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*Image Analysis of Collective Cell Migration Through Posts**Usama Ismail***ABSTRACT:**

The relationship between the presence of an obstacle in a cell's migration path, in this case a series of posts, is attempted to be correlated with the migration speed of a cell sheet as they pass through the posts. Using photolithographic methods, a post-laden substrate was previously constructed in the lab. Cell sheet was imaged through a microscope, as it migrated through the post structure. Using image analysis techniques, it was found that the average cell migration speed for six different post sizes, 25x25, 50x25, 50x50, 50x100, 100x25, and 500x25 microns, did not differ significantly. This lack of difference among post sizes suggest either that the experimental set up is flawed and thus will not show the correlation between post size and cell speed, or that the grouped cells have the ability to seamlessly navigate physical obstacles of different sizes. These questions will continue to be resolved through ongoing research in the Pathak Lab.

INTRODUCTION:

The extracellular matrix (ECM), and more importantly, how the cells interact with this environment, is a key factor to understanding cell migration. The ECM is defined as the material that surrounds the cells. It is primarily composed of different proteins and sugars that make the scaffolding which supports cellular structure. The ECM is crucial to cell migration as it allows cells to adhere to it. The ECM not only provides the structure for the cells to move on, but also provides a traction for their movement. Structures in the ECM can help to signal cells how and where to move in order to maintain a state of homeostasis (1).

It is important to understand how a migration sheet of epithelial layer navigates physical obstacles. The Pathak Lab has engineered obstacles in the form of posts in a device. Two-dimensional single cells migration occurs in three distinct steps. The first of these steps is known as the protrusion or extension phase of cellular movement. In this phase, the front of the cell protrudes filaments known as Lamellipodia or Filopodia into the ECM. These extrusions serve to create attachments between the cell and the ECM, creating a mechanical connection through which forces can propagate and cause movement. These extensions into the ECM are almost always coupled with actin polymerization which aids in the adherence of the cell to the ECM. The second step is the generation of the contractile forces that will push the cell along the matrix. This is done primarily through the contraction of the actin filaments connecting the cell to the ECM. This contraction allows the cell to move forward, almost as if it were travelling on tracks. Finally, the third and final step is that the rear of the cell release the adhesions it has created and either reuses them to move the cell forward more or degrades them to stop movement. One of the most important conclusions from this model of cell movement is that cells must be polarized to move. In other words, they must have a distinct front, center, and rear. (2)

However, the model of cell movement listed above applies to single cell movement in two dimensions and not the behavior of cells that move collectively, as they might in an epithelial monolayer (3). There are three general modes of collective cell migration: sheet migration, sprouting and branching. The mode of most interest to this study is sheet migration. Put simply, sheet migration is the process through which the leading edge of a sheet of closely adhered cells senses a gap next to it in the ECM. As one side of the leading-edge cell does not have cells next to it, it becomes polarized and now has a distinct front and rear of the cell.

Freeing of the cells next to the open space and the subsequent polarization of the monolayer allows for cell migration (4).

It remains unknown how cell sheets navigate physical obstacles, posts on a substrate in this case. By examining whether the cells retain the properties and characteristics of epithelial cells or whether the cells by the posts revert to a single cell model could provide a framework for how cells move away from their native environments and into new ones of different topography.

A logical first step in trying to classify and uncover the migration behavior and interaction of the cells exposed to posts is to see how the presence of the posts will affect the velocity of the cells as they migrate through an otherwise homogenous environment. By understanding how the presence of the posts will affect migrations speeds the underlying forces and attractions that the posts change can begin to be elucidated.

METHODS:

The first step in conducting this study was creating negative silicon master plates of the posts using photolithography. Next a PDMS gel mixture was plated over the master and then removed allowing posts to be formed. A 20:1 ratio of base (Sylgard 184) to crosslinker was used when creating the gels.

