A Biomimetic Hydrogel System to Study Tumor Angiogenesis

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Statement of Purpose: Blood vessel recruitment to a tumor and the role the microenvironment plays in this process are not well understood. Thus the goal of this research is to build a model of tumor angiogenesis in a tunable biomimetic matrix that enables the incorporation of desired bioactivity and mechanical properties allowing for a high degree of control over cell-matrix interactions. The tumor component of the model is composed of a murine model cell line of metastatic lung adenocarcinoma derived from subcutaneous metastases (344SQ) that forms spherical lumenized epithelial structures *in vitro* when cultured in 3 dimensions and responds to TGF-β1 by undergoing an epithelial-to-mesenchymal transition (EMT) and losing epithelial structure[1]. The angiogenesis component of the model is composed of human umbilical vein endothelial cells (HUVEC) and human pericytes (HP) that spontaneously form tubule structures that mimic nascent vasculature when cultured in bioactive proteolytically degradable poly(ethylene glycol) (PEG) hydrogels[2].

Methods: To understand the angiogenic factor release profile of the cancer cells, VEGF secretion from 344SQ after treatment with 5 ng/mL TGF-β1 was assessed via ELISA. Next, cells were encapsulated in PEG hydrogels that were rendered bioactive by reacting monoacrylate-PEG succinimidyl carboxymethyl with peptides: RGDS for cell adhesion and GGGPQGIWQGK (PQ) for cell-mediated proteolytic degradation and thus migration through the hydrogel. Cancer Gels: 344SQ were encapsulated alone to study their morphology when cultured in hydrogels. With 5% PEG-PQ-PEG serving as the backbone and 3.5 mM PEG-RGDS, 344SQ were incorporated at 1.5 x10^6 cells/mL into the pre-polymer suspension, which was crosslinked with white light using eosin Y as the photoinitiator (Fig.1B, top). Angiogenic Gels: Utilizing the same encapsulation methods as in the cancer gels, HUVEC and HP were incorporated into the polymer pre-polymer solution at 30 x10^6 cells/mL in a 4:1 ratio, respectively (Fig 1B, middle). Dual Gels: To study the interactions of the cancer cells with angiogenic cells, two pre-polymer solutions, one with angiogenic cells and one with cancer cells, were photopolymerized in contact with each other (Fig. 1B, bottom). Imaging was performed on a Zeiss Axiovert 135 inverted fluorescent microscope and a Zeiss LIVE 5 Confocal microscope.

Results: The results of the VEGF ELISA revealed a 12-fold increase in the VEGF secretion of 344SQ when treated with TGF-β1, indicating a pro-angiogenic phenotype following EMT (Fig. 1A). In cancer gels, 344SQ formed spherical lumenized epithelial aggregates that stained positively for polarity markers (Fig. 1C). In angiogenic gels, tubule formation was observed (Fig. 1D). In dual gels, cells from the vessel gel invaded the cancer gel and appeared to penetrate 344SQ spheres at the gel interface (Fig. 1E).

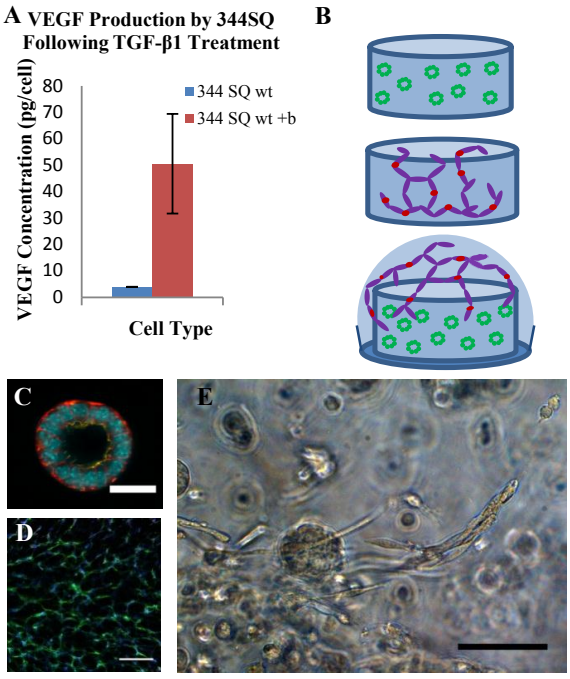

Figure 1. (A) VEGF secretion from 344SQ, (B) schematic of cancer gels (top, 344SQ=green), angiogenic gels, (middle, HUVEC=purple, pericytes=red) and dual gels (bottom), (C) lumenized sphere of 344SQ in a cancer gel stained with polarity markers, ZO-1 (yellow) and β-catenin (red) (sb = 20 μm), (D) angiogenic gel with cells forming tubules stained with PECAM (green) and DAPI (blue) (sb = 200 μm), and (E) phase contrast image of dual gels showing angiogenic cells invading a cancer gel (sb = 100 μm).

Conclusions: The increased VEGF production from 344SQ after EMT induction supports the notion that these cells are pro-angiogenic, making them interesting to study in a tumor angiogenesis model. Furthermore, when brought into contact, cells of the vasculature have an affinity for interacting with cancer cells in a biomimetic PEG matrix. Thus, this tumor angiogenesis model serves as a platform with which the vessel recruitment capacity of cancer cells of varying metastatic capacities can be assessed. Future work includes treating the cells of the dual gel with TGF-β1 and evaluating changes in their interactions. Ongoing work also includes incorporating different adenocarcinoma model cell lines into the model in order to further elucidate the role of angiogenesis as a pre-malignant process.

References: 1. Gibbons, DL. Genes and Dev. 2009; 23: 2140-2151.
2. Moon, J. J. Biomater. 2010; 31: 3840-3847.