Test System for Modeling Cancer Cell Behavior as a Function of Substrate Stiffness

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Statement of Purpose: It has been well established that cancer development and tissue stiffness are correlated [1,2]. More recently it has been suggested that regions of high extra-cellular matrix (ECM) stiffness promote the growth of tumors (versus healthy tissue), specifically aggressive malignant tumors (versus benign cancer tissue) [3]. However, it is still not known whether a) a stiffer substrate attracts aggressive cancer cells or b) aggressive cancer cells modify the stiffness of their environment. The objective of this work is to develop a method for assessing cancer cell behavior and modeling its relationship to substrate stiffness.

Methods: MCF-7 (human adenocarcinoma line) cells were cultured within hydrogels composed of agarose (1% or 2% w/v) and collagen (2.9mg/mL) in a 1:1 ratio in 12-well plates. Cells were stained with a long-term fluorescence dye (CFSE) and imaged daily by fluorescence microscopy (n=3). Additionally, metabolic activity measurements were performed daily on separate samples (n=6) over the 14-day study. MCF-7 cells were subsequently seeded into a monolayer on hydrogels consisting of agarose (1, 1.25, 2, 2.25, 2.5, 3% w/v) and gelatin in a 1:1 ratio in 24-well plates (n=3). Following cell attachment, barrier hydrogels (2% w/v agarose) were added. The single monolayer of cells was imaged daily over two weeks via light microscopy in spatial reference to a marker dot on the bottom of each well. Image processing was performed to isolate cells and statistical image analysis was performed to gather information regarding cell density, position, and morphology.

Results: MCF-7 cells in suspension in 2% (strong) agarose/collagen gels demonstrated aggregate formation, as is common for the cell line in suspension culture. However, cells in the 1% (weak) gel failed to form aggregates. The 2% gel samples demonstrated rapidly increasing metabolic activity until stabilization around Day 6.

Within the cell monolayer experiment, exploratory data analysis suggests that the total coverage for the stiffer (higher agarose) gels tends to increase in time whereas that for the "weakest" gel remains relatively constant. In this preliminary analysis cell aggregation is quantified by the measured total coverage area for cell aggregates that are larger than the threshold of 15,000 pixels. This measure is referred to as *coverage area over threshold* (COT). The increase in COT between Days 6-8 is quite well modeled by a quadratic function of stiffness (agarose%) with a "multiple correlation" of R = .91(Figure 1). Also, COT is found to be a surprisingly good fit to a regression model involving stiffness, quadratic terms in time, and their statistical interactions:

$$COT=-111695+67945*Stiffness+2362*Day^2+4660*Stiffness*Day^2+Error$$

This model has a multiple correlation value of R = .89. While this is very encouraging, the model is based on assumptions (such as constant variances) which may not strictly hold, and there may be "overfitting" of the model due to the small amount of data on which it is based. However, it certainly suggests a starting point for future analyses. Modeling cell coverage across all days is extremely complex in view of day to day changes, especially because of medium exchange on certain days. This requires a more comprehensive model and of course more data for estimation of the effects.

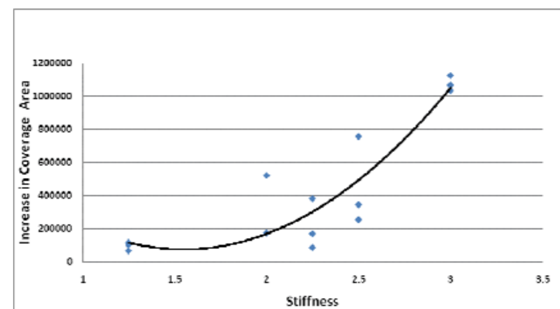

Figure 1. Increase in coverage area over threshold from Day 6 to 8.

Conclusions: Suspension culture of MCF-7 cells produced two cell types – large aggregates (in strong gel only), and single cells in suspension (in both weak and strong gels). Live/Dead staining (Day 14) demonstrated that both aggregates and single cells remained viable, but activity assays suggest that single cells are metabolically inactive. Substrate stiffness was shown to have a strong influence on cancer cell fate. Image processing techniques and data analysis methods were effective in producing spatial and temporal quantitative data which can be used to analyze and model cell behavior as a response to stiffness. The model is intended to measure and predict factors such as aggregate growth rate, migration, and cell:cell and cell:matrix binding, as a function of substrate stiffness. Future work will include spatial quantitative measurements of cellular metabolism and viability for correlation to morphology, as well as measurement along substrates with non-uniform stiffness to predict the migration of cancer cells *in vivo*.

References:

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