U251 Glioblastoma Multiforme Durotaxis

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Introduction

- Glioblastoma multiforme (GBM) comprises 77% of all malignant brain tumors[1] and usually results in death in the first 15 months after diagnosis[2].
- Glioma cell motility must be understood to develop treatments to limit the spread of GBM in the brain.
- The motor clutch model has been used to relate cellular migration to a force balance through a simple mechanism of molecular clutches and motors.
- 10 cell types have been observed to display durotaxis, which is the preferential migration in the direction of increasing substrate stiffness.
- It is hypothesized that U251 glioma cells will exhibit durotaxis due to a force balance posited by the motor clutch model.

Methods

Creation of Gradient Substrates

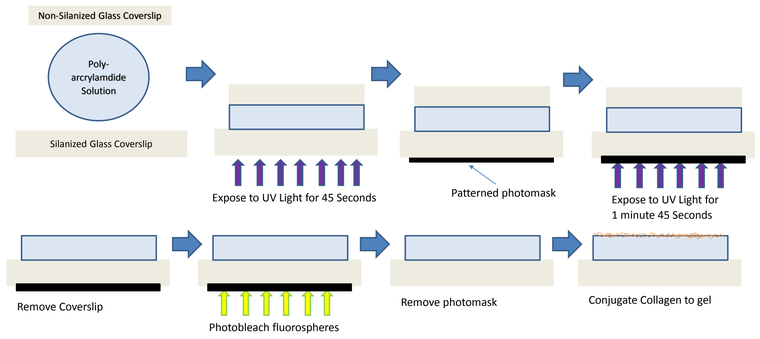

Figure 1. Photopolymerization method for creation of stiffness gradients in polyacrylamide hydrogels.

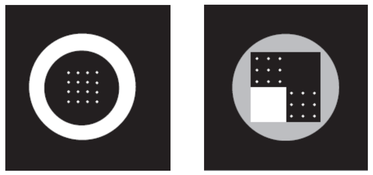

Figure 2. Photomask patterns used for gradient gel creation, viewed with GFP bead fluorescence.

Computational Modeling

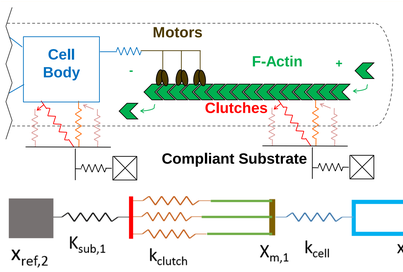

Figure 2. Diagram of motor clutch model for cellular traction[1]. Myosin motors pull on F-Actin filaments, which transmit force to the substrate through molecular clutches.

Figure 3. Diagram of the cell schematic used in the stochastic cell migration model.

A two-dimensional migration model created by a member of the lab group was modified to function in one dimension with a stiffness gradient. The cell clutches were modeled as a collection of springs pulled on by myosin motors towards the center of the cell[4]. Clutch binding and unbinding events were determined using a Gillespie algorithm, where Equation 1 was used to determine the time to the next event.

$$t_{event,i} = \frac{-\ln(URN_i)}{k_i} \quad (1)$$

A periodic Gaussian function was used to create a two-fold stiffness gradient over ~100 um.

Results

Gradient Gel Cell Experiments

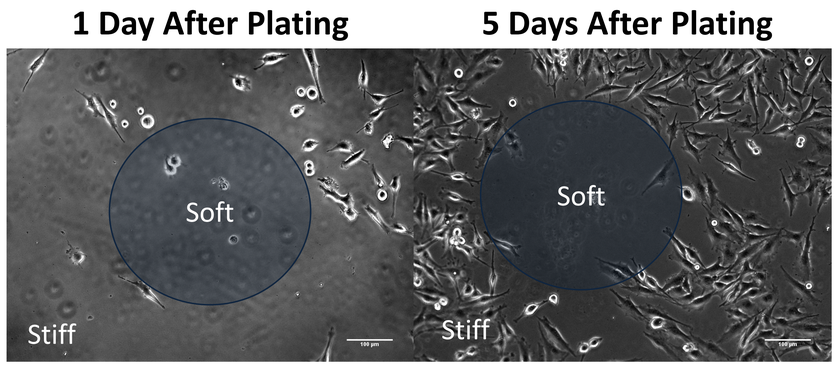

Figure 4. Images of gradient region with a soft island in a stiff (~20 kPa) sea.

