MORPHOLOGICAL CHANGES TO BREAST CANCER CELLS INDUCED BY LONG-TERM CULTURE ON RIGID SUBSTRATES

by

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A thesis submitted to Johns Hopkins University in conformity with the requirements for the degree of Master of Science in Engineering

Baltimore, Maryland

May 2019

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Abstract

Metastasis occurs when malignant cancer cells disseminate from a primary tumor and colonize a secondary site in the body. It is attributed to over 90% of all cancer-related deaths, and there is only a 27% 5-year survival rate for metastatic breast cancer as opposed to 99% for localized cancer. Tumorigenesis, which is the formation of tumors, is characterized by extracellular matrix (ECM) remodeling and stiffening. While the characteristics regarding cancer formation are still largely unknown, the rigidity of the ECM is commonly accepted as an important regulatory factor for tumor invasion and metastasis, for an increase in ECM stiffness is proven to enhance cell growth and survival and promote migration. There has been many studies done on the correlation between ECM stiffness and cancer cell morphology. However, the impact of long-term culture on stiffness substrate remains unexplored, for most studies only culture the cells for up to 72 hours. In this study, we observe the morphological changes in breast cancer cell lines caused by long-term culture on substrates of varying rigidity. We find that although the correlation between cell area and substrate stiffness depends on the individual properties of each cell line, there is a general increase in elongation as culture time increases. Additionally, we found that the morphology of cells is elastic, for we observed changes in cell shape and area even after 4 weeks and 8 weeks of culturing. Finally, our data implies that the effect of substrate stiffness on cell morphology is dependent on the length of culturing time.

Advisor: Dr. Denis Wirtz

Reader: Dr. Pei-Hsun Wu

Acknowledgments

First and foremost, I would like to thank Dr. Denis Wirtz for being a wonderful mentor and advisor. He has always inspired me to be the best I can be and pushed me to think outside of the box as well as to believe in my own abilities. I would also like to thank Dr. Pei-Hsun Wu for basically guiding me through this entire project (and life) for the past 3 years. Without him, this report would still be blank. He taught me that things can always be better, and the importance of being meticulous and organized. The rest of Wirtz Lab deserves a big thank you too, especially Tiffany Chu and Qianyue Huang for being so supportive and encouraging. Kyu Sang Han also deserves a special call out for being my first lab partner at Wirtz Lab, and helping me with bits and pieces of this project. Finally, I'd also like to thank John Del Toro, Zachary Rodriguez, Steven Doria, and all my other friends and family who have supported me this far. I couldn't have done it without you guys.

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Introduction

The extracellular matrix (ECM) stiffness is commonly accepted as an important regulatory factor for tumor invasion and metastasis. However, the impact of long-term stiffness effects on cells remains unexplored. When cultured for 24 hours, MDA-MB-231 cell area increases dramatically with increasing stiffness (Fig. 1)[1].

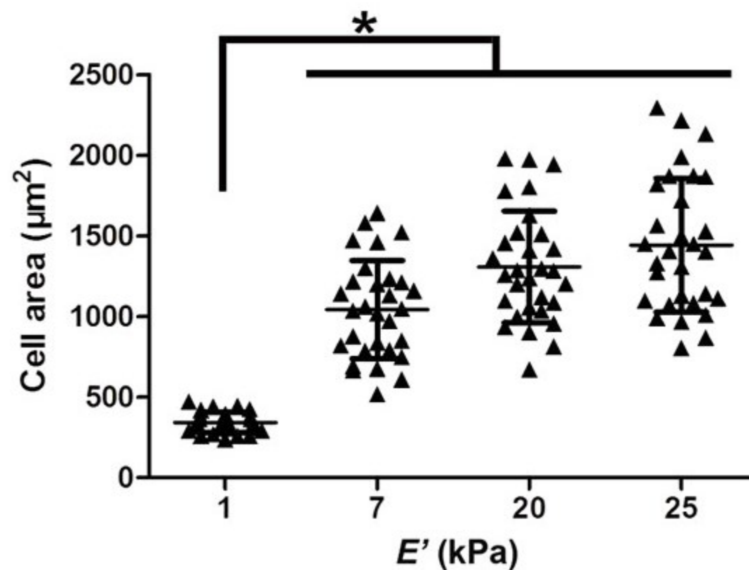

Figure 1: Cell areas of MDA-MB-231 on PAA gel substrates[1]. After 24 hours of culturing, there is a positive correlation between cell area and stiffness

Yet, when cultured for 6.5 hours, a different correlation is observed. MDA-MB-231 appears to have a very slight increase in cell area at 7.2 kPa but as substrate stiffness increases the cell area decreases back to the same levels as they were on 2 kPa substrate (Fig. 2)[2]. This suggests that the effect of rigidity on cell morphology largely depends on the length of time the cells are cultured on the substrate.

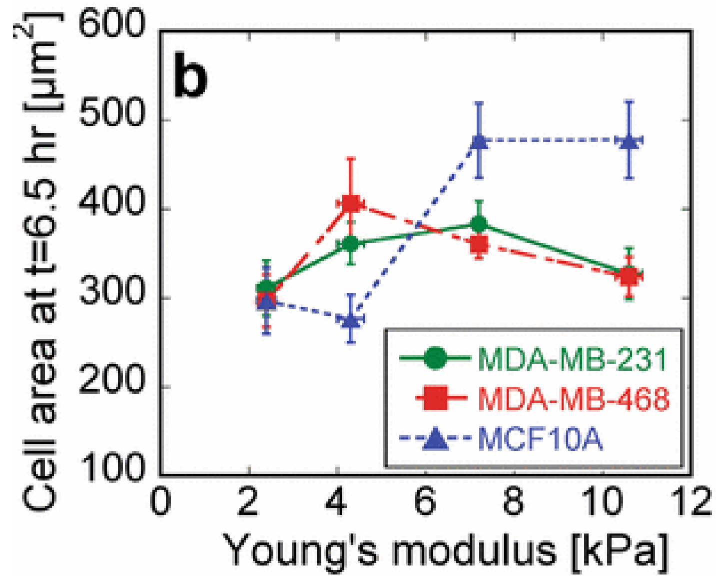

Figure 2: Averaged cell area of MDA-MB-231 on PAA gel substrates[2]. After 6.5 hours of culturing, the area of MDA-MB-231 shows a decrease after an initial increase.