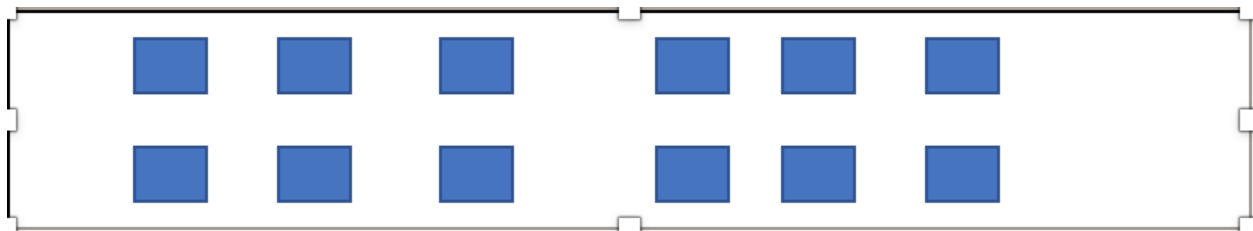


Figure 1. Post diagram and Location of Cell Seeding.

After the pattern was created with PDMS, a layer of collagen was applied to the gel. The gel and posts were laid out as shown in figure 1. With the blue rectangles representing the posts and the white space representing the PDMS/collagen on which the cells were seeded on and on which they migrated through. The cells, a secondary cell line of human mammary epithelial cells (MCF10A), were seeded in the middle of the two groups of posts and were allowed to migrate in either direction.

The migration of the cells was tracked using a 10x objective on the confocal microscope. With images being taken every hour and each set of posts was imaged for 90 hours. An example of the type of image captured is shown below in Figure 2 (5).

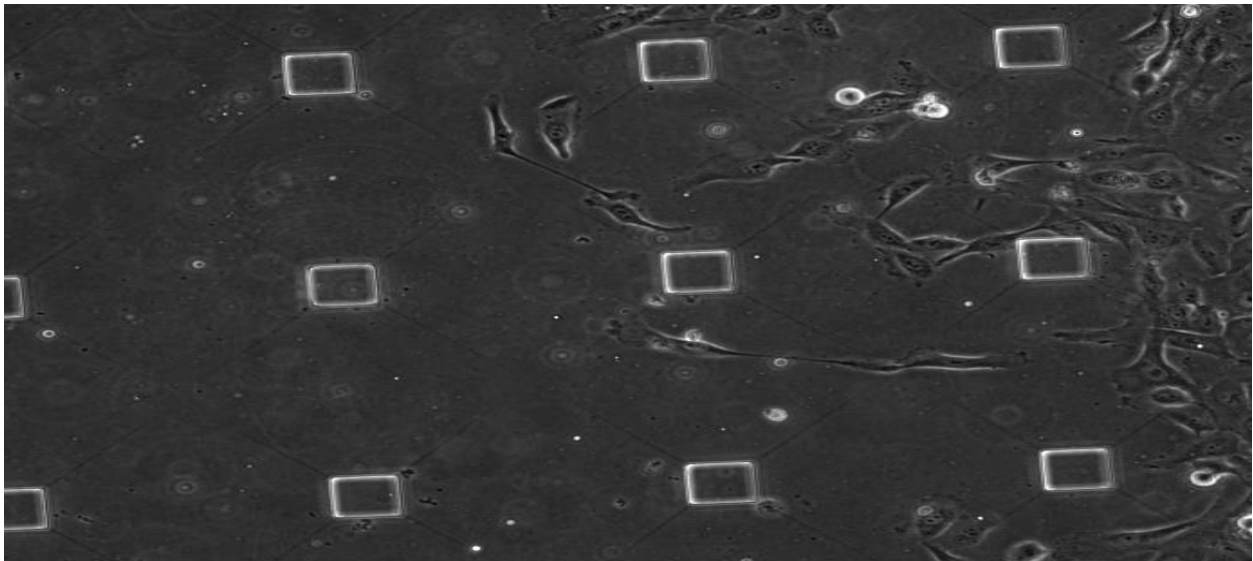


Figure 2. Example of image captured using the microscope.

