Bacterial Cellulose as a Potential Scaffold for an *in vitro* Cancer Tumor Model

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Statement of Purpose: Recent advances in tissue engineering have allowed for the formation of three-dimensional (3D) cellular co-cultures that are invaluable for studying the specific response of cancer in a controlled environment. A 3D *in vitro* cancer tumor model would provide significant insight into the mechanisms that direct tumorigenesis and the role of environmental factors on cancer growth. The objective of this study was to analyze the use of bacterial cellulose as a potential scaffold for a novel tissue engineered *in vitro* tumor model. The model consists of a 3D, cancerous tumor grown around an endothelialized scaffold in a perfusion bioreactor. Selection of an appropriate scaffold material to facilitate cancer growth and tumorigenesis is an essential component of the design. A scaffold consisting of a tubular vascular graft is needed which can withstand physiological pressures and support the attachment, viability, and proliferation of endothelial cells on the lumenal surface and cancer cells on the outer surface. This model will support the growth of a variety of cells to allow for the study of tumorigenesis in different types of cancer. Bacterial cellulose is a novel biomaterial proven to have adequate properties for use as a scaffold in both blood vessel and cartilage tissue engineering. It was selected as a potential scaffold for the *in vitro* cancer tumor model due to studies showing chondrocyte and smooth muscle cell adhesion and proliferation on bacterial cellulose (Bäckdahl, H. Biomaterials. 2006;27:2141-2149), (Svensson, A. Biomaterials. 2005;26:419-431). We hypothesize that human prostate cancer cells and murine renal cancer cells will adhere and proliferate on bacterial cellulose, expressing growth factors and angiogenic markers necessary to promote tumorigenesis. Results of the study will identify if bacterial cellulose is an appropriate scaffold for use in the *in vitro* tumor model.

Methods: Bacterial cellulose (BC) was synthesized by *Acetobacter xylinum* subsp. *sucrofermentas* BPR2001. A pellicle of bacterial cellulose was statically grown in corn steep liquid media using Roux flasks, purified by boiling, and steam sterilized (Bäckdahl, H. Biomaterials. 2006;27:2141-2149). Cell lines in this study include a human androgen-independent prostate cancer cell line, PC3, purchased from American Type Culture Collection (Manassas,VA) and a murine renal cancer cell line, RENCA, provided by Dr. Heather Hatcher (Wake Forest University Baptist Medical Center). Cell viability and proliferation were measured using an alamarBlue®assay (Invitrogen). Bacterial cellulose was cut into scaffolds of area 0.75cm^2, placed in 48 well non-treated tissue culture polystyrene plates, and seeded with 10,000 cells each of either RENCA or PC3. Cells were incubated with the reagent for 6 hours and proliferation was observed over 7 days. Cell adhesion and in growth on the bacterial cellulose were analyzed using field emission scanning electron microscopy (SEM) (Leo Zeiss 1550).

Results: Investigation of cell viability and proliferation on bacterial cellulose scaffolds revealed that PC3 cells grow more favorably than RENCA cells (Fig. 1).

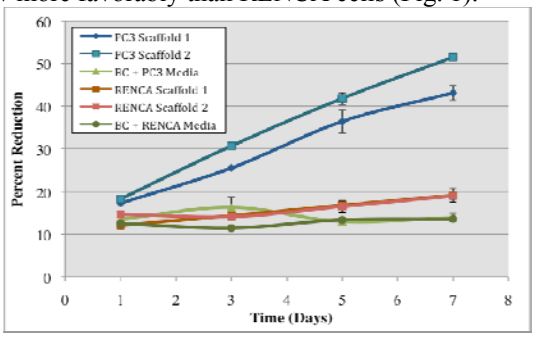

Figure 1. Cell Viability and Proliferation

The scaffold was non-toxic to both cell lines and amenable to cell adhesion. Over the duration of the experiment, both cells continued to grow and divide, reducing the dye as a function of time. PC3 cells reduced the dye at a greater rate than the RENCA cells, and the final percent reduction was more than double that of the RENCA cells. SEM of PC3 cells cultured on BC revealed the morphology of the cells and adhesion on the scaffold (Fig. 2).

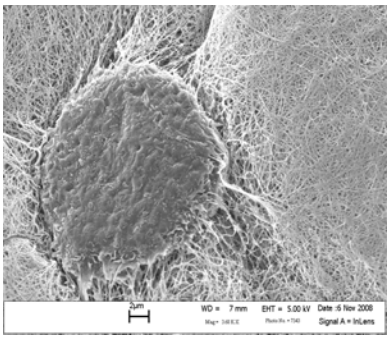

Figure 2. PC3 Adhesion on BC

Conclusions: Human prostate cancer cells showed significant proliferation on the BC scaffolds. SEM demonstrated that the cell type easily attaches to form functional cellular morphology for growth and proliferation. Murine renal cancer cells had a slower growth rate and showed less adhesion and proliferation on the scaffold. Possible modifications of the scaffold to encourage cellular adhesion and proliferation include pretreatment with laminin or fibronectin, prior to cell seeding. Additional cancer cell types, such as human breast cancer cells, will be examined to investigate the broad utility of BC as a scaffold for *in vitro* cancer growth. Verification that the BC scaffolds do not inhibit protein expression or angiogenic markers using qRT-PCR will further substantiate the scaffold as an appropriate conduit to facilitate 3D tissue growth in the perfusion bioreactor. It is important that cellular in-growth of the scaffold is sufficient for cell-cell communication and tumorigenesis pathways. This exploratory study will continue through investigation of other polymeric materials, such as electrospun poly(ε-caprolactone) and collagen type I fibers, and decellularized porcine carotid arteries as potential scaffolds for the *in vitro* tumor model.