Sphere-Templated Hydrogels for 3D Observation of Prostate Cancer Cell Growth and Tumor Formation

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Statement of Purpose: Prostate cancer is the leading cause of cancer death among men, and according to the American Cancer Society, about one in six men will be diagnosed with prostate cancer during his lifetime. The study of a disease as complex as prostate cancer requires a tumor model that can accurately represent growth, development, and metastasis. These parameters are defined by the local tumor microenvironment. The extracellular matrix (ECM) components of that microenvironment are particularly critical in the regulation of tumor progression, as changes in expression levels of ECM proteins such as laminin have been shown to correspond to increased rates of cellular detachment and migration. In addition, soluble growth factors can also have strong effects on tumor properties. Thus, it is of clear interest to develop a tumor model using an inert scaffold such that tumor development is influenced only by the cells within it. An inert scaffold would allow for precise manipulation of the tumor microenvironment in a controlled way through cell line comparison. Tumor models should re-create conditions in three dimensions that capture accurate representations of cell proliferation, signaling, and cell-matrix interactions. Additionally, the model should mimic the overarching tumor architecture, which features a luminal center and some degree of angiogenesis for material transport. Not only could a 3D tumor model be used to study tumorigenesis, it could also be used to evaluate cancer therapeutics under physiologically relevant conditions. Here, we propose a 3D porous poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel as an inert scaffold for a prostate cancer model. We chose pHEMA due to its well-characterized biocompatibility. Our lab has shown previously that a precisely tuned pore size can be used to enhance angiogenesis *in vivo* within biomaterial implants. Thus, our porous pHEMA system would serve as an ideal prostate cancer tumor model because of its controllable and pro-angiogenic microenvironment.

Methods: Sphere-templated scaffolds were prepared by first sintering closely-packed spherical, monodisperse poly(methyl methacrylate) (PMMA) beads. HEMA was then polymerized and cross-linked around the sintered beads. Collagen was embedded in the pHEMA as a cell attachment substrate. The polymer was washed with dichloromethane to remove the PMMA beads, leaving a network of spherical pores interconnected at the regions where beads were sintered together. The scaffolds were sterilized in ethanol before being re-hydrated and punched into 8 mm discs with a thickness of 1 mm for *in vitro* studies. Scaffolds were pre-incubated in media for one hour at 37ºC prior to cell seeding. M12 prostate cancer epithelial cells and C4-2 LNCaP subline cells were from Dr. Stephen Plymate. Cells were seeded into the scaffolds by repeated application of capillary force, layering 50 µL at a time of a $1x10^7$ cells/mL suspension on top of each scaffold and allowing capillary forces to draw the cells into the pores. Cells within the scaffolds were cultured for up to 14 days before fixation and histological processing. Alamar Blue® (AB) (AbD Serotec) was used as an indicator of cell metabolic activity for preliminary proliferation studies. To test whether cells were altering their microenvironment, sections of paraffin-processed materials were stained for laminin-1 (AbCam), a common basement membrane constituent whose expression is changed in prostate cancer cells.

Results: Capillary force seeding allowed for better cell distribution within our scaffolds when compared to centrifugation, controlled release of IGF-1 for chemotaxis, and vacuum seeding methods. Seeding success depended most strongly on pore throat diameter, which was a function of PMMA bead sintering time. The optimal pore throat size for seeding was cell-line dependent based on cell diameter. M12 and C4-2 cells attached and spread along the pore walls (see Fig. 1).

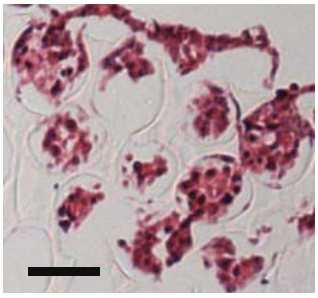

Figure 1 Histological section showing 7 days growth of M12 cells seeded by capillary force within a sphere-templated pHEMA/collagen scaffold with 80 µm pores and stained with hematoxylin and eosin (scale bar 80 µm)

AB proliferation studies indicated an increase in viable cell number for M12 and C4-2 cells grown for seven days after seeding, and a decrease in cell number between seven and fourteen days. Preliminary immunohistochemistry (IHC) results suggested that both cell lines were depositing laminin-1 within their immediate pore microenvironment.

Conclusions: Sphere-templated pHEMA scaffolds are promising for use as a 3D xenograft model to study prostate cancer due to their inert, controllable composition and potential to induce angiogenesis. We have demonstrated capillary force seeding as an effective method to distribute prostate cancer epithelial cells within the scaffolds. M12 and C4-2 cells attach after seeding and show evidence of modifying their microenvironment through deposition of laminin-1. Both cell lines also display a proliferation pattern consistent with existing models for 3D cancer cultures. IHC confirmation of differential ECM component expression in the cellular microenvironment based on seeding modified cell lines will allow for future studies of the effect of these changes on tumorigenesis *in vivo*.