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Stiffness and Modulus and Independent Controllers of Breast Cancer Metastasis

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STIFFNESS AND MODULUS AND INDEPENDENT CONTROLLERS OF BREAST CANCER METASTASIS

A Thesis Presented

by

DANNIELLE ALEK'SANDRA RYMAN

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Molecular and Cellular Biology

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Molecular and Cellular Biology

DEDICATION

This work is dedicated to my mother Donna Lea' Spadachene, without her love, strength and determination I would not exist.

To my brother Nicholas Ryman and my PaPa for their love and support

ACKNOWLEDGEMENTS

First, I would like to thank and acknowledge my graduate advisor Dr. Shelly Peyton. I am eternally grateful to have had her as my advisor and mentor. Her passion and enthusiasm for scientific research is not only admirable but contagious. I was fortunate to be one of her first graduate students at the University of Massachusetts Amherst and to be able to participate and observe her and her lab develop over the years. I have tremendous respect for Dr. Peyton's willingness to help others and her unwavering drive to push her students to produce nothing but their best. I appreciate her guidance and for giving me the opportunity to grow as a researcher. I am grateful for her trust and given independence when needed. In addition, allowing me to mentor undergraduate researchers in her laboratory. Dr. Peyton had thankfully accepted me into her lab even with a non-engineering background which has given me the experience, passion and desire to continue biomedical engineering research. I am extremely grateful for the opportunities given and the experiences I have had working with her.

I am thankful for the relationships and collaborations I have developed with Dr. Crosby's lab in Polymer Science at The University of Massachusetts Amherst. Despite my learning curve in material science I was fortunate to have wonderful graduate students in his lab guiding me through my research endeavors. Thank you to Yuri Ebata especially for all her help, patience and friendship. Second, I would like to thank Yujie Liu for her help and dedication to the "Nano Lines" project. Last, I would like to thank Dr. Crosby for his mentoring and support throughout our collaborative projects, not only to myself but some of my undergraduates. I was fortunate to experience his kindness and knowledge and hope the relationships I have acquired with him and his lab members with continue in the years to come.

I have to acknowledge and thank all of the wonderful and talented undergraduates I have had the honor of working with and mentoring. I have had the pleasure of mentoring eight undergraduates with varying backgrounds and some were for summer internships whereas others were UMass regulars. I would especially like to thank Max Nowak, Aidan Gilchrist, and Prateek Katti, I was fortunate to mentor them the longest and they contributed the most to my lab and personal life. I am proud of them all and wish them nothing but the best for all their future endeavors.

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Thank you to all of my committee members Dr. Peyton, Dr. Crosby and Dr. Schneider for their support, patience and time.

ABSTRACT

STIFFNESS AND MODULUS AND INDEPENDENT CONTROLLERS OF BREAST CANCER METASTASIS

SEPTEMBER 2013

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One out of eight women in the United States will develop breast cancer during their lifetime. Ninety percent of cancer related deaths are due to metastasis. Metastasis is the biological process where individual or aggregate cancerous cells break away from the primary tumor site and colonize distant, non-adjacent locations throughout the body. It is my objectives to study how mechanical, topographical and biochemical cues affect metastatic breast cancer metastasis at an early developmental stage. ECM components have previously been shown to affect cell motility via ligand-receptor interactions, and physical cues, such as matrix stiffness and protein density. The primary tumor site significantly stiffens during tumor progression. The ability cells have to sense and respond to these matrix features influences and facilitates cell invasion. It is now widely accepted that mechanical properties of the ECM can regulate cell migration; however, presently, tissue *modulus* and *stiffness* have been used interchangeably. It is unknown if cell responses are sensitive to a bulk tissue modulus or stiffness on the geometric length scale of the cell. It is my objective to create tunable biomaterials from known materials to independently parse the roles of stiffness and modulus upon the migration of breast cancer cells.

I have created a variety of tunable biomaterials which I can parse the roles of mechanical properties and observe their affect upon cell mechanosensing. All systems were coated with collagen I, which is the most abundant ECM protein during tumor development. I was able to quantify the migration along with other parameters of the metastatic breast cancer cell line MDA-MB-231. My results show that the highly metastatic MDA-MB-231 is stiffness sensitive among all biomaterial models. Cells maximum cell speeds are at high concentrations of collagen I on the polymer microlenses and show a biphasic response dependent on stiffness. On poly (ethylene glycol)- 2-Methacryloyloxyethyl phosphorylcholine (PEG-PC) hydrogels cells favor intermediate modulus and show stiffness dependency at low protein concentrations. Cells on Cd/Se and polydimethylsiloxane (PDMS) samples are influenced by the topographical cue more so than the stiffness or modulus of the material. By controlling mechanosensing via force transduction signaling pathways, and determining the appropriate length-scale by which mechanical properties regulate cancer metastasis, I hope to eventually uncover novel therapeutics to block cell invasion.

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INTRODUCTION

Breast cancer is the second leading cause of cancer-related deaths in women, despite a steady decrease in death rates over the last twenty years (American Cancer Society, 2013). These improvements in survival rate can be attributed to a number of factors: more treatment options, an increased understanding of risk factors, an increase in frequency of screening, and the improvement of screening technology. These combine to give patients a five year relative survival rate of ninety-eight percent for localized breast cancer, yet it is metastasis of breast cancer, the spread of cancer cells throughout the body, that is the most prevalent obstacle in effective treatment (American Cancer Society, 2013). There is a need to better understand the process by which cells translocate from the primary tumor and spread to secondary locations of the body (Weigelt et al., 2005). It is essential to be able to study the basic principles behind metastasis in order to create more efficient methods of detection, prevention, and treatment.

The extracellular matrix (ECM) surrounding the tumor is responsible for many of the cues that act as invasive signals to the cancerous cells (Friedl et al., 2003; Friedl and Wolf, 2004; Wang et al., 2002). As the tumor progresses it alters the surrounding ECM, remodeling the deposition, composition, and reorganization of collagen fibers, aid in cancer proliferation and local invasiveness (Schedin and Keely, 2010). The process of cells sensing the mechanical properties of their environment which influences intercellular biochemical reactions is known as mechanotransduction. The modification of the ECM into a stiffer environment and subsequent mechanosensing results in the increased expression of genes identified as proliferation signatures in human breast

carcinoma (Provenzano et al., 2009). The study of mechanotransduction via mechanical cues and nanotopography is essential for understanding how the altered ECM affects breast cancer metastasis. The study of cancer metastasis and how mechanical, biochemical and topographical cues influence individual cells can yield insight to the *in vivo* phenomena; therefore, gaining more understanding behind the cause of breast cancer metastasis and potential therapeutics.

Cell Migration

Understanding the fundamentals of cell migration is important to know so one can understand how cells move in their surrounding environment. This can be broken down into three separate cellular components: the leading edge protrusion, adhesion of the leading edge and releasing of the trailing edge, and cytoskeletal contraction. Migration is accomplish by the organization of three distinct biopolymers that form the cytoskeleton: actin, microtubules and intermediate filaments. The process of movement begins with the reaction to an external signal such as a physical, chemical, diffusible or non-diffusible signal (Lauffenburger and Horwitz, 1996; Sheetz et al., 1998; Horwitz and Parsons, 1999; Lauffenburger and Wells, 2001).

When a signal is received, actin polymerization is prompted in the frontal region of the cell body. In combination with actin polymerization, focal adhesions are created Focal adhesions are pertinent to adhering the cell to the substrate. They originate as clusters of integrins which are directly tethered to the ECM proteins on the substrate. The actin polymerization force creates a protrusion. Once the leading edge makes contact with integrin-binding proteins on the substrate surface more focal adhesions are created. The focal adhesion sites at the trailing edge of the cell are dissembled. This activity creates a rigid leading edge, with a trailing edge that is rapidly losing rigidity due

to disassembly of actin bundles. The tension between the leading and trailing edge results in the contraction of the cytoskeleton, moving the cell forward in the direction of the leading edge (Ananthakrishnan and Ehrlicher, 2007).

Mechanotransduction

A cell has the capability to respond to its physical, mechanical environment, using cues from adjacent cells, external stress, and the ECM to turn mechanical signals into active biochemical signaling pathways. This feature is known as mechanotransduction and is accomplished using integrins, focal adhesions, and myosin-based contractility. All of these interact with extracellular signals and create a pathway for intracellular reaction (Chen 2008). The stiffness of the substrate can be sensed by cells and affect its morphology, proliferation, migration and differentiation, as suggested by several studies utilizing polyacrylamide gels and collagen gels (Peyton and Putnam, 2005; Engler et al., 2004).

Cells constantly probe the mechanical properties of their environment by adhering and contracting, sensing the resistance to induced deformations; this process creates mechanical signals that feedback and regulate cytosketal reorganization and actomyosin contractility which feed into the cells' ability to "mechanosense" the surrounding environment. This is dependent upon integrins and focal adhesions to interact with the mechanical properties of the substrate which they are upon (Discher et al., 2005). When a focal adhesion is formed, the force can influence the shape, size, and composition of the focal adhesion, resulting in either stabilized or destabilized protein-protein interactions, along with exposure of active sites (Galbraith et al., 2002). Focal adhesions, integrins, and cytosketal organization are essential to cell-matrix adhesion complexes, which are necessary for efficient cell migration and invasive behaviors of

metastatic cells. Since these factors can be induced without any soluble chemical stimuli, cell migration can be based exclusively on the mechanical compliance, or stiffness of the substrate. This phenomenon is called durotaxis or durokinesis, depending on if the substrate has a gradient and is likely connected to the ability of cancer cells to invade and become metastatic (Lock et al., 2008; Lo et al., 2000).

Tumor Progression

During the progression of a breast carcinoma, the surrounding extracellular matrix undergoes transformation. As the tumor develops, the ECM deposition, composition, and organization are all altered. While it is not clear whether or not matrix stiffening causes cancer formation, it is apparent that stiffening of the ECM occurs alongside the carcinoma. This stiffening is associated with a change in the ECM deposition, specifically the concentration of collagen V is increased which alters the structure of collagen I fibrils (Barsky et al., 1982; Berendsen et al., 2006; Breuls et al., 2009). There is an increase in collagen signaling and an alteration in the formation of collagen fibers results in a mechanically stiffer ECM (Paszek et al., 2005; Provenzano et al., 2009). This remodeling of collagen fibers and consequent stiffening of the ECM has been shown to increase proliferation and local invasiveness of cells (Schedin and Keely, 2010). The progression of a tumor can be determined based on local stiffness variations and from ECM reorganization. For example, the identification of tumor progression can be made from the surrounding collagen fibers.

There are several distinct forms of collagen organization collectively known as Tumor Associated Collagen Signatures (TACS) that correspond to varying phases of the tumor (Provenzano et al., 2006). TACS-1: During the initial phases of tumor progression, when the tumor size is minimal, the collagen structure is unorganized and not

polymerized; however there is an increase in collagen density around the tumor. Within this global increase of collagen there are also pockets of local high density collagen near the surface (Provenzano et al, 2006). TACS-2: As the tumor size increases the collagen fibers stretched around the tumor and become more rigid. The fibers are straight, stretched, and aligned towards the tumor surface. It is believed this structure constrains sections of the tumor via compression restraint as well as produce stimuli that activate fibroblasts (Provenzano et al., 2006). TACS-3: The tumor mass contains invasive properties such as activated fibroblasts along with further tumor growth. The tumor begins to form an irregular shape with collagen fibers arranged in a radial pattern from the tumor boundary. These fibers point in the direction where invasiveness is observed (Provenzano et al., 2006). During TACS-3 collective and single cell migration is observed (Hegerfeldt et al., 2002; Friedl et al., 2004; Friedl and Wolf, 2003; Wang et al., 2002).

Stiffness and Modulus

In order to quantify substrate rigidity, measurements of Young's modulus or bulk modulus are taken through methods such as compression testing. Young's modulus is the ratio of stress along a given axis to the strain along that same axis. Stress is the force per unit area, while the strain is the change in length of the object divided by the original length of the object. Young's modulus is therefore given in units of pressure. Bulk modulus is the ratio of differential change in pressure to the differential change in volume all multiplied by the opposite of the volume and is also given in units of pressure. Although they are useful in describing the physical systems being examined to understand the role of rigidity in cancer and migration, both of these measurements fail to take into account the local differences in material properties, like living tissue. More importantly, they also do not account for the heterogeneity of composite materials like

organs. They have significant limitations in their description of ECM compliance on a cellular level. Unlike modulus, which is an intrinsic material property, stiffness is geometrically dependent and can therefore more accurately describe the mechanical properties of the tumor microenvironment, as seen in work conducted by Provenzano et al. localized collagen fibers contribute to stiffening. Stiffness is defined as the resistance of an object to deformation along a given degree of freedom and given in units of force per length. When used in literature, however, the term “stiffness” is often used to describe compliance or rigidity. Changes in the material modulus can result in proportional changes in stiffness when the geometry remains constant. Literature uses these two terms interchangeably and further misperceives our understanding of the role of modulus and stiffness in cell-material mechanosensing.

Work by Buxboim et al. supports this notion of a difference between stiffness and modulus (Buxboim et al, 2010). Using polyacrylamide gels filled with monodispersed silica microbeads, Buxboim created thin gels on top of functionalized glass coverslips. Despite making gels of the same composition and thus modulus, Buxboim found that cell spreading area varied based on the height of the gel, and thus the stiffness. Cells generally spread more and are more contractile on substrates with higher moduli, and these same phenotypes were observed on thinner gels of the same modulus, suggesting that it is stiffness rather than modulus which controls cell behavior. Table 1 highlights the different modulus and stiffness values from tissue samples and previous research conducted on various materials.