This leads us to the question of what the effects of long-term culture on stiffness substrates could be. Most of existing literature only culture cells for 48 hours and under, at most 72 hours. However, most cancers are not detected until 3 to 6 years after tumors form[3]. Thus, the morphology of cancer cells at 72 hours may not be the same morphology observed after 3 years.

Additionally, metastatic cells are exposed to varying levels of elastic moduli depending on the organs they arrest to. For example, a breast cancer cell that metastasizes to the lungs would first be surrounded by breast tissue that is around 800 Pa to a tumor environment greater than 23 kPa, then to lung tissue which is only 200 Pa. Thus, we will also observe the effect of culturing breast cancer cells on soft substrate then letting them “recovery” on stiff substrate.

Background

2.1 Breast Cancer Overview

Breast cancer is the most common cancer in women, with an estimated 41,760 deaths and 269,600 new cases of invasive breast cancer diagnosed in the US in 2019[4]. Despite improvements in early detection and treatment, it is still the 2nd leading cause of cancer related deaths for women (Fig. 3). Old age and being a woman are the strongest risk factors for breast cancer, followed by having a family history of breast cancer, high breast tissue density, and high-dose radiation to the chest at a young age. While 5-year relative survival rate for women with invasive breast cancer appears to be high, at 90%, the location of the cancer greatly impacts the survival rate. Localized breast cancer, which accounts for 62% of cases, has a 5-year survival rate of 99%. However, distant breast cancer only has a 27% 5-year relative survival rate[5].

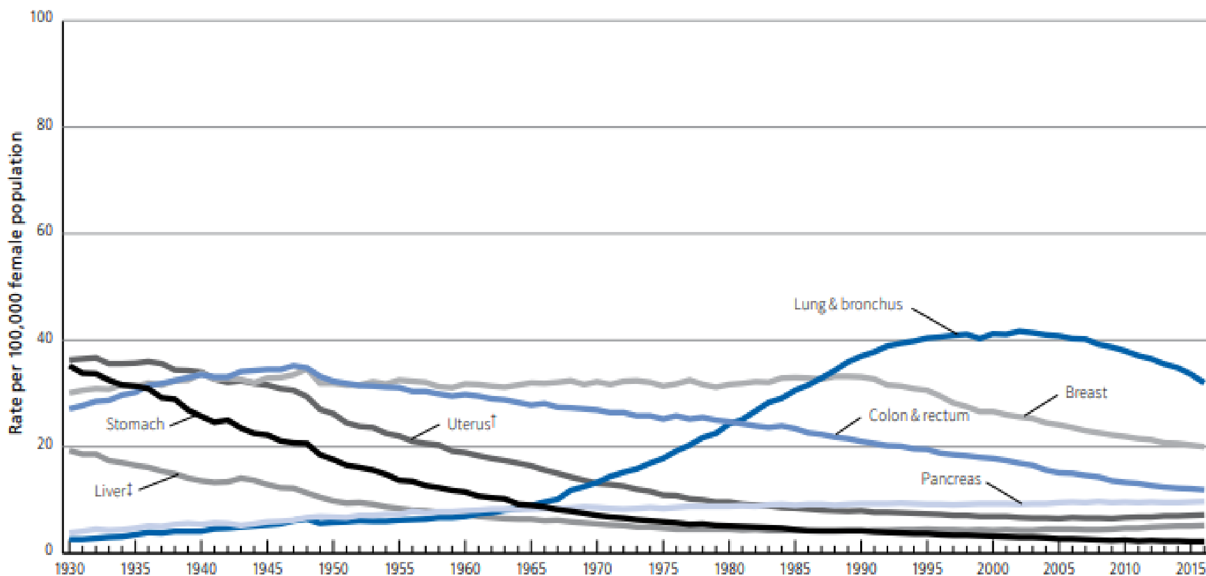

Figure 3: Trends in age-adjusted cancer death rates by site, females, US, 1930-2016[4]
According to the American Cancer Society, breast cancer is the second leading cause for death by cancer. Age is adjusted to the 2000 US standard population and excludes deaths in US territories.

2.1.1 Metastasis

Metastasis occurs when malignant cancer cells disseminate from a primary tumor and colonize a secondary site in the body. Over 90% of mortality from cancer is attributed to metastases: while surgery and adjuvant therapy can cure well-confined tumors, metastatic disease is largely incurable because of its systemic nature[6]. Metastases are formed following a series of cell-biological events: (1) A primary tumor is formed, (2) cancer cells invade surrounding extracellular matrix (ECM) and stromal cell layers, (3) intravasation into the blood or lymphatic vessel through the basal membrane, (4) travel and survive through the circulation, (5) arrest at a distant organ site, (6) exit the circulation and into the parenchyma of distant tissues, (7) form micrometastases in the tissue, and finally (8) proliferate and colonize into clinically detectable macroscopic metastases, becoming another tumor (Fig. 4)[7]. Theoretically, cancer cells can disseminate to a variety of secondary location. However, individual carcinoma types are observed to form metastases in a limited subset of target organs. For example, breast cancer disseminates to only the brain, lungs, liver, and bone[8].

