.81 ± 1.81
High	131.16 ± 22.51	18.50 ± 3.43
Low	41.18 ± 1.39	5.99 ± 0.35

Table 1. *Rheology data reported as storage modulus (G') and loss modulus (G'').*

The ConA group showed a trend towards increased VEGF secretion but the result was not statistically significant for these replicates. Morphologically, though, we observed a vast increase in network extent. The extent of branching among other groups was inversely related to VEGF levels, as increased VEGF is traditionally linked to increased network development; the exception, again, is the ConA group where branching and VEGF are positively correlated.

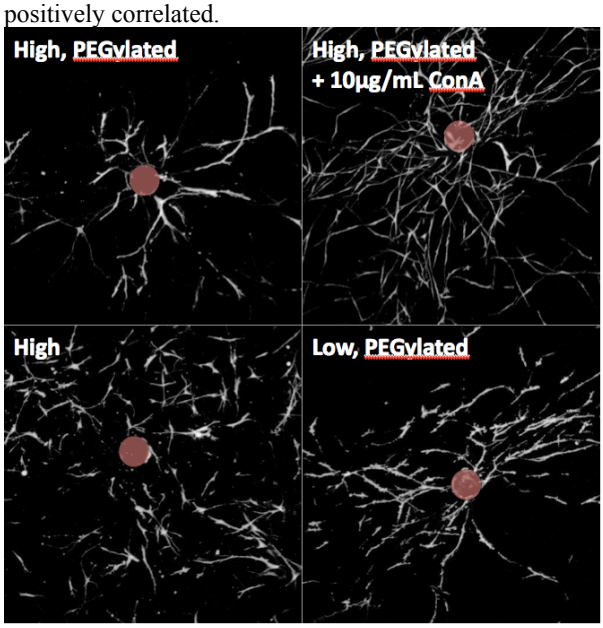

Figure 2. *Z-projection of confocal image stacks. Superimposed red dot indicates location of bead.*

Conclusions: Based on the biomechanical and secretory profile data, we would conclude that PEGylation of fibrinogen does not substantially alter the biomechanical properties of the gel. Morphologically, however, clear differences are evident. In fibrin-only groups, hMSCs completely migrate off of the bead and fail to form linked networks; PEGylated fibrin groups, on the other hand, successfully form vascular networks in a fibrinogen concentration-dependent manner. Thus, 3D morphology of our cells introduces new context for data interpretation, which is quantified through our confocal Z-stacks. In addition, ConA successfully perturbed the cells as noted in the increased VEGF and vascular development, highlighting the importance of the VEGF-MMP2 pathway in our system. Future work aims to compare the biology and morphology of our angiogenic model (with Cytodex beads) to our vasculogenic model (without beads) in hope of clarifying mechanistic and VEGF-MMP2 pathway-related details.

References: 1. Rytlewski J. Biomedical Engr Society Annual Meeting, Oct 2010. 2. Zhang G. Acta Biomaterialia, 2010;(6):3395-403.