Tested Sample	Testing Method	Measured Parameter	Measured value	Stiffness *
Mammary Gland[1]	Unconfined Compression Test	Elastic Modulus of Normal to Premalignant to Invasive Cancer	200~2000 Pa	0.004-0.04 N/m
Human Breast Carcinoma[2]	MR elastography (MRE)	Elastic Modulus	60-100 Pa	1.2-2 N/m
Normal Breast Tissue Active State [3]	Indentation, MRI	Elastic Modulus	0.4-2 kPa 4-12 kPa	0.008-0.04 N/m 0.08-0.24 N/m
PEGDMA gels[4]	Tensile Compression Test	Tensile Modulus	13.7-423.9 kPa	0.06-2 N/m
Polyacrylamide gels[5]	Compression Test	Young's Modulus	1.0-308 kPa	0.06-0.62 N/m

Table 1: Modulus and Stiffness Values of Biological and Material Samples

[1]Levental, K. R., Yu, H., Kass, L., Lakins, J. N., Egeblad, M., Erler, J. T., Fong, S. F. T., et al. (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell, 139(5), 891-906. Elsevier Ltd. doi:10.1016/j.cell.2009.10.027

[2]Van Houten, E. E. W.; Doyley, M. M.; Kennedy, F. E.; Weaver, J. B.; Paulsen, K. D. Initial in vivo experience with steady-state subzone-based MR elastography of the human breast. *Journal of Magnetic Resonance Imaging* 2003, 1, 72-85.

[3]Engler, A. J., Sen, S., Sweeney, H. L., & Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. Cell, 126(4), 677-89. doi:10.1016/j.cell.2006.06.044

[4]Peyton, S. R., Raub, C. B., Keschrumrus, V. P., & Putnam, A. J. (2006). The use of poly(ethylene glycol) hydrogels to investigate the impact of ECM chemistry and mechanics on smooth muscle cells. *Biomaterials*, *27*(28), 4881-93. doi:10.1016/j.biomaterials.2006.05.012

[5]Peyton, S. R., & Putnam, A. J. (2005). Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. *Journal of cellular physiology*, *204*(1), 198-209. doi:10.1002/jcp.20274

*Modulus and stiffness was added by authors. Modulus value was multiplied by the cell diameter or testing probe diameter.

CHAPTER 2

OBJECTIVES

The objective of my thesis was to develop highly tunable biomaterials in which to quantify the independent effects of stiffness and modulus on breast cancer cell migration. First I sought to verify that stiffness and not solely modulus is the primary mechanical regulator of migration. This would expand on the implications of the work of Buxboim et al. studying cell spreading area using thin polyacrylamide gels into more clinically relevant phenomena such as migration. By using multiple protein concentrations, it would also be possible to show that the work by Peyton and Putnam on SMCs translates to breast cancer cells as well, and breast cancer cells also respond in a biphasic fashion to stiffness and the biphasic response shifts depending on cellular ligand availability and or protein concentration. I sought to create models with tunable and independent modulus and stiffness which has yet to be done in previous research. As a result I can identify length scales for which mechanical properties influence cell behavior. In addition, the objectives of these projects are to develop an understanding for cell-matrix interactions and to initiate inspiration for future biomaterials in order to bridge the gap between 1D, 2D and 3D models.

CHAPTER 3

PEG-PC HYDROGELS WITH TUNABLE MODULUS AND STIFFNESS WITH CONTROLLED HEIGHTS

Introduction

The first model system I created consists of poly (ethylene glycol) (PEG)-based hydrogel, (Herrick et al, 2013) with which I can control the hydrogel thickness (on the order of tens of microns), which regulates the stiffness of the system. Independent of the height, I can also tune the crosslinker concentration, which regulates the modulus of the system. The crosslinker content controls the hydrogel modulus, whereas the thickness of the gel overlaying a rigid glass coverslip controls the stiffness. This system is novel in its ability to control and tune stiffness and modulus independently; whereas previous work has only tuned one parameter or the other. In addition to controlling the stiffness and modulus collagen I was applied to the gels to enhance cell adhesion to the substrate. I used two concentrations of collagen I to further elucidate the relationship between protein density, stiffness and modulus and their independent effect upon cell migration.

Hypothesis

Similar to work done by Buxboim et al., I aimed to identify if cells can sense stiffness and modulus as separate mechanical properties. I hypothesize the biomaterials I use can independently tune and quantify stiffness and modulus and their effect upon cell migration. Our PEG-PC model is set up to tune both modulus and stiffness independently. I believe cell migration will be influenced by both mechanical properties and exhibit a biphasic response at intermediate to low modulus. As shown by Peyton et al., I believe our model will display similar trends. The biphasic curve will shift as a function of protein concentration where lower concentrations will exhibit peak

migration at stiff values whereas higher protein concentration will peak at lower stiffness (heights).

Methodology

Coverslip Preparation

To prepare methacrylate-functionalized coverslips, glass coverslips were placed into a UV ozone oven and cleaned for ten minutes in order to sterilize and expose hydroxyl groups. The coverslips were placed in a 95% ethanol solution at pH 5, adjusted by glacial acetic acid, to which 2% (V/V) 3-(trimethoxysilyl) propyl methacrylate was added. This mixture was stirred for two minutes, then rinsed three times thoroughly with 200-proof ethanol then incubated at 120°C for 15 minutes. The coverslips were stored in a desiccator if not used immediately. Preparation for Sigmacote® (Sigma-Aldrich) treated coverslips initially the coverslips were immersed in Sigmacote® solution and shaken for 20 minutes. They were then washed three times in 200-proof ethanol, then placed on aluminum foil and dried in an oven at 120°C for 15 minutes. These coverslips were also stored in a desiccator if not used immediately.

PEG-PC Preparation

The hydrogel solution was made by adding 20% (W/V) 2-Methacryloyloxyethyl phosphorylcholine (PC, Sigma-Aldrich) to 1X phosphate buffered saline (PBS, Sigma-Aldrich). Poly (ethylene glycol) dimethacrylate. (PEG, Sigma-Aldrich) was then added by volume to achieve the desired concentration. The entire solution was degassed under nitrogen, then combined with a radical solution of 10% (W/V) Igracure 2959 (BASF) in 140 proof ethanol. The radical and PEG-PC solutions were combined in a 1:16.5 (V/V) ratio. The hydrogel was then prepared by cross-linking the hydrogel solution between a

methacrylate and sigmacote coverslip separated by spacers. A methacrylate coverslip is placed on the bottom, two spacers (Kapton® polyimide film, DuPont) of the desired height are placed on either side of the slip, a sigmacote coverslip is placed on top, and the entire sandwich is clipped together using binder clips. Once the sandwich was prepared, the gel solution was pipetted between the two coverslips. The gel was polymerized under UV light for 7 minutes, then immediately placed in filtered PBS to allow for swelling overnight. If the sigmacote coverslip did not float off of its own accord, it was removed manually using tweezers to yield the complete the biomaterial system. (Figure 1. A) shows the experimental design of our PEG-PC with controlled height system. The final gel has a set height of 127µm. (Table 2) illustrates the mechanical testing done by Herrick et al. on PEG-PC sytem and the chosen PEG concentrations and their respective moduli.

A.

Figure 1: PEG-PC Experimental Design. (A) Photo taken of PEG-PC experimental design with treated coverslips. Final gel is set to be 127μm in height.

[PEG-PC] (wt %)	0.55	1.1	1.6
E (kPa)	0.9	2.8	8.2
SD (kPa)	0.2	0.8	1.5
N	7	6	9

Table 2: Mechanical testing of PEG concentrations (Herrick et al. 2013)

PEG-PC Protein Treatment

In order to prepare for protein absorption the methacrylate coverslips with gel adhered were epoxied (5 Minute Epoxy ®) into a 12-well plate and covered with 1X PBS. The gels were covered with 1mL per well of a 0.2mg/mL solution of sulfo-SANPAH (Thermo Scientific). The plates were then placed under ultraviolet light for 15 minutes. The gels were washed once with 50mM HEPES (Invitrogen) at pH 8.5, covered with fresh Sulfo-SANPAH solution, and UV treated for another 15 minutes. The gels were then washed three times with 1X PBS and covered with collagen I diluted in 0.02 M acetic acid at the desired theoretical concentration. The plates were placed in the fridge overnight to allow proteins absorption. The gels are then washed with sterile 1X PBS and allowed to sterilize in the tissue culture hood for 30 minutes. The gels are washed once with sterile 1X PBS, immersed in DMEM with 10% FBS and 1% PS, and incubated until cells are ready to be seeded into the plate.

Cell Culture

For all experimentation, MDA-MB-231immortalized breast carcinoma cells were used. The cells are obtained already transfected with cytosolic green fluorescence protein. They are cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin–streptomycin (PS) added, at 37°C and 5% CO2. Cells were obtained from Dr. Shannon Hughes, MIT.

Microscopy

Cells are seeded at a density of approximately 8,000 cells per well and allowed to adhere overnight prior to microscopy experiments. All experimentation used 10 x objectives on a Zeiss Axio Observer Z1 microscope with a motorized piezo X-Y stage and an incubation chamber (Carl Zeiss Ltd.). Prior to the setup of a migration experiment, the incubation chamber was set to 37°C and 5% CO2. Once the microscope incubation chamber was equilibrated, the plate was placed in the chamber and allowed to equilibrate once more. Time lapse are set up so that pictures were taken of the same location at 15 minute intervals for 12 hours using both bright field and the FITC channel. AxioVision (Carl Zeiss Ltd.) software was utilized during all microscopy experiments. Upon completion of the experiment, the videos created from this series of pictures were exported for analysis.

Data Analysis and Statistics

Using Imaris (Bitplane) software, cells were tracked using a Brownian algorithm built in. Single cells which did not touch other cells, divide, or interact with gel imperfections were used to collect data in order to ensure minimization of confounding factors. Once position data for individual cells was gathered using Imaris and placed into Excel files, a MATLAB code (Aaron S. Meyer, MIT 2009) was utilized to calculate the

speed, displacement and persistence of each individual cell using a modified persistent random-walk model, as well as consolidate this data on the basis of the conditions (modulus, gel height, and theoretical protein concentration) that each of these cells was experimentally conducted under. The data gathered using the MATLAB code was then input into Prism v5.0a (Graphpad) for organization, graphing, and statistical analysis. Data is presented as the mean value for the set of conditions and error bars represent standard error. In addition, combination of a one-way analysis of variance (ANOVA) and a Newman-Keuls post-test was done using a P-value of less than 0.05 to denote statistical significance.

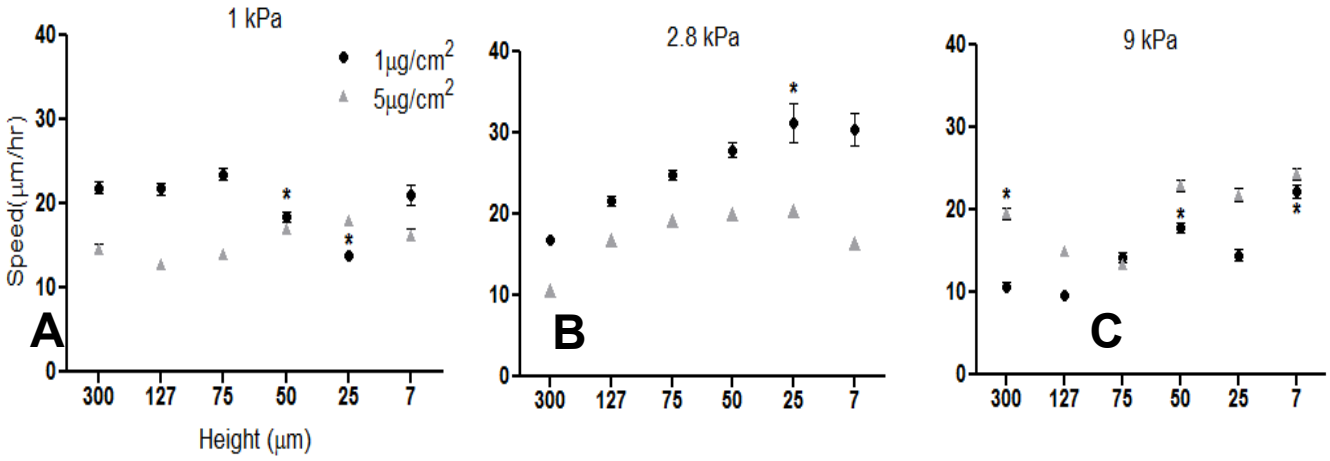
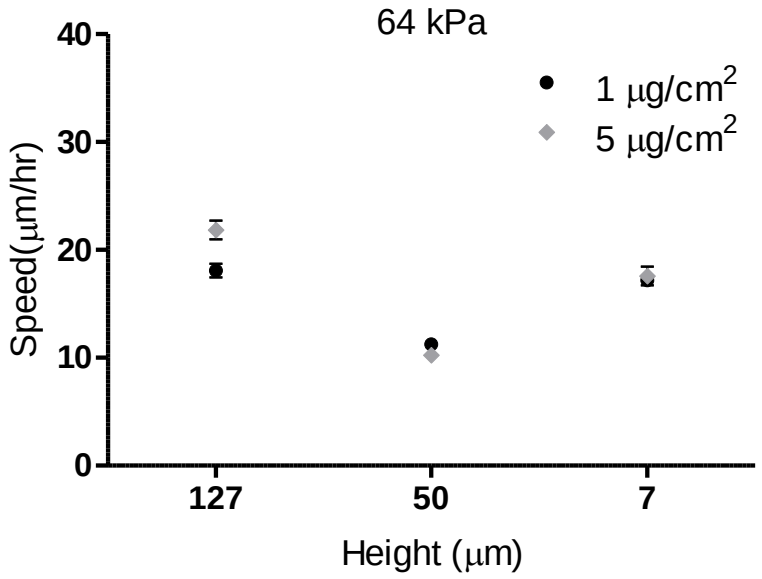


Figure 2: Average cell speed on 1, 2.8 AND 9kPa modulus with gel heights/stiffness ranging from 7-300μm. PEG-PC with 1 and 5 μg/cm^2 collagen I. Asterisk denotes averages which are significantly greater than the rest in that series. N= 80+ cells. SEM values set as error bars.