The leading-edge velocity for the different post sizes imaged was calculated using ImageJ and Microsoft Excel software programs. The calculation of the leading-edge velocity was accomplished by using ImageJ's multipoint tool to mark the leading edge of the cells in frame. Then the leading-edge coordinates were taken for another frame. The center of mass of the two data sets were found and then subtracted from each other to find how far the cell front had

migrated between the two frames. This distance was then converted from pixels to microns and then divided by the time step, one hour per frame, thereby finding the velocity values for the cells.

The equation used to calculate the center of mass for a collection of point masses is:

$$X_{cm} = \frac{M_1X_1 + M_2X_2 + M_3X_3 + \dots + M_nX_n}{M_1 + M_2 + M_3 + \dots + M_n} \quad (1)$$

where M represents the mass of the point and X is its x-position in a Cartesian coordinate system. However, this equation was simplified by assuming that the mass of all the points recorded were equivalent. Thus, the center of mass equation simplified to

$$X_{cm} = \frac{X_1 + X_2 + X_3 + X_n}{n} \quad (2)$$

which is simply just the average of the points position coordinates.

The positions of the points that were recorded were before the leading edge passed through the first set of posts, after the first set, after the second set, after the third set, and if needed after the fourth set of posts.

Results:

They were five sizes of posts studied in this experiment (all sizes are in microns): 25x25, 50x25, 50x50, 50x100, 100x25, 500x25, where the first value is the width of the post and the second the length of the post perpendicular to the direction of cell migration. The averages of all the velocities found for each video associated with a specific post size and shape is shown below in

Figure 3. As it can be seen from the graph most of average velocity values fall between .003 microns/hr and .004 microns/hr.