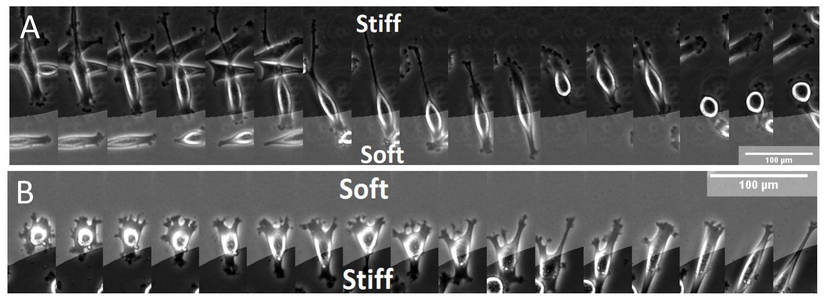

Figure 5A. Kymograph of U251 cell approaching stiffness gradient from the stiff side (time step = 15 minutes). Stiff = ~15 kPa. Figure 5B. Kymograph of U251 cell approaches stiffness gradient from the soft side (time step = 15 minutes). Stiff = ~15 kPa.

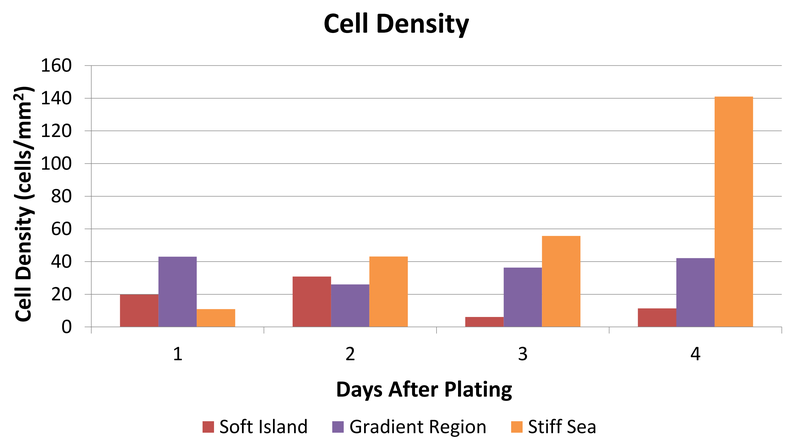

Figure 6. Cell accumulation after plating on a substrate with a stiff sea stiffness of ~20 kPa.

Results (cont.)

Computational Modeling

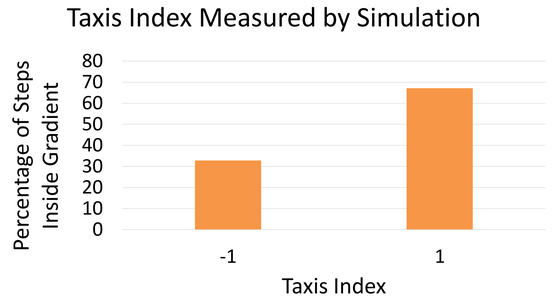

Figure 7. Taxis index, calculated as the ratio of the displacement for each time step to the ratio of the displacement in the direction of the gradient, for 24 simulations.

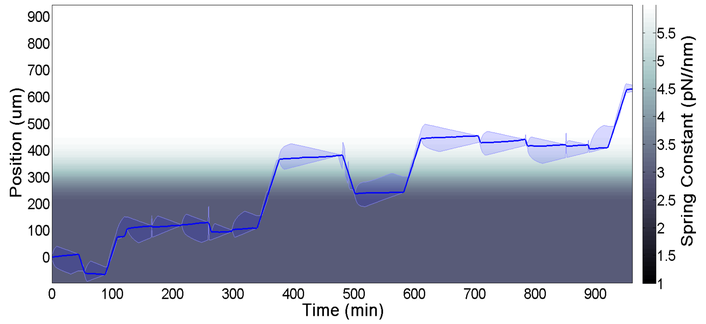

Figure 8. Simulated glioma cell migration in one dimension over a modified Gaussian stiffness gradient.

Conclusions

- U251 glioma cells preferentially migrated in the direction of preferential stiffness and accumulated on stiff regions of the substrate.
- The motor-clutch model based 1D migration simulator predicted biased movement in the direction of increasing stiffness.
- Unpublished work from our lab suggests there exists an optimal stiffness for traction force generation in U251 cells. Future experiments aim to determine whether U251 cells migrate towards the optimum stiffness or always in the direction of increasing stiffness.

References

I thank Ben Bangasser for creating the stochastic motor-clutch cell migration model which was adapted for this study and for his patience with answering my never-ending questions. In addition, I thank Professor David Odde for his guidance on the project and trust in letting a freshman work in his lab. Finally, I thank Ghaidan Shamsan for gel stiffness measurements. In addition, this work was partially funded by an Undergraduate Research Opportunities Program grant.

1. Wax, A. (2012, May 7). Brain Cancer and Gliomas. Retrieved February 22, 2013, from WebMD: http://www.webmd.com/cancer/brain-cancer/malignant-gliomas
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