A



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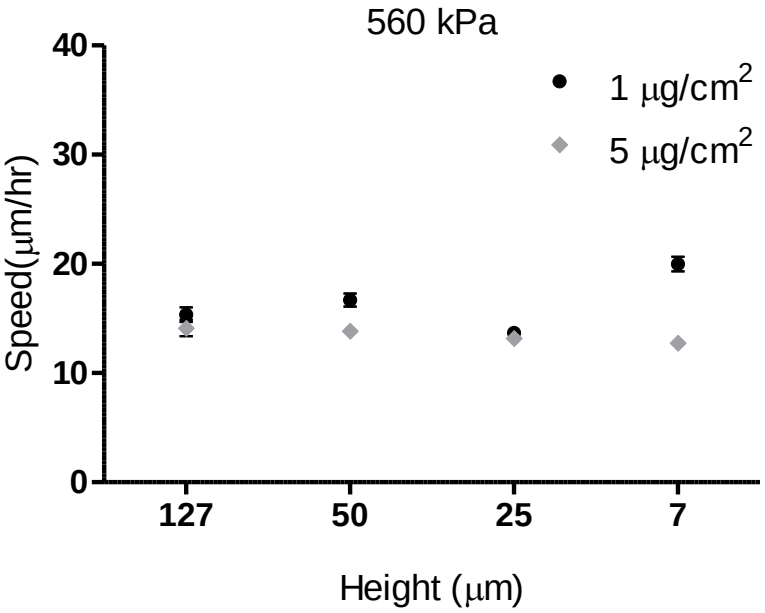


Figure 3: Average cell speed on 4 (A) and 10% (B) PEG-PC with 1 and 5 μg/cm^2 collagen I. N= 25+ cells. Error bars are SEM values.

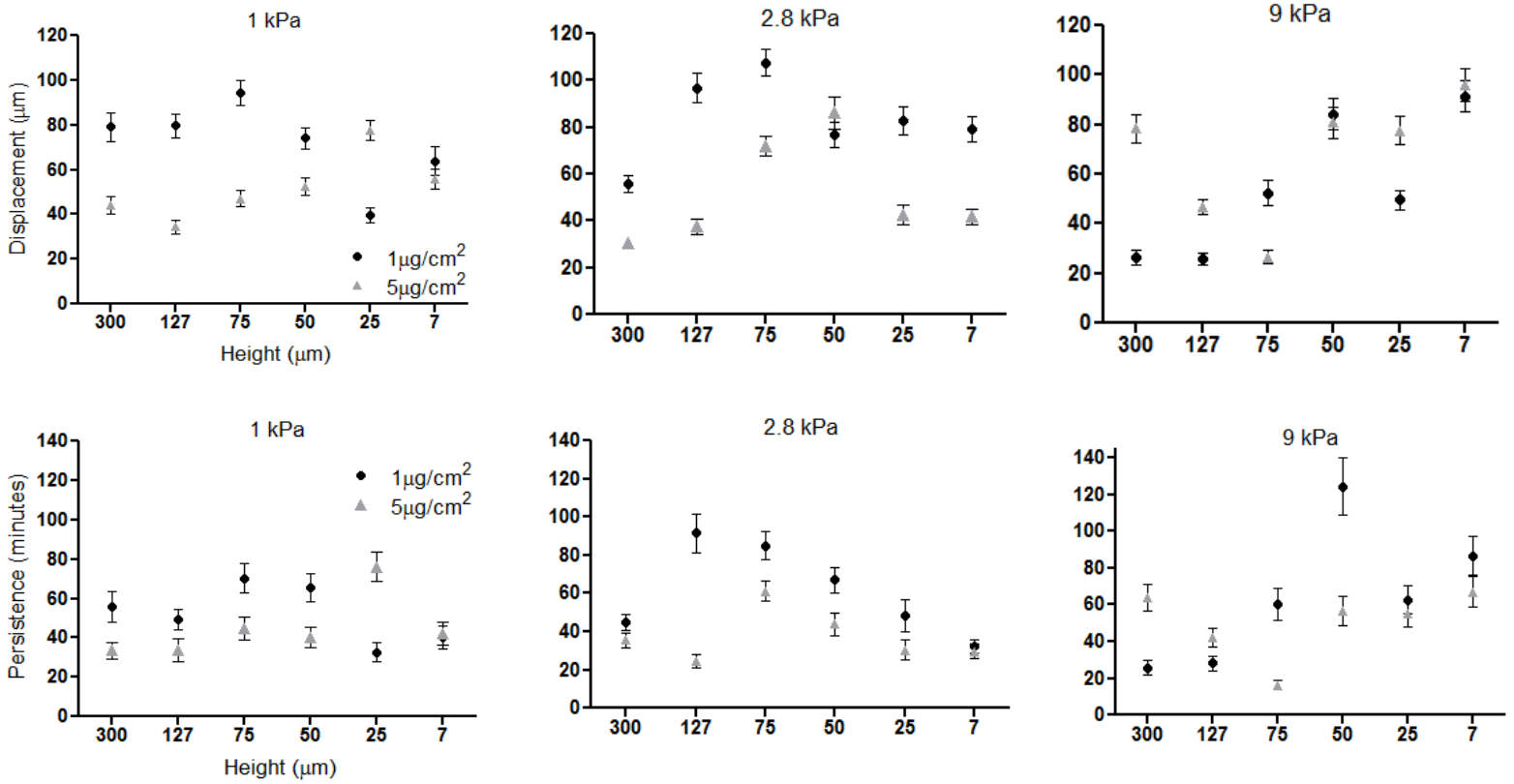


Figure 4: Average cell displacement and persistence on 0.5, 1 and 1.5% PEG-PC with 1 and 5 µg/cm^2 collagen I.

Results and Discussion

The data shown in (Figure 2), shows cell speeds at varying PEG-PC concentrations which regulates the substrates modulus. Speed is shown verses gel height (stiffness) at 1 and 5μg/cm^2 collagen I concentrations. A biphasic response is shown at both protein concentration and at intermediate modulus (2.8 kPa). If the modulus is increased or decreased as shown in (Figure 2), 1 and 9 kPa modulus has less influence upon cell migration. This is further shown in (Figure 3) with higher PEG-PC concentrations of 4 and 10%. Data from (Figure 3) was conducted before data collected in (Figure 2).Due to the minimal cell response to the conditions presented in (Figure 3) we sought to lower the modulus range in compliance with previous modulus ranges studied. (Figure 4) illustrates persistence and displacement values. Persistence at 2.8 kPa has a shifted biphasic response at lower stiffness; whereas, the displacement for 2.8 kPa follows a similar pattern as the cell speed shown in (Figure 2). These results suggest within this model both modulus and stiffness influences cell migration at intermediate modulus and intermediate stiffness. I applied a broad range of both modulus and stiffness in order to further elucidate the independent roles of stiffness and modulus. Stiffness is influential upon cell speed and persistence at intermediate moduli.

Cell migration is influenced by cellular tension and focal adhesions. Focal adhesions form upon integrin-protein binding and aid in the cell's attachment to the substrate. If there an excess of focal adhesion, there is an increase in cellular tension, resulting in the inability for cells to efficiently migrate (Ananthakrishnan and Ehrlicher, 2007). If a substrate is too soft a cell cannot generate the appropriate amount of focal adhesions to generate enough traction to migrate forward quickly. Inversely if a substrate is too stiff the cell generates more focal adhesion generating excess tension therefore the cell cannot migrate rapidly. ECM protein concentration also influences a

cell's ability to generate tension and motility. At our intermediate modulus and intermediate stiffness cell speed is at its maximum. As the stiffness increases or decreases, from the intermediate stiffness range, the speed decreases regardless of protein concentration (Peyton et al, 2005). This is due to the substrate being either too soft/stiff for the cells to generate traction and migrate as efficiently. Collagen I aids in cell adhesion and migration. Integrins bind to the collagen I forming focal adhesions which generate tension and depending on the substrate mechanical properties, making it soft/stiff, the collagen influence the efficiency of the migration due to the increase/decrease in integrin binding and focal complexes forming.

Future Directions

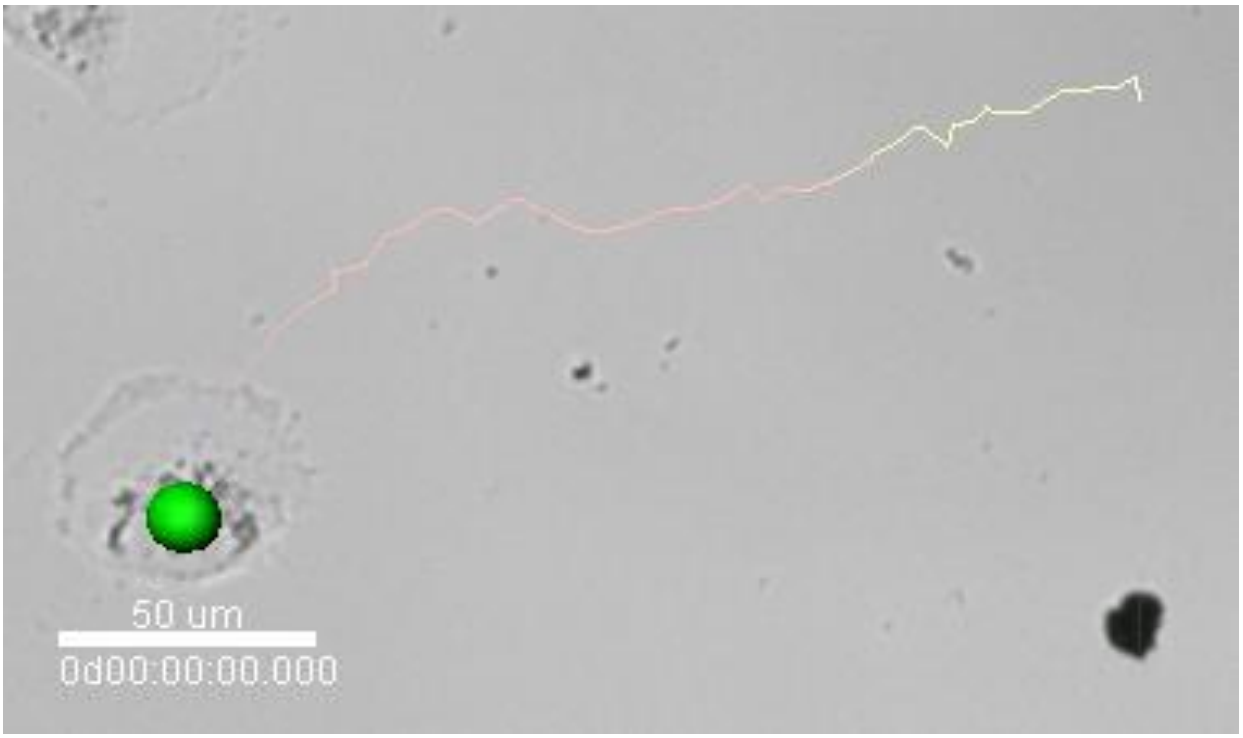
One of the most significant challenges is characterizing these biomaterials to find their stiffness as it relates to cells length scale. Some calculations assume the cell can feel the glass coverslip underneath the gel regardless the final gel height, which seems biologically unlikely. Developing a method or way of rheology testing to obtain stiffness as it relates to cell length scale would be especially useful to compare data from different conditions. If there was a method validated, I could use different combinations of height and modulus and verify this response because we would know what stiffness each combination corresponds to.

Previous work conducted by ICE-REU undergraduate Chaz Chuckler, shown in (Figure 5 and 6), examined cell area over time with varying stiffness and protein concentrations and identified cell adhesion is independent of gel height/stiffness (with these height values, more can be applied) but is dependent on collagen I concentration. It is unknown if adhesion is modulus dependent. Only one PEG concentration (10%, 560kPa) was used throughout these experiments. The lower collagen I concentration

result a faster rate of cell spreading. In addition cell polarization was observed over time with the same conditions as cell area. The percentage of cells polarized at each condition by the end of the 160 min time interval is greatest for the cells exposed to the higher collagen I concentration. These experiments would be beneficial to repeat with conditions described in (Figure 2). Initial cell-matrix contact and how the stiffness and modulus affects the cell shape and spreading rate with the combination of protein quantification chosen from select pathways (i.e. FAK, and MAPK) could gain insight on mechanosensing at the intercellular level at various contact stages of the cell to biomaterial. Additional further characterization of the ECM protein upon PEG-PC would aid in validating theoretical density and aid in future experiments in increasing or decreasing protein concentrations.

In combination to the external factors in the ECM that control cell behavior, there are numerous internal mechanisms that have a role in how the cell behaves and responds to external factors. Identification of common cellular signaling such as Rho, ROCK and MAPK in response to these biomaterials would be a way to further understand the cause of the cell-matrix response. This system has validated my objective to create a material with tunable stiffness and modulus. It would be advantageous to create three dimensional experiments with a PEG base or copolymer with PEG. A modified version of this system could be used to study how 2D properties affect 3D migration, and then identify what influences cancer invasion.