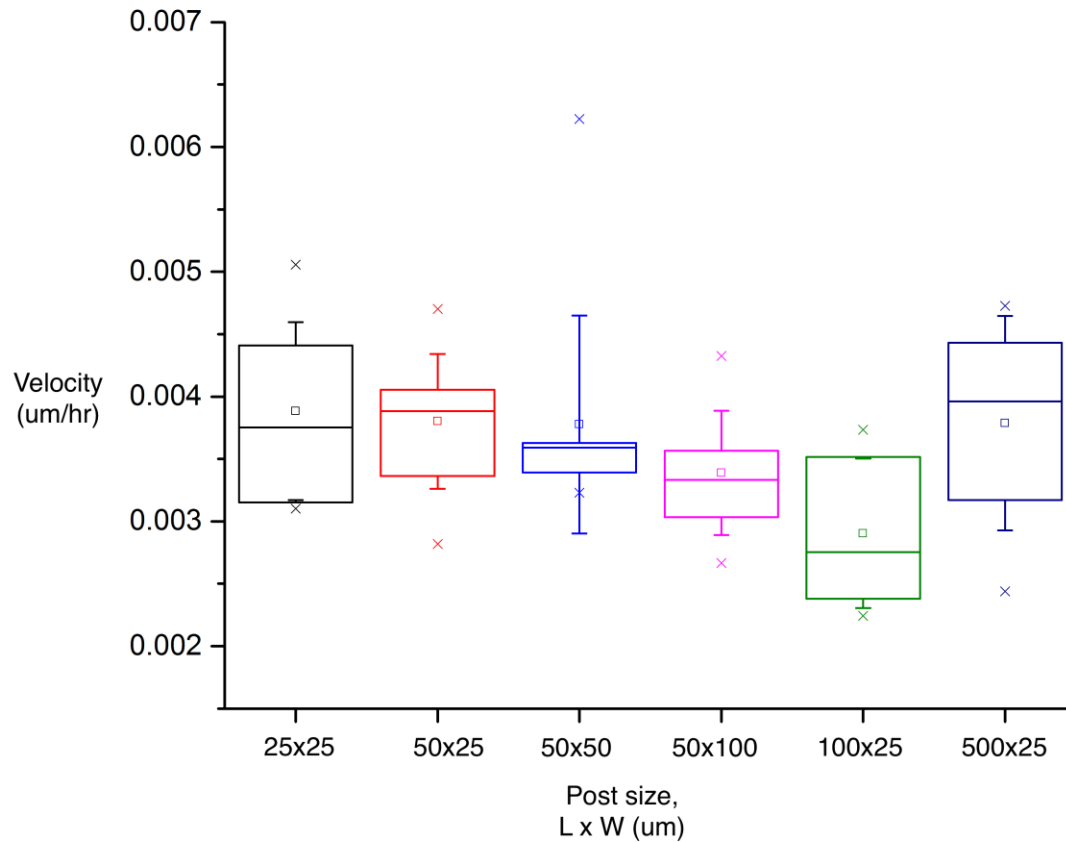


Figure 3. Box and Whisker Plot of Velocities of Cell Migration Through Varying Post Sizes. The small boxes in these plots represents the average velocity value for each post size, the main boxes represent the range into which most of the data lies, the small x's are the minimum and maximum value of the data, and the whiskers represent one standard deviation of the data.

DISCUSSION:

By examining the data from the plot, it can be seen that there is no real correlation between the size of the post and the velocity of the cells as they migrate through the posts. While, a slight downward trend can be seen in the velocities for the first 5 posts sizes no real

conclusion can be reached. The values of the data are too similar from post size to post size to definitively draw a conclusion about how post size affect migration speed.

This lack of definitive results can be due to a number of reasons. Chief among these is that the velocities calculated consider the position of all of the cells over time. It may be the case that only the cells that interact with and adhere to the posts have their velocities affected by them. By calculating an overall center of mass for the leading edge and using those coordinates to calculate velocity of the cells the number of points that are not affected by the posts outweigh those that are and as a result the migration speed more closely match cells that are not in contact with posts. Thus, the average velocity from post size to post size would be similar and would not especially show how cell migration speed is affected by differing post size.

If the above statements are true it could suggest that interacting with an obstacle like the posts polarizes the monolayer even further by separating the cells into those that have adhered to the posts and those that have migrated by the posts. This further classifies the cells along with the previous classification of leading edge cells and cells that are constrained in the monolayer. This could suggest that a cellular mechanism exists in epithelial cell layers that allow it to travel at a given overall speed even when hindered by obstacles in its migration path by changing the intercellular interactions between those cells that have attached to or interact with an obstacle and those cells that are free to move.

In order to further explain the calculations that were already made, a control plate should have been made to see how long the cells take to migrate across an identical PDMS gel while no posts were impeding its path. The lack of a control makes it difficult to know against what values the current velocity values should be calculated.

CONCLUSION:

Overall, no definitive conclusions can be made from the data that was collected and the procedures that were used in this study. The effect of post size on the migration speed of a cell monolayer could not be deduced from the methods used. Through this research, several potential questions are emerging. How do cells interact with one another as they migrate its way through a series of posts? Can cell navigate physical obstacles of different sizes without changing their speed?

Some changes should be made to the experimental procedure to better examine how velocity is affected by the posts. First, a gel of smaller area should be made around the post so as to maximize the possibility that the cells that are passing by the post are affected by it and therefore, increasing the possibility that there is a change in velocity compared to the normal migration speed. Second, the experimental set up should be revised so as to see how the imaging process or the image analysis process can be changes so as to look out for and record what cells interact with the posts and how their behavior is affected by this interaction. Third, a control plate and control velocity should be made so as to be able to quantify how the mere presence of a post will affect the velocity of the cells. Lastly, the process of finding a center of mass for the points on the leading edge for two different frames and using the distance in between them and the time-step in between the two frames them to calculation velocity should be examined. This method could unfairly weight cells that aren't affected by the posts and thus skew the data.

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