Wicking Fiber Approach for Cell Separation and Isolation

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Statement of Purpose: A major challenge for developing tissue-engineered test systems for diseased microenvironments is the ability to identify and isolate pathological cells from patient biopsies or donors. Conventional approaches to identify normal or altered cell types involve detecting biomarkers and analyzing gene expression[1]. Cancer is a complex disease containing a heterogeneous population of altered cells with varying degrees of metastatic potential[2]. The use of biomarkers to identify cancer cells is unreliable, lacks specificity, and provides limited information regarding the metastatic potential of the cell[3].

Other less developed techniques to identify pathological cells based on cell function involve cellular filters or microfluidic systems to identify and isolate cancer cells. Cellular filters have low purity efficiency and microfluidic systems are limited by the specificity of the system, the amount of sample the system can analyze, and the ability to isolate viable cells.[4,5] We have developed a novel technique to identify and separate pathological cells using modified synthetically engineered wicking fibers, with non-circular cross-sections. Specifically, we developed a wicking fiber construct with optimal wicking properties that can separate and isolate viable cancer cells from a mixture of normal and cancer cells.

Methods: The transport properties of the wicking fiber construct, modified by proprietary methods, and unmodified wicking fibers were evaluated by comparing the rate of wicking and amount of fluid absorbed over time. The vertical wicking rate was determined by analyzing the change in height of the liquid front over time after placing the samples vertically in a well containing dye-solution. The volume of absorbed fluid was determined by measuring the amount of fluid remaining in the well after 1 hour. The custom designed wicking fiber construct was used to separate and isolate cancerous mammary epithelial cells, MCF-7 (ATCC), from a mixture of benign mammary epithelial cells MCF-10A (ATCC) and MCF-7 cells. To track the separation of the cell lines, benign and cancerous cells were stably transfected with Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP), respectively. The separation along the construct was evaluated at 3 hours, 24 hours, and 48 hours with fluorescent microscopy. After 24 hours the cells were removed from the constructs and the percentage of benign and cancerous cells were determined using Guava EasyCyte™ flow cytometry (Guava Technologies) by evaluating the percentage of red and green fluorescent count. Positive and negative controls of known cell densities were used to calibrate the machine before measuring the treatment groups.

Results: The modified wicking construct showed enhanced fluid transport properties. The rate of the fluid front moving vertically was significantly greater in modified samples. The amount of fluid absorbed after 12 hours and 24 hours was greater in modified wicking constructs compared to unmodified wicking constructs. Fluorescent images of MCF7-RFP and MCF10A-GFP demonstrate separation of cancerous cells along the construct. The percentage of isolated MCF7-RFP was significantly greater than MCF10A-GFP isolated from the modified wicking construct.

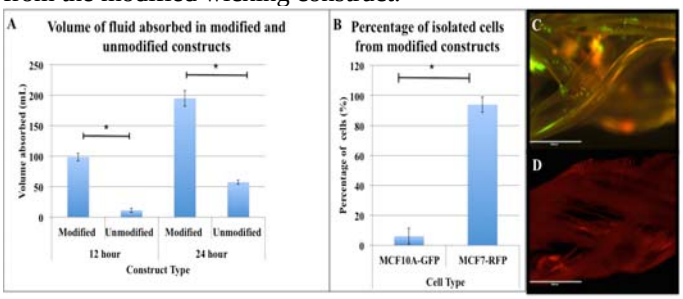

Figure 1. (A) Demonstrates the volume of fluid absorbed in modified and unmodified wicking fiber constructs, asterisksindicate significant differences ($p<0.05$) (B) Depicts a high percentage of cancerous MCF7-RFP cells are isolated from the constructs, asterisks indicate significant differences ($p<0.05$) (C) Fluorescent image illustrates a mixture of benign breast cells fluorescing green and cancerous breast cells fluorescing red initially along a region of the construct (D) Fluorescent image depicts separation of cancerous and benign breast cells, only showing MCF7 cells expressing red fluorescent protein on the construct

Conclusions: Our preliminary results indicate the modified wicking fiber construct has enhanced transport properties that can be used for cell separation of normal and pathological cells. The results suggest the device can separate and isolate pathological cells from a mixture of cells in solution with high purity and efficiency. This device provides a rapid and label-free approach to isolate various cell types for pathological tissue test system applications.

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