A



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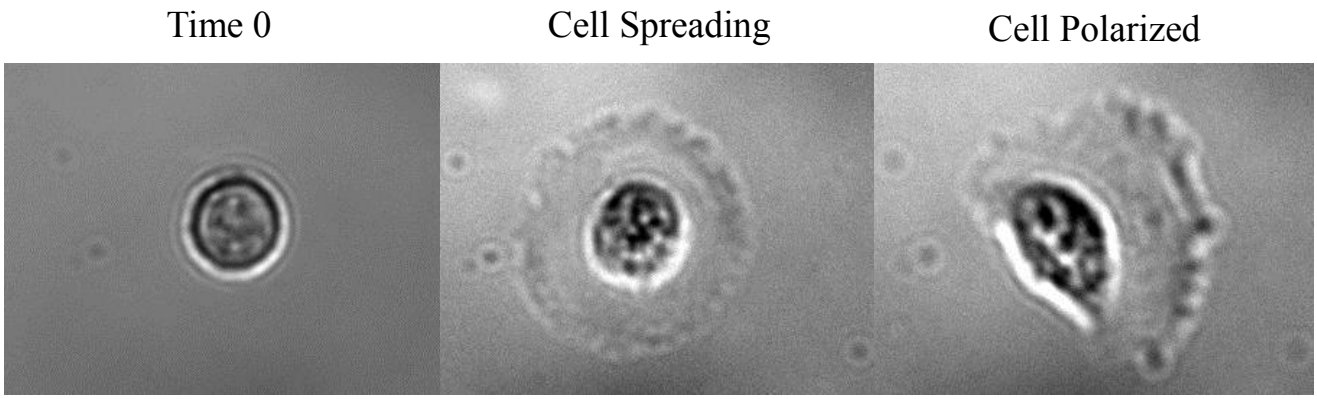


Figure 5: MDA-MB-231 cell migration path as shown in Imaris (A) Cell spreading and polarizing over time (B).

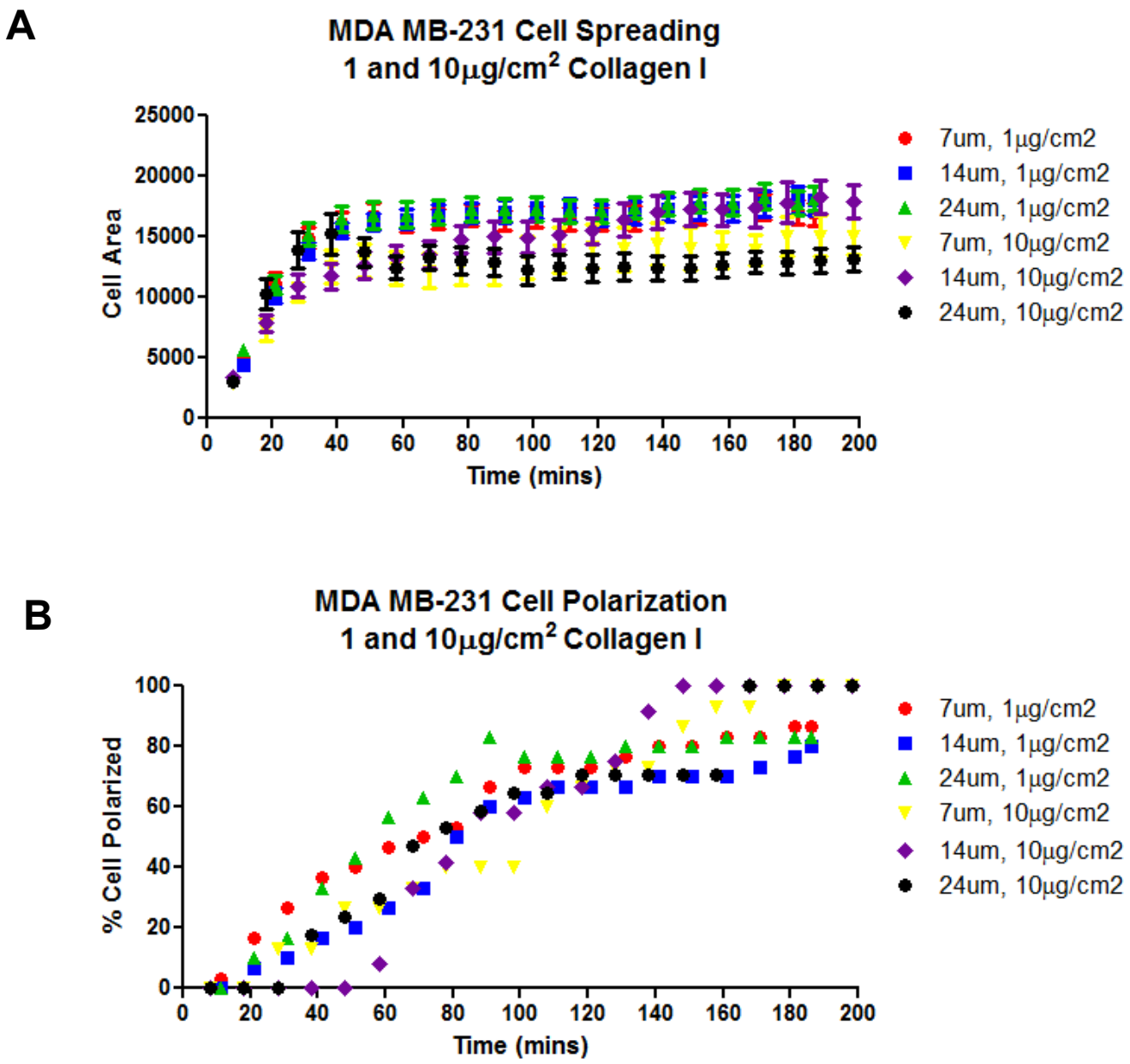


Figure 6: Cell Spreading (A) and polarization (B) percentage on 10% PEG-PC (560kPa) gels with varying collagen I concentrations.

CHAPTER 4

PATTERNED AND NON PATTERED PDMS-PS COMPOSITES WITH CONTROLLED HEIGHTS

Introduction

Using a combination of soft and photolithography I modified a polymer microlense system that contains a polydimethylsiloxane (PDMS) base, which has a patterned array of cylindrical wells, coated with a thin, spun coated film of polystyrene (PS) (Miquelard-Garnier, 2010). Cells only directly interact with the PS layer, but by controlling the geometry of both the PS and the PDMS, I can independently tune the stiffness and modulus the cells experience. The inherent polymer architecture of the PDMS and PS controls the geometry-independent modulus, while the PDMS well diameter and thickness of the PS independently control the geometric stiffness. The completed microlenses consist of “compliant” regions of a thin film of PS and “stiff” regions where the PS film is supported by the PDMS. Using non-template PDMS with spin-coated PS we aimed to vary the height of the PS controlling the stiffness. Changing the PDMS concentration enabled us to control the modulus. After analysis with a patterned template we sought to use non-template PDMS and applying varying heights of PS on top. The height of the PS regulates the composite stiffness whereas the crosslinker of the PDMS regulates the modulus.

Hypothesis

Similar to work done by Buxboim et al. we aim to identify if cells can sense stiffness and modulus as separate mechanical properties. I hypothesize the biomaterials I use can independently tune and quantify stiffness and modulus and their

effect upon cell migration. I believe cell migration will be influenced by both mechanical properties and exhibit a biphasic response at intermediate to low modulus. As shown in Peyton et al. I hypothesize our model will exhibit similar trends. The biphasic curve will shift as a function of protein concentration where lower concentrations will exhibit peak migration at stiff values whereas higher protein concentration will peak at lower stiffness (heights).

Methodology

Preparation of Microlenses

Utilizing the microlense protocol from (Miquelard-Garnier, 2010), Sylgard 184 (Dow Corning) polydimethysiloxane (PDMS) was mixed in varying ratios then cast into the silicon wafer and cured at 70ºC overnight. The PDMS template was then covered with a 2% (w/v) polystyrene PS thin film creating a PDMS-PS composite. Samples were placed in a 24-well plate where they were plasma treated to sterilize and expose hydroxyl groups. Samples were covered with a mixture of collagen I diluted in .02M acetic acid at the desired theoretical concentration. The plates were left at room temperature for 2 hours to allow protein absorption then Pluronic F127 was added to each sample for blocking and left at room temperature overnight. The gels are then washed with sterile 1X PBS and allowed to sterilize in the tissue culture hood for 30 minutes. The gels are washed once with sterile 1X PBS, immersed in DMEM with 10% FBS and 1% PS, and incubated until cells are ready to be seeded into the plate. (Figure 7) illustrates measurements of microlenses along with bright field image of MDA-MB-231 cells on microlense. PDMS for the non-template samples followed the same protocol as the template PDMS except initially the PDMS is cast into a petri dish then cut into smaller squares to fit into a 24-well plate. (Figure 8) is a schematic of the PDMS-PS

design with measurements. Table 3 illustrates the modulus and height values for the top layer PS.

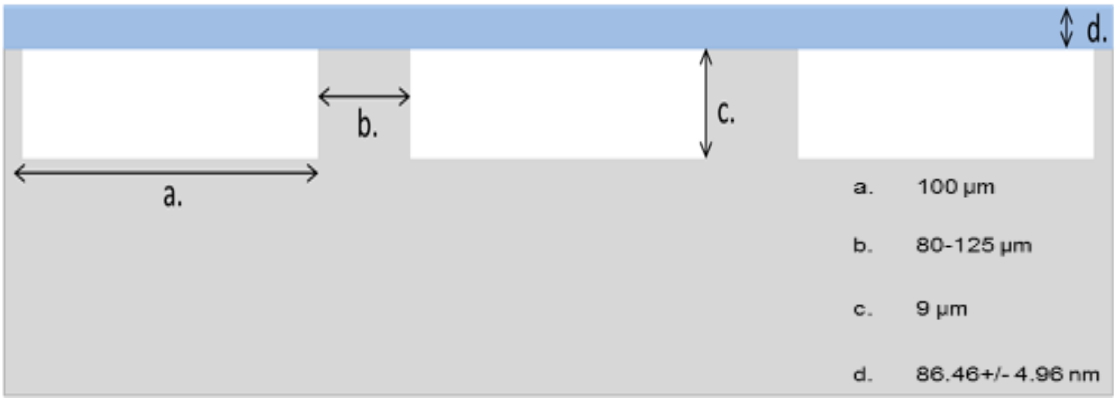
Cell Culture

For all experimentation, MDA-MB-231immortalized breast carcinoma cells were used. The cells are obtained already transfected with cytosolic green fluorescence protein. They are cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin–streptomycin (PS) added, at 37°C and 5% CO2. Cells were obtained from Dr. Shannon Hughes, MIT.

Microscopy

Cells are seeded at a density of approximately 8,000 cells per well and allowed to adhere overnight prior to microscopy experiments. All experimentation used 10x objective on a Zeiss Axio Observer Z1 microscope with a motorized piezo X-Y stage and an incubation chamber (Carl Zeiss Ltd.). Prior to the setup of a migration experiment, the incubation chamber was set to 37°C and 5% CO2. Once the microscope incubation chamber was equilibrated, the plate was placed in the chamber and allowed to equilibrate once more. Time lapse are set up so that pictures were taken of the same location at 15 minute intervals for 12 hours using both brightfield and the FITC channel. AxioVision (Carl Zeiss Ltd.) software was utilized during all microscopy experiments. Upon completion of the experiment, the videos created from this series of pictures were exported for analysis.

A.



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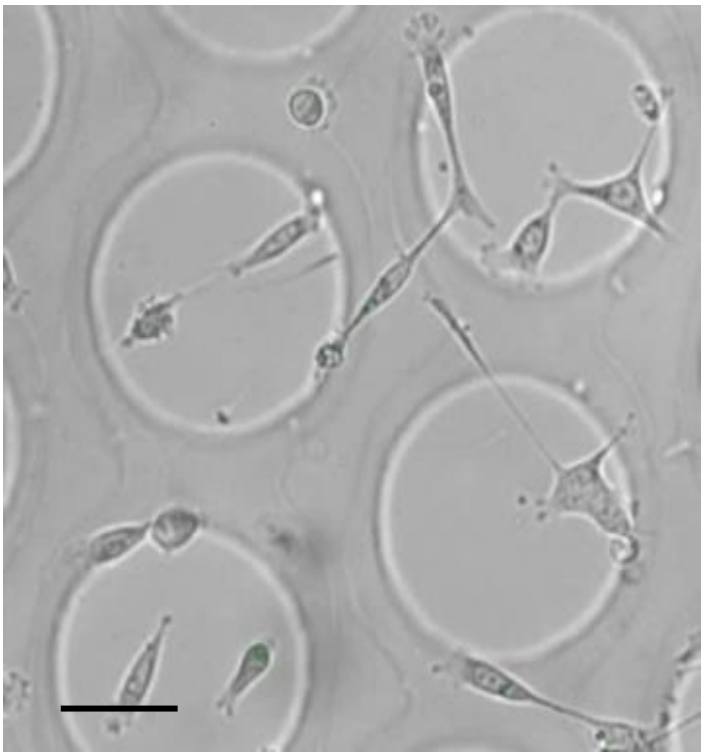

Figure 7: Polymer Microlense Dimensions(A). Bright field image of microlense with MDA-MB-231 cells, 10x magnification, scale bar 50µm (B)

Figure 8: Schematic of PDMS setup.

PDMS [wt/vol]	E (kPa)	Polystyrene [wt/vol]	Polystyrene Height
10:1	1230+/- 0.08	2%	86.46 +/- 4.96 nm
20:1	710+/- 0.05	3%	153.8 +/- 3.41 nm
30:1	420+/- 0.04	4%	226.4 +/- 5.12 nm
40:1	120 +/- 0.01		

Table 3: Mechanical results for elastic modulus of PDMS along with profilometer measurements of PS

Data Analysis and Statistics

Using Imaris (Bitplane) software, cells were tracked using a Brownian algorithm built in. Single cells which did not touch other cells, divide, or interact with gel imperfections were used to collect data in order to ensure minimization of confounding factors. Once position data for individual cells was gathered using Imaris and placed into Excel files, a MATLAB code (Aaron S. Meyer, MIT 2009) was utilized to calculate the speed, displacement and persistence of each individual cell using a modified persistent random-walk model, as well as consolidate this data on the basis of the conditions (modulus, PS concentration, and theoretical protein concentration) that each of these cells was experimentally conducted under. The data gathered using the MATLAB code was then input into Prism v5.0a (Graphpad) for organization, graphing, and statistical analysis. Data is presented as the mean value for the set of conditions and error bars represent standard error. In addition, combination of a one-way analysis of variance (ANOVA) and a Newman-Keuls post-test was done using a P-value of less than 0.05 to denote statistical significance. Additional analysis was conducted looking at individual cells on various parts of the template, over the well, the border of the well and the flat/PDMS-PS region. Using a Matlab code (Aidan Gilchrist, 2012) individual cell speeds were obtained. (Table 3) displays the theoretical calculations obtained from the Crosby lab at UMass to obtain stiffness values for the polymer microlense locations.

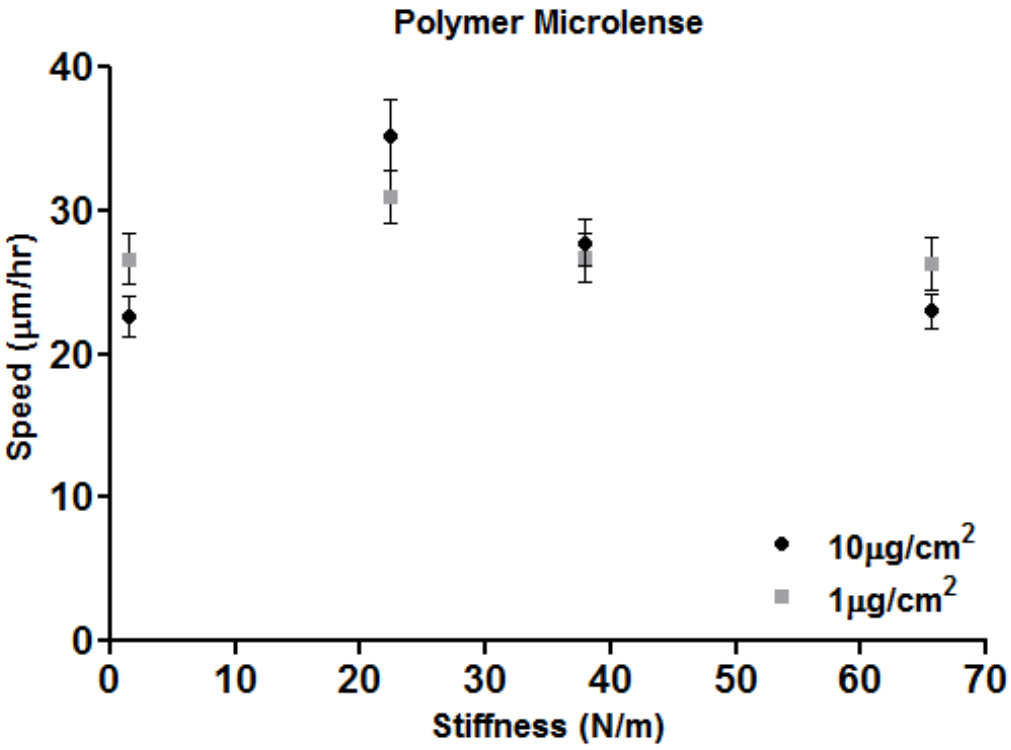

Figure 9: Average speed verses stiffness of MDA-MB-231 cells on Polymer Microlense. N= 50+ cells, error is SEM value.

Polymer Microlenses	Stiffness
Well (Polystyrene Only)	$K=\frac{2\pi E_{PS} t^2}{a_0}$
$E_{PS/PDMS}$= Elastic/Bulk Modulus of PS/PDMS	
Flat (PDMS & PS)	$K=\frac{8E_{PDMS}L}{3}$

Table 4: Equations used to calculate stiffness for microlense system. E= elastic modulus, t= height of PS, a= diameter of well, L= length of cell.

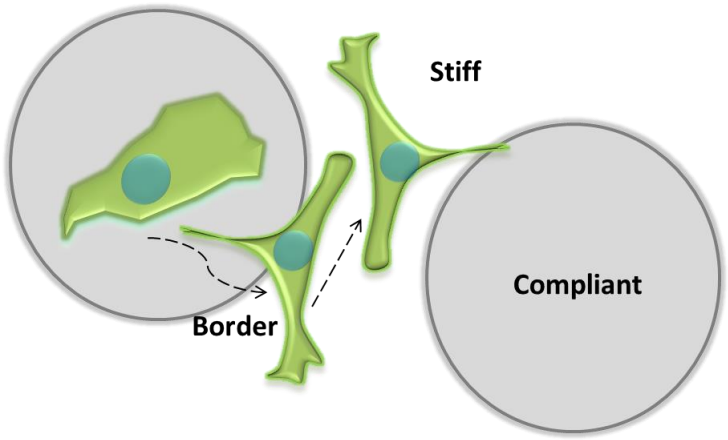

Figure 10: Illustration of MDA-MB-231 cells on polymer microlense with varying locations

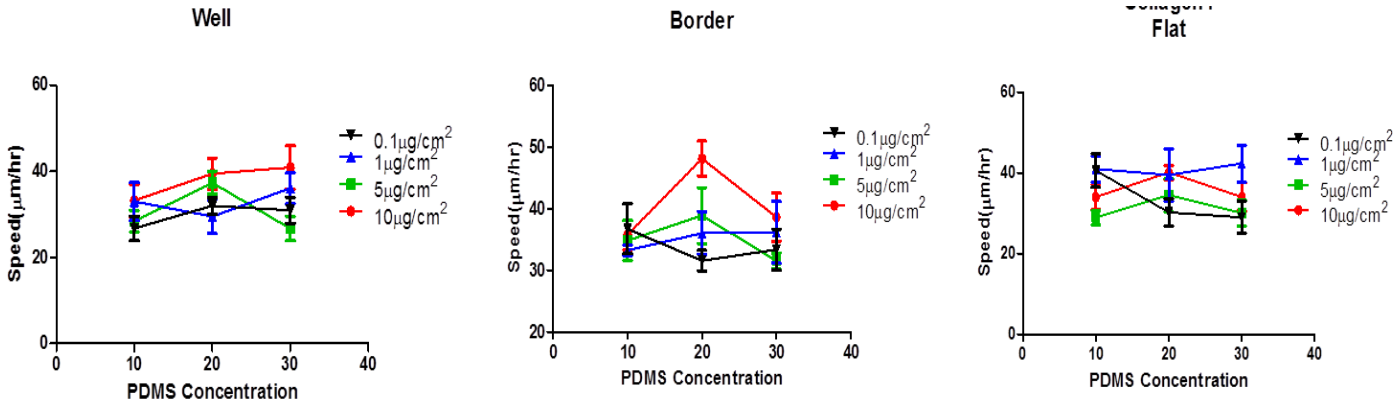

Figure 11: Average speed verses stiffness of individual cell on varying microlenses locations. N= 20+ cells, error bars are SEM value

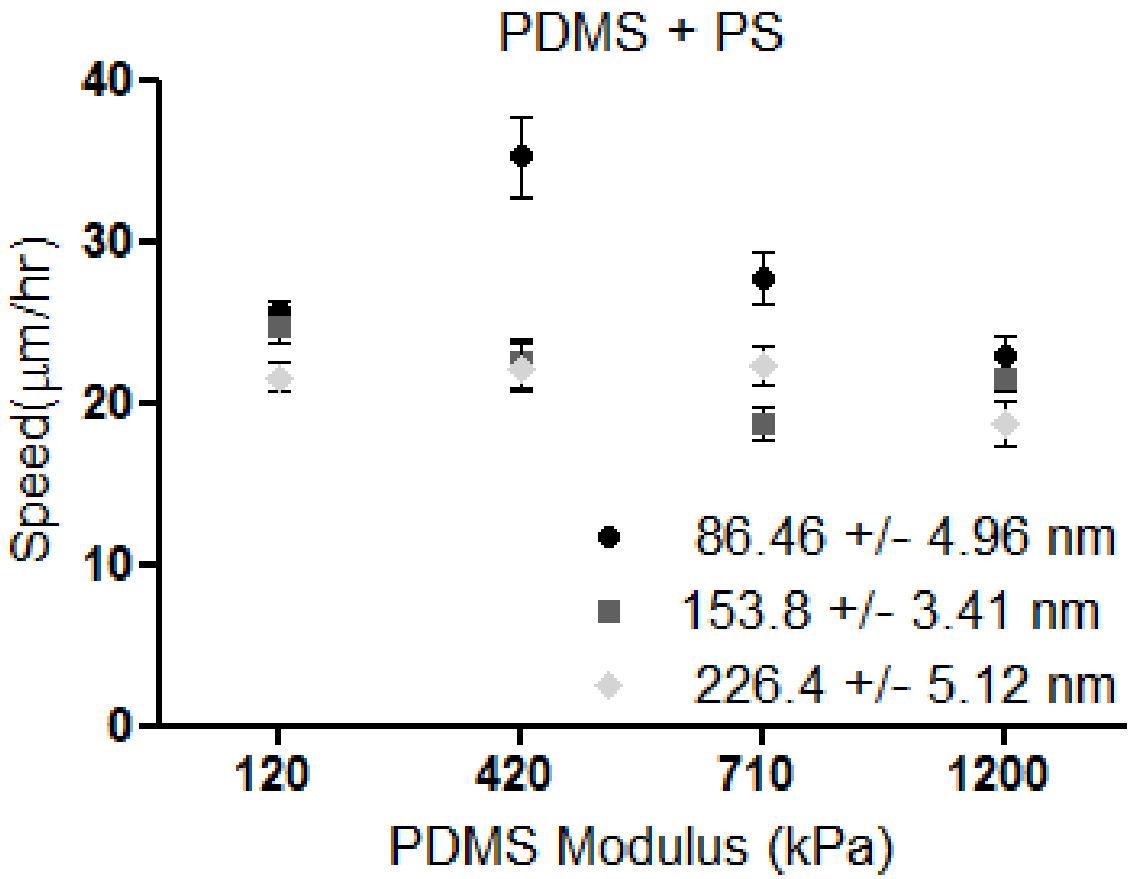

Figure 12: PDMS-PS average speed with varying PS heights. N=50+ error bar are SEM value.

Results and Discussion

Similar to our PEG-PC system, patterned PDMS-PS cell speed has a biphasic response at intermediate stiffness at both high and low collagen I concentrations in (Figure 9). This can be contributed to differences in protein absorption and a higher modulus than the PEG-PC system. Individual cell response to changes in stiffness due to the template allows us to identify a cell‘s response on a smaller length scale and shorter time interval. Data shown in (Figure 11) displays individual cell speed is not significantly influenced by changes in location on the microlenses which are relative to changes in stiffness. This could be due to the high modulus of the PS in addition collagen. Non-templated PDMS-PS (Figure 12) exhibited similar response to pattered PDMS-PS at 2% PS concentration with a biphasic curve at intermediate stiffness. When the PS height was increase there was a decline in cell speed and was not stiffness dependent. This arguably again could be due to the high modulus of the PS and differences in protein absorption upon the PS top layer than that of our PEG-PC model.

Future Directions

Due to the high modulus of PS and Sylgard 184 PDMS it would be advantageous to use another Sylgard such as 527 to lower the modulus and repeat select condition to identify if the modulus of the PDMS is a contributing factor to the cells insensitivity to the PDMS-PS composite. Collagen I density quantification can give insight if the theoretical amount on the material is sufficient or if other concentration would yield more efficient results. Especially since there is no pre-treatment it is not known how much collagen is being “passively absorbed”.

Photolithography as a tool would be valuable to create new patterns with different well diameters and shapes to see if cells sense changes in stiffness and topography.

Instead of using PS as a top layer one could spin coat another material such as a higher modulus PDMS to yield a thin sheet over the PDMS template yet a lower modulus. PS has a high modulus around 3 GPa. Last, similar to the PEG-PC system identification of signal pathways, cell adhesion, shape and protein composition would be advantageous to identify at various locations of the patterned substrate and on non-patterned PDMS.

CHAPTER 5

CD/SE NANO LINES AS A TOPOGRAPHICAL AND STIFFNESS CUE FOR CELL MIGRATION

Introduction

The last system I created was a composite of Cd/Se nano lines in between two layers of PDMS. By changing the he top layer of PDMS I can control the stiffness. The modulus was controlled by the PDMS crosslinker ratio for both the top and bottom layer. The addition of the nano lines yield topography cue which the cells sense. This system specifically aims to mimic TACS during tumor progression. Paszek and Keely both demonstrated the ability for collagen fibers to contribute to tumor stiffness and assist cell motility and extravasation from the primary tumor sit *in vivo.* I was able to create a composite material which we can tune stiffness of the top layer by varying the height, change the modulus of both layers by crosslinker ration and tune the stiffness and topography of the lines in-between.

Hypothesis

For this project I sought to create a composite material with topographical cues embedded. Similar to work done by Buxboim et al. we aim to identify if cells can sense stiffness and modulus as separate mechanical properties. I hypothesize the biomaterials we use can independently tune and quantify stiffness and modulus and their effect upon cell migration. The addition of nano lines add another stiffness and topographical cue which I hypothesize the cells will be able to sense at low heights and as the top PDMS height increases the cells will be less influenced by the topographical cue.

Methodology

Preparation of Bottom PSMS Substrate

The bottom most section of the sample is entitled the base substrate and is made up of poly (dimethylsiloxane). Sylgard® 527 Dow Corning in a two part solution, stored as Part A and Part B. Each part is mixed in a ratio of 1:1 per the manufactures instructions. When prepared as such the modulus of the resulting PDMS is 5.05 ±0.37 kPa as measured by Palchesko et al. using the slope of a stress-stain curve throughout the linear regime (2012). In order to create the base substrate, a thick layer of PDMS (exceeding 150 μm) needs to be adhered to a circular glass coverslip of 18 mm diameter. To do so a glass microscope slide is coated with a thin layer of poly (acrylic acid), PAA, over which a layer of PDMS is poured. The thin layer of PAA is created by making a 2 wt% solution of PAA in water. The slides are cleaned by being placed in a soap water bath and sonicated for 15 minutes, then washed with an acetone bath and isopropyl, and a final sonication for 15 minutes. After the final wash the slides are placed in the UV/ozone oven for 20 minutes; after which they are placed on the spin-coater. The slides are then covered in a comprehensive layer of the 2 wt% PAA solution and spin-coated at 2000 rpm for 1 minute. The PAA coated slides are allowed to dry for a minimum of 2 hours to allow any moisture to evaporate. After drying the slides are placed in a square petri dish and the pre-prepared mixture of the Sylgard® 527 gel is poured over the glass slides. The gel was prepared using equal parts A and B, thoroughly mixed, and then degassed in a vacuum chamber for several minutes or until all bubbles have disappeared. The PDMS is cured overnight at 70^{o}C. The cured PDMS is transferred to a sterile glass coverslip. The sun-coated PDMS is floated off in a water bath onto the PDMS-coverslip.

Preparation of Cd/Se lines

The fabrication of the quantum dot Cd/Se lines was performed by the Crosby Research Group. Polystyrene-functionalized cadmium and selenium quantum dots were dissolved in a toluene solution and then placed beneath a polyethylene terephthalate blade (PET). This blade was flexible and came to a sharp edge. Due to slip motion of the blade, as controlled by a computer program, Quantum dot stripes were left behind in the space where the blade's edge once occupied. By altering the tempo of the blade's stick-slip motion, different line spacing's can be created. The Cd/Se QDs were then cross-linked using UV light at 365 nm for 30 minutes (Kim et al., 2010). After the lines had been formed, they were floated off their substrate using water. The floating lines were then deposited upon the base substrate described above.

Preparation of PDMS Top Layer

To complete the sample a top layer of Sylgard® 527 gel PDMS is added atop the lined base substrate. This layer will range from 6 μm to 80 μm and is floated onto the lined base substrate after being spin-coated onto a silicon wafer. To do so the Si wafers are cleaned in the same manner as the aforementioned glass microscope slides and glass coverslips. After being cleaned and dried the Si wafers are treated with UV/ozone for 20 minutes and then immediately spin-coated with a solution of 2 wt% PAA, prepared in the same manner as mentioned above. The spin-coating is done at 2000 rpm for 1 minute and then the PAA covered Si wafers are allowed to dry for a minimum of 2 hours.

A solution of PDMS is made using the procedure described above with Sylgard® 527 with a ratio of 1:1 and a period of degassing in a vacuum. The PAA covered Si wafers are covered in a layer of the PDMS and spin coated at various rpms. Each rpm correlates to a different thickness of the final PDMS layer as shown in the table below.

The thickness of each rpm-related PDMS layer was determined using an Optical Profilometer. Once the Si wafers were coated in a layer of PDMS the samples were allowed to cure in a 70^{o}C oven for a minimum of 4 hours. After curing, the PDMS is cut into squares of approximately 1 cm. To each of these squares is added the lined base substrate. The entire Si wafer, coated in PAA and PDMS, topped with the lined base substrate, is immersed in water. The PAA layer dissolves in water allowing the thin PDMS layer on the Si wafer to float off adhered to the lined base substrate.

Protein Chemistry

Before the samples are ready for cell seeding they must first be prepped; cells are unable to adhere and develop well on PDMS alone. The samples were epoxied onto a 12-well plate using 5 minute Epoxy®, and covered with 1x PBS. Each well contained one sample and was covered in approximately 1 mL of 0.2 mg/mL sulfo-SANPAH from Thermo Scientific in a 50 mM HEPES (Invitrogen) solution at pH 8.5. The plate was then exposed to UVt light for a minimum of 15 minutes or until the sulfo-SANPAH solution had turned from a bright red to a dark brown color. The samples were then washed once with 50 mM HEPES at pH 8.5, covered again with 0.2 mg/mL Sulfo-SANPAH solution and exposed to UV light for an additional 15 minutes; after which the samples were washed 3 times in 1x PBS.

After the wash cycle is complete the samples are covered in a solution of collagen I in 0.02 M acetic acid at the desired theoretical concentration of collagen. The samples were then placed in a 20^{o}C refrigerator overnight to allow the proteins to adhere to the exposed amine groups of the Sulfo-SANPAH without degradation. Following protein coating the samples were washed in sterile 1x PBS and sterilized in low

frequency UV light for 30 minutes. The cells are then ready to be seeded onto the samples immersed in DMEM with 10% FBS and 1% PS.

Cell Culture

For all experimentation, MDA-MB-231immortalized breast carcinoma cells were used. The cells are obtained already transfected with cytosolic green fluorescence protein. They are cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin–streptomycin (PS) added, at 37°C and 5% CO2. Cells were obtained from Dr. Shannon Hughes, MIT.

Microscopy

Cells are seeded at a density of approximately 8,000 cells per well and allowed to adhere overnight prior to microscopy experiments. All experimentation used 10 x objectives on a Zeiss Axio Observer Z1 microscope with a motorized piezo X-Y stage and an incubation chamber (Carl Zeiss Ltd.). Prior to the setup of a migration experiment, the incubation chamber was set to 37°C and 5% CO2. Once the microscope incubation chamber was equilibrated, the plate was placed in the chamber and allowed to equilibrate once more. Time lapse are set up so that pictures were taken of the same location at 15 minute intervals for 12 hours using both bright field and the FITC channel. AxioVision (Carl Zeiss Ltd.) software was utilized during all microscopy experiments. Upon completion of the experiment, the videos created from this series of pictures were exported for analysis.

Data Analysis and Statistics

Using Imaris (Bitplane) software, cells were tracked using a Brownian algorithm built in. Single cells which did not touch other cells, divide, or interact with gel

imperfections were used to collect data in order to ensure minimization of confounding factors. Once position data for individual cells was gathered using Imaris and placed into Excel files, a MATLAB code (Aaron S. Meyer, MIT 2009) was utilized to calculate the speed, displacement and persistence of each individual cell using a modified persistent random-walk model, as well as consolidate this data on the basis of the conditions (modulus, gel height, line spacing, and theoretical protein concentration) that each of these cells was experimentally conducted under. The data gathered using the MATLAB code was then input into Prism v5.0a (Graphpad) for organization, graphing, and statistical analysis. Data is presented as the mean value for the set of conditions and error bars represent standard error. In addition, combination of a one-way analysis of variance (ANOVA) and a Newman-Keuls post-test was done using a P-value of less than 0.05 to denote statistical significance.

Results and Discussion

Initial experiments conducted by Ravi Yelleswarapu shown in (Figure 13) quantified cell migration on nano lines placed on glass coverslips and lines placed on PEG-PC gels. Cell migration was higher on PEG-PC gels than glass. This could be due to the lower modulus and PEG-PC than of glass. Prateek Katti conducted a series of experiments to identify what growth factors, ECM (fibronectin) and media (plus or minus serum) affected cell survival. Cell survival time increased significantly when fibronectin was added. With these results we sought to make a composite material with the lines embedded in between two layers. We first conducted optical profilometer testing to identify what range we could acquire with spin coating PDMS. (Figure 16) illustrates the rpm and respective heights of PDMS spun-coated onto glass slides. With the composite

model shown in (Figure 15) we conducted migration experiments with a top layer of 6µm and varied the line spacing. (Figure 17) displays minimal changes in cell migration and persistence due to changes in line spacing. We visualized changes in cell morphology and could identify through videos cells follow the lines yet have yet to quantify. Peggy Yuen (ICE-IGERT REU) observed throughout the time-lapse videos various cell behavior such as protrusions increased with lines spacing yet overall identification of cell percentage following lines did not change between the two line spacing (Figures 18 and 19). Overall cells show they sense the topographical cue of the lines yet it is unclear what the threshold is for the top layer PDMS until the cells are insensitive to changes in stiffness and topography.

Work conducted by Buxboim et al showed cell were sensitive to heights ranging from 3.1 to 18.9 µm, at low modulus. By altering the heights maybe we will see changes in cell migration and persistence. We set the top height to 6 µm; therefore our results are incomplete and we could be in an intermediate height where cells sense the topographical cue underneath them.

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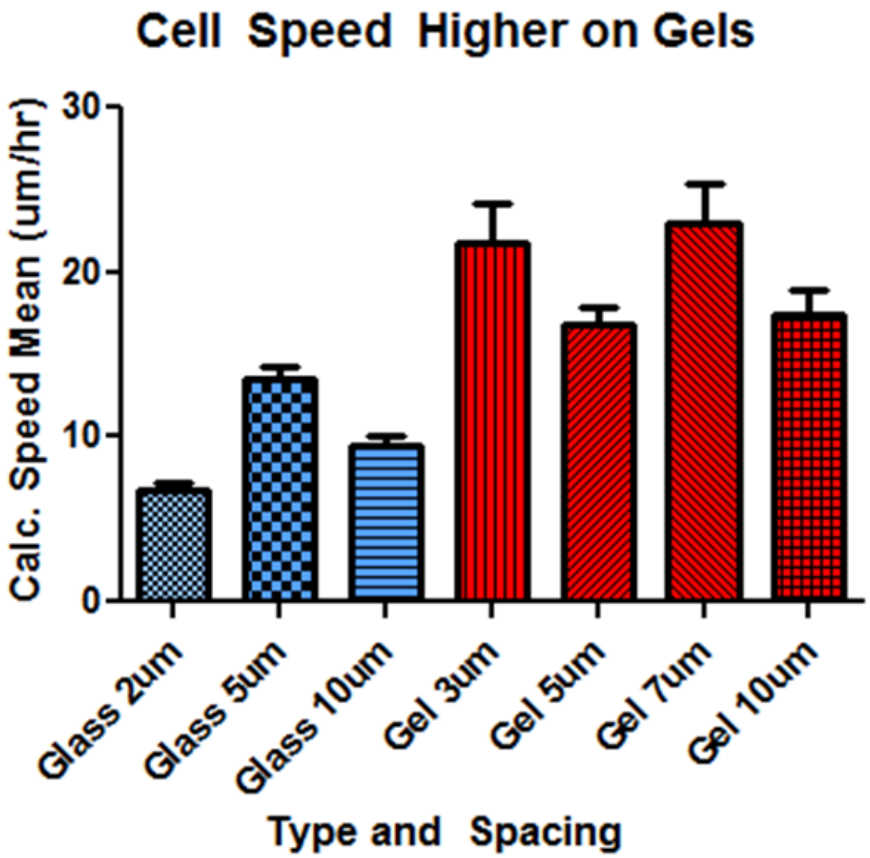

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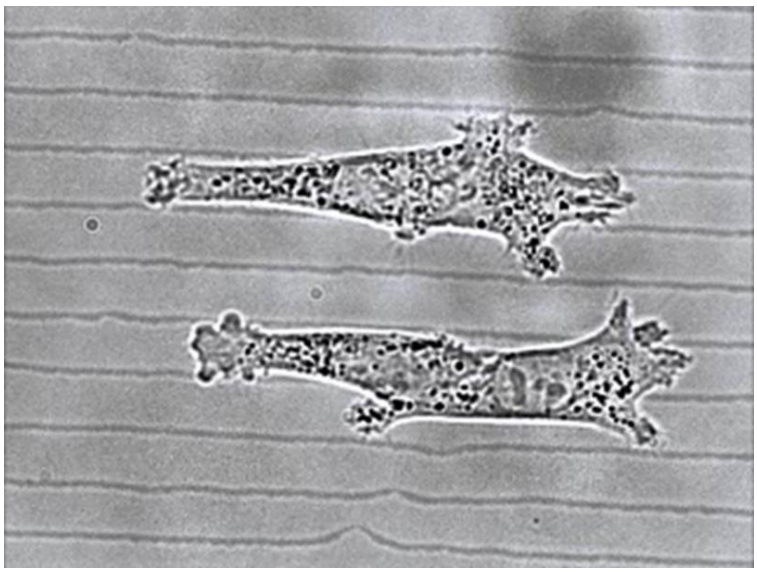
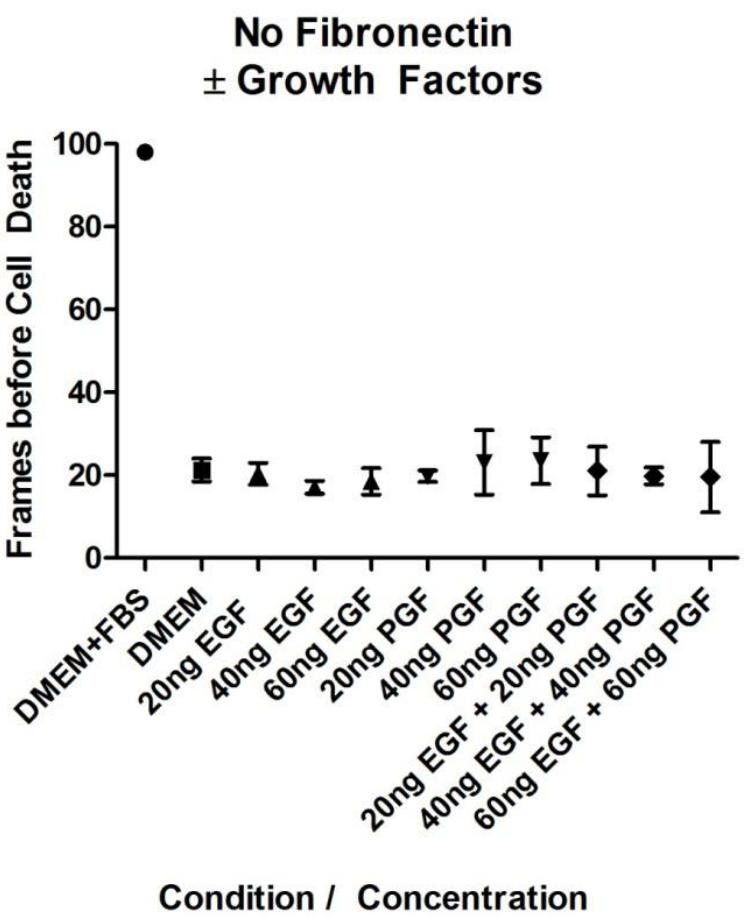


Figure 13: Cell speed on Nano lines-glass coverslips. (A) Bright field image of lines and MDA-MB-231 cells. 63 x (B).



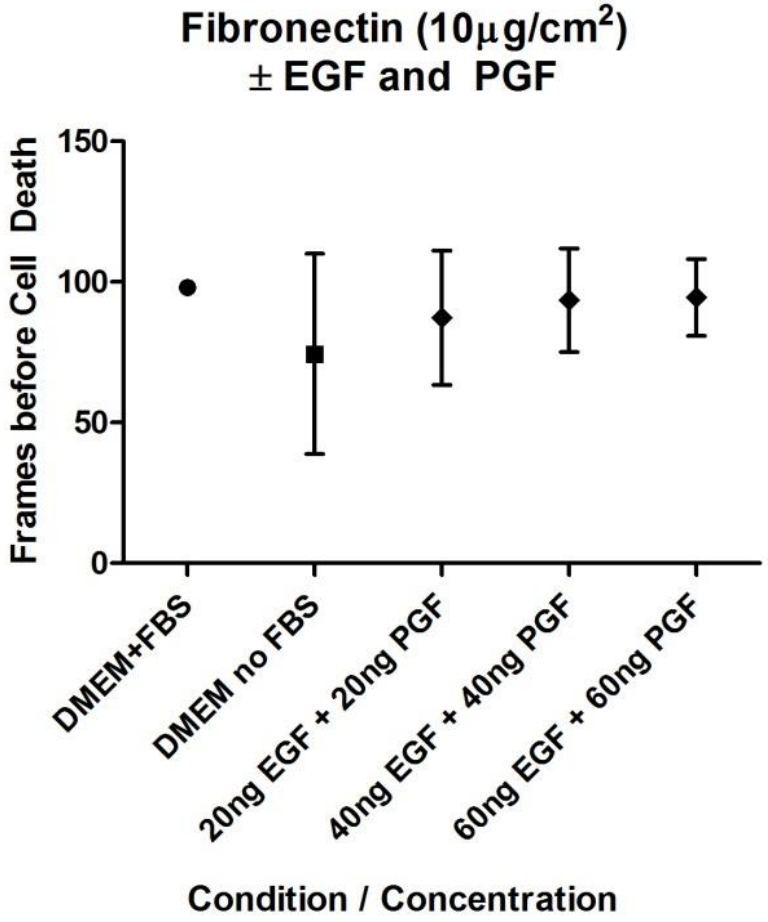


Figure 14: Cell survival with and without Fibronectin and growth factors

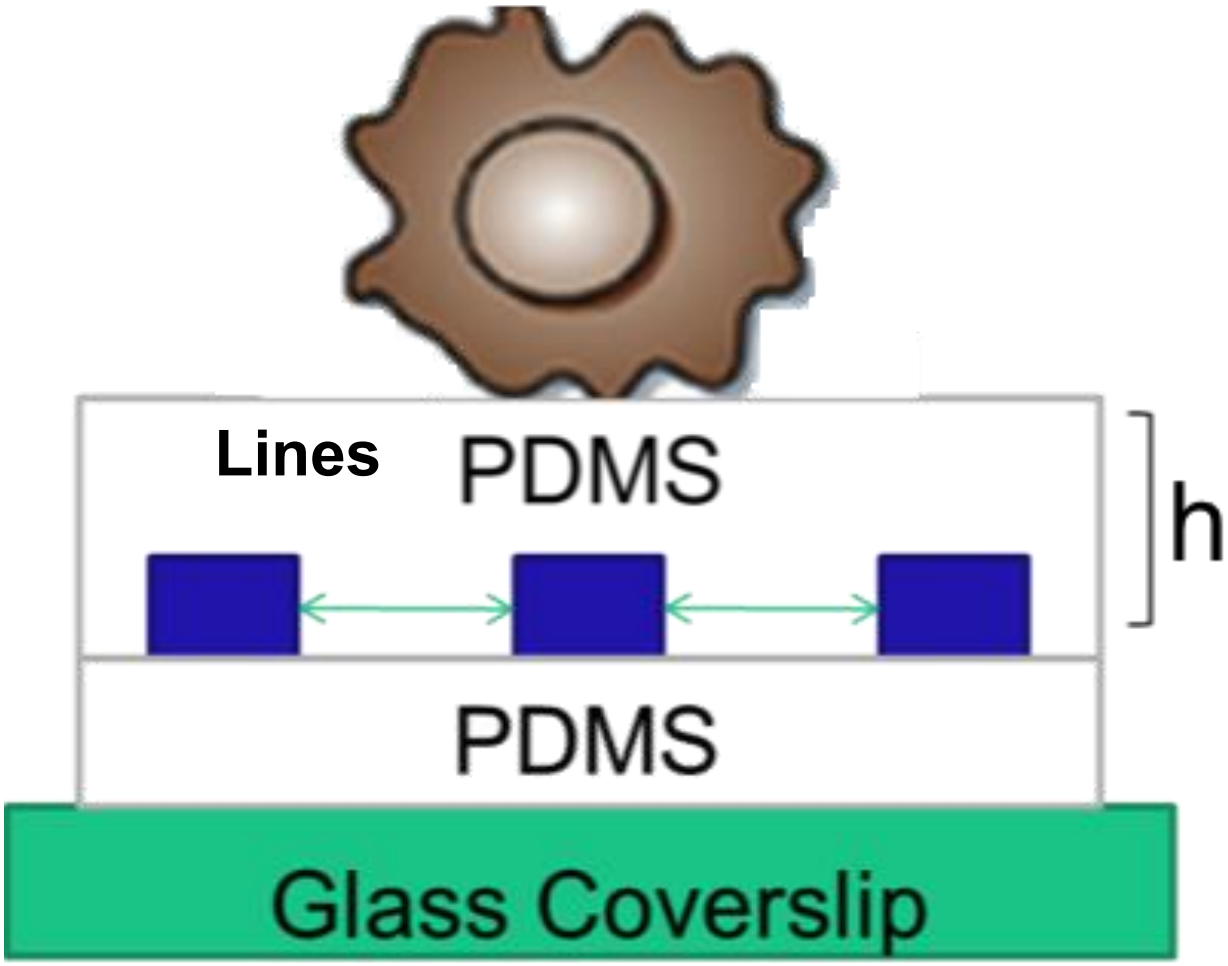


Figure 15: PDMS-PS Experimental Design. Schematic of PDMS setup.

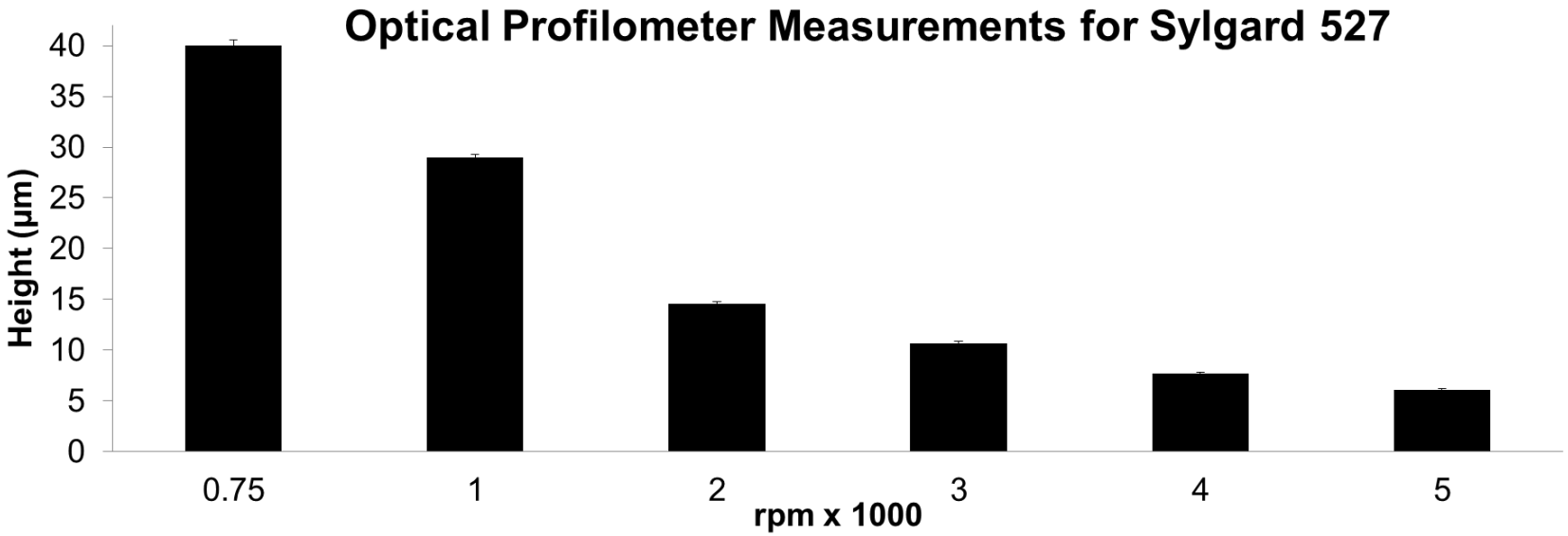
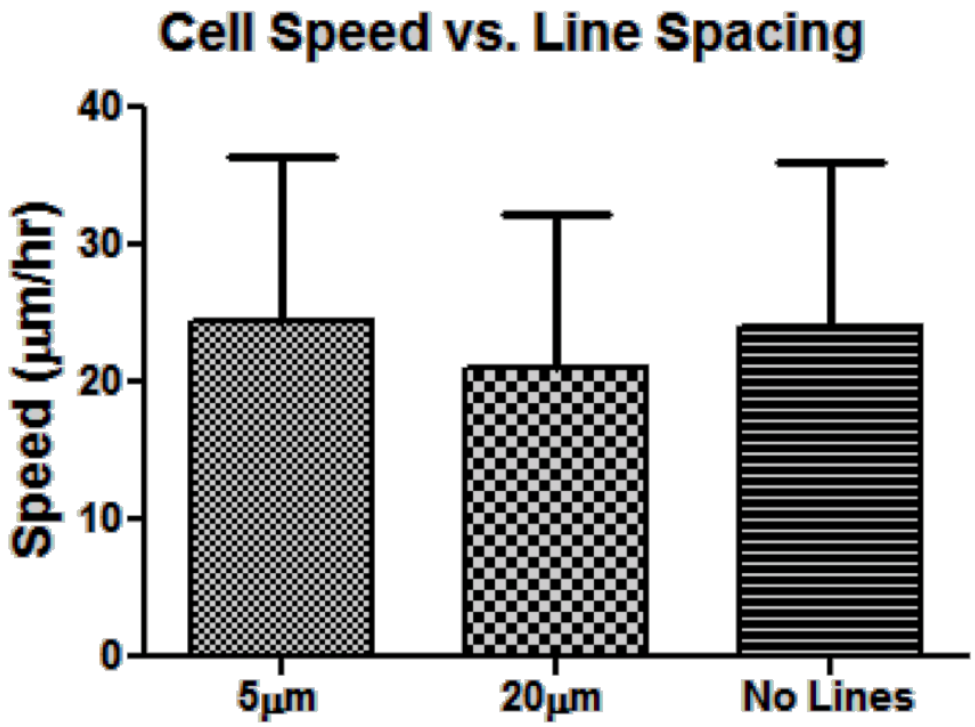


Figure 16: Optical Profilometer testing of PDMS sin coated heights.

A



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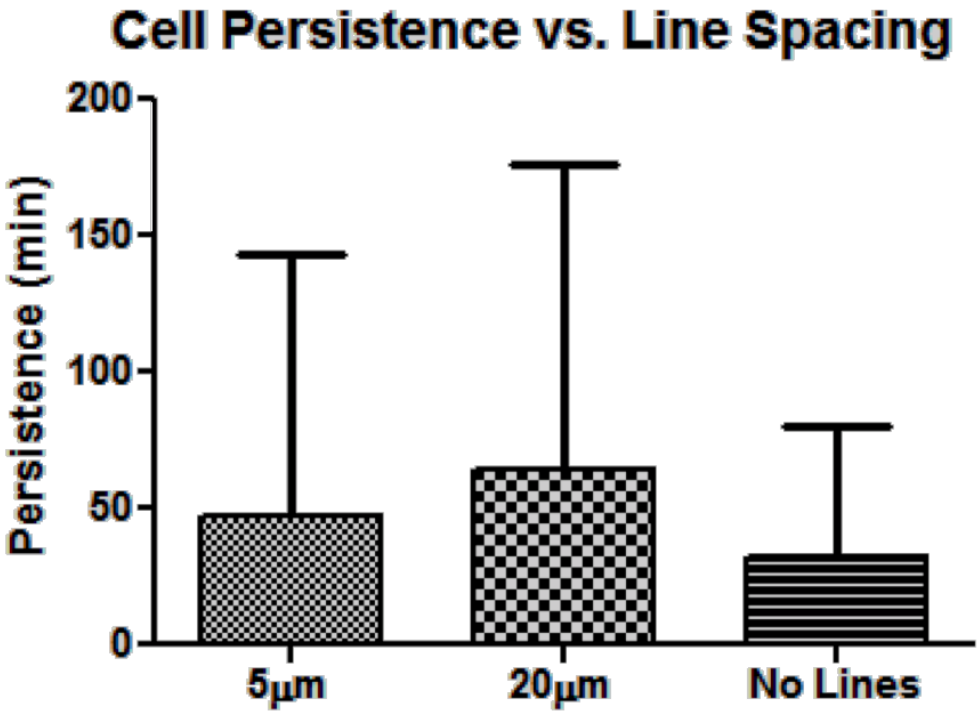
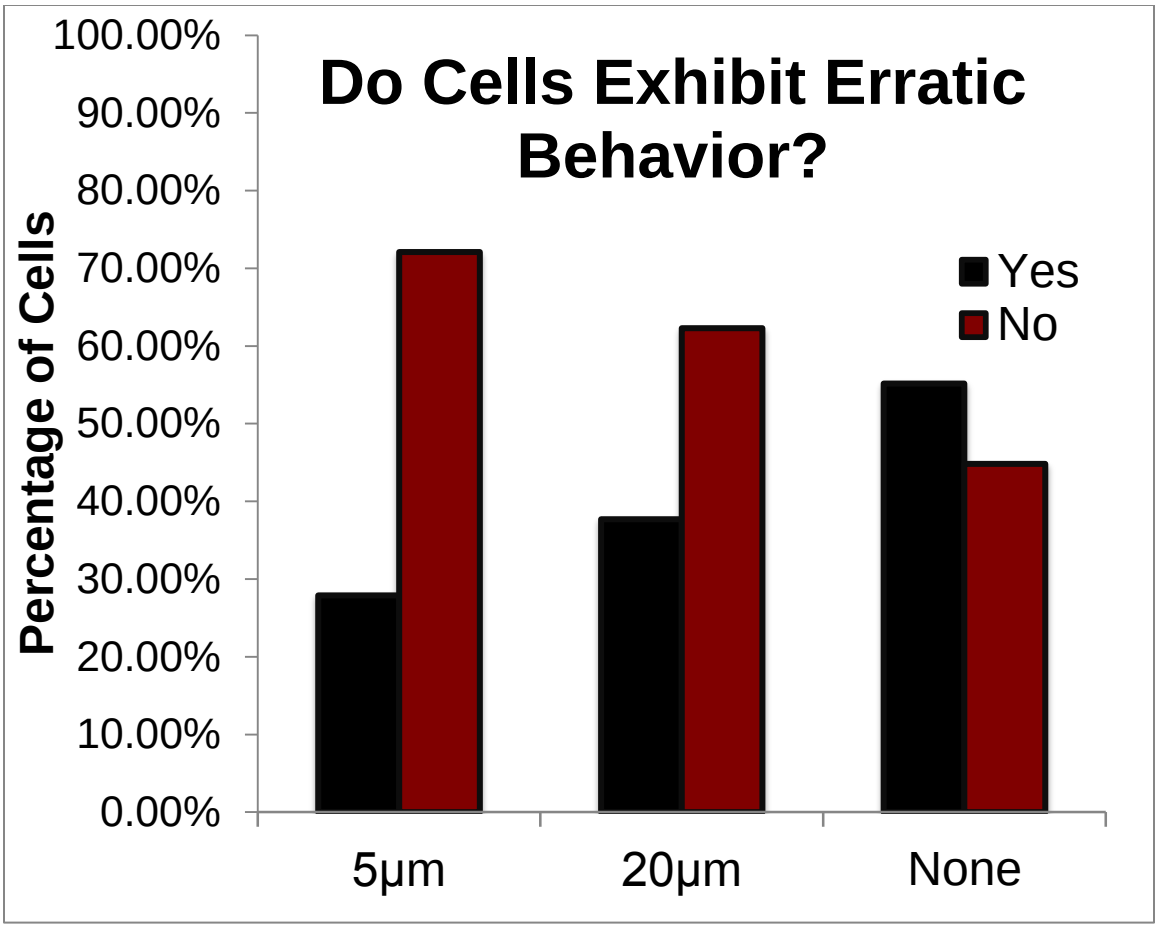


Figure 17: Cell speed (A) and persistence (B) on composite PDMS with lines.

A



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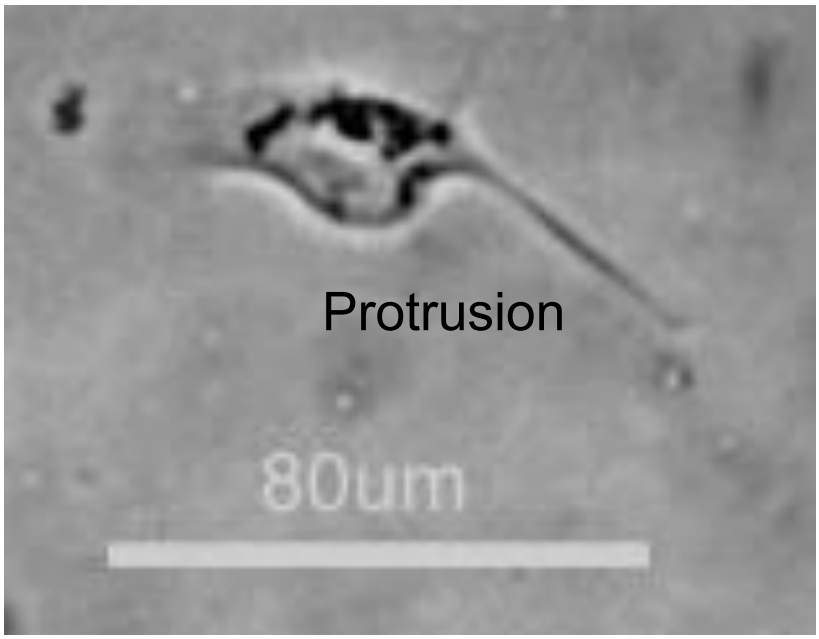

Figure 18: Cell behavior on lines (A). Brightfield image of MDA-MB-231 cell with protrusion on composite PDMS with lines (B).

A

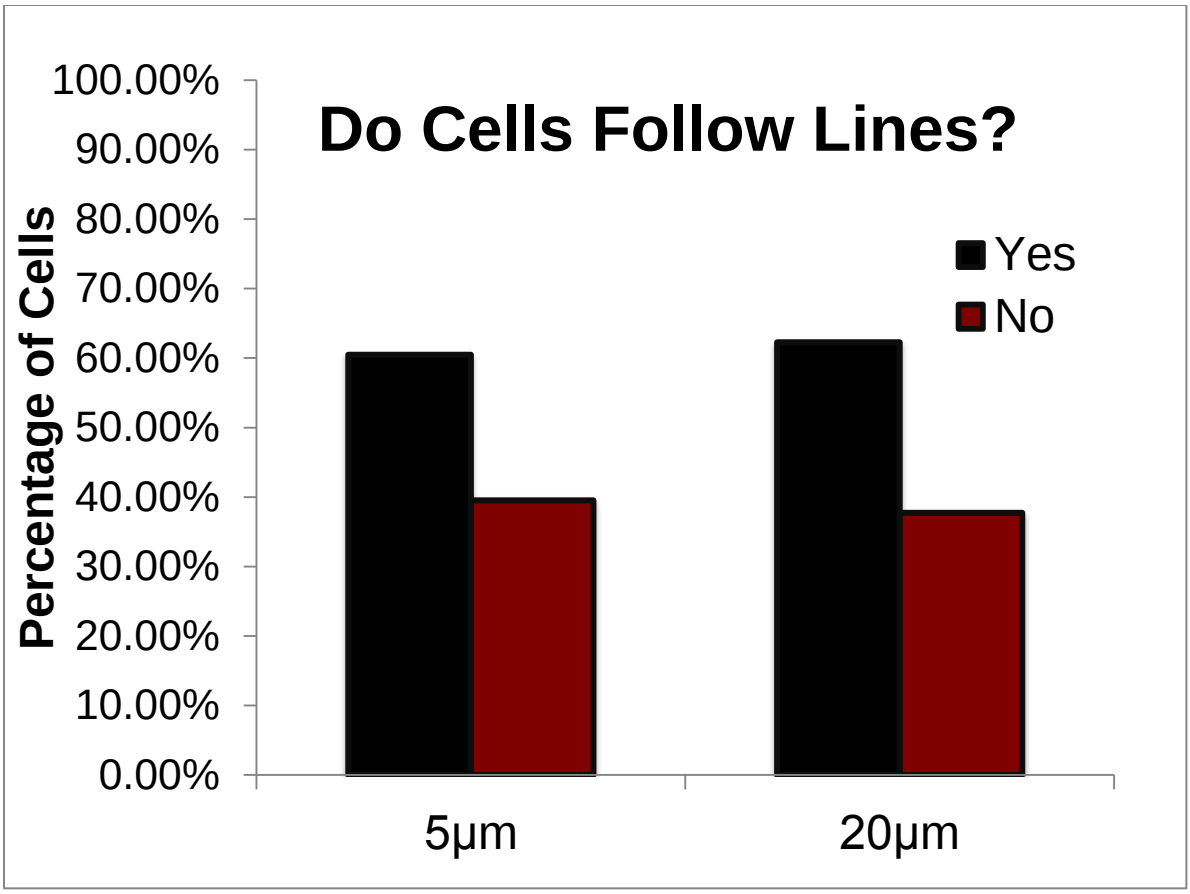

Figure 19: Percent cells follow lines (A) Brightfield image of MDA-MB-231 cells on PDMS composite. with lines (B).

Future Directions

The goal of this project is to understand what the length scale is cells sense the surrounding mechanical cues. We have developed a system where we can tune the modulus by PDMS crosslinker, stiffness by top PDMS height and embed nano lines which add a stiffness and topographical cue. It would be advantageous to investigate how varying the height of the top layer PDMS influences cells to sense the lines underneath. Varying the line spacing and complimenting with staining of focal adhesions will illustrate phenotypic cellular response and “sensing” of the topography. It has been a struggle to quantify how persistent or aligned the cells follow the lines other than by eye. Although we are using a lower modulus of PDMS (5 kPa) we cannot vary the modulus as easily as we can with other material such as the PEG gels. Instead of using PDMS one can attempt to use PEG-PC gels again and try to print or float lines onto the gel then backfill with additional PEG-PC thus embedding the lines.

Once a concrete model has been established, as suggested with the PEG-PC and PDMS-PS systems, identification of signal pathways, cell adhesion, shape and protein composition would be advantageous to identify at various time points. Future objectives would be to identify “real-time” cell-matrix interactions and intercellular components which facilitate the initial and long term mechanosensing.

CHAPTER 6

CONCLUSION

Herein I sought to study how mechanical, topographical and biochemical cues affect metastatic breast cancer. I sought to understand how the extracellular matrix (ECM) influences individual cell migration at the primary tumor site. ECM components have previously been shown to affect cell motility via ligand-receptor interactions, and physical cues, such as matrix stiffness, modulus and protein density. The primary tumor site significantly stiffens during tumor progression. The ability cells have to sense and respond to these matrix features influences and facilitates cell invasion. It is now widely accepted that mechanical properties of the ECM can regulate cell migration; however, presently, tissue *modulus* and *stiffness* have been used interchangeably. It is unknown if cell responses are sensitive to a bulk tissue modulus or stiffness on the geometric length scale of the cell. It is our objective to create tunable biomaterials from known materials to parse the roles independently of stiffness and modulus upon the migration of breast cancer cells. We created and utilized several biomaterials which we can tune both the modulus and stiffness independently of one another.

On PEG-PC cell migration has a biphasic response at various protein concentrations at intermediate stiffness and modulus. With our PDMS-PS system, like the PEG-PC system, we saw peak cell migration and a biphasic response at intermediate modulus and stiffness, at high protein concentrations. In addition, as PS height increases there is less cell response and a decrease in cell speed Last, our Cd/Se lines proved cells can sense the line topography embedded within layers of PDMS yet unlike the previous models cell speed is not as influenced by the changes in

line spacing. Further testing should be conducted to determine if changes in PDMS height and modulus affect cell migration which could agree or disagree with the PDMS-PS and PEG-PC models.

All of these systems complement each other by having tunable properties of stiffness and modulus. Cells adhere and respond to these materials differently yet are sensitive to the independent parameters we have addressed. It is with future directions these models can be used for further experiments to identify various signal pathways and associative proteins related to cell-matrix mechanosensing. In addition to initiate inspiration for future biomaterials in order to bridge the gap between 1D, 2D and 3D models, which will gain knowledge to improve cancer drug delivery and therapeutics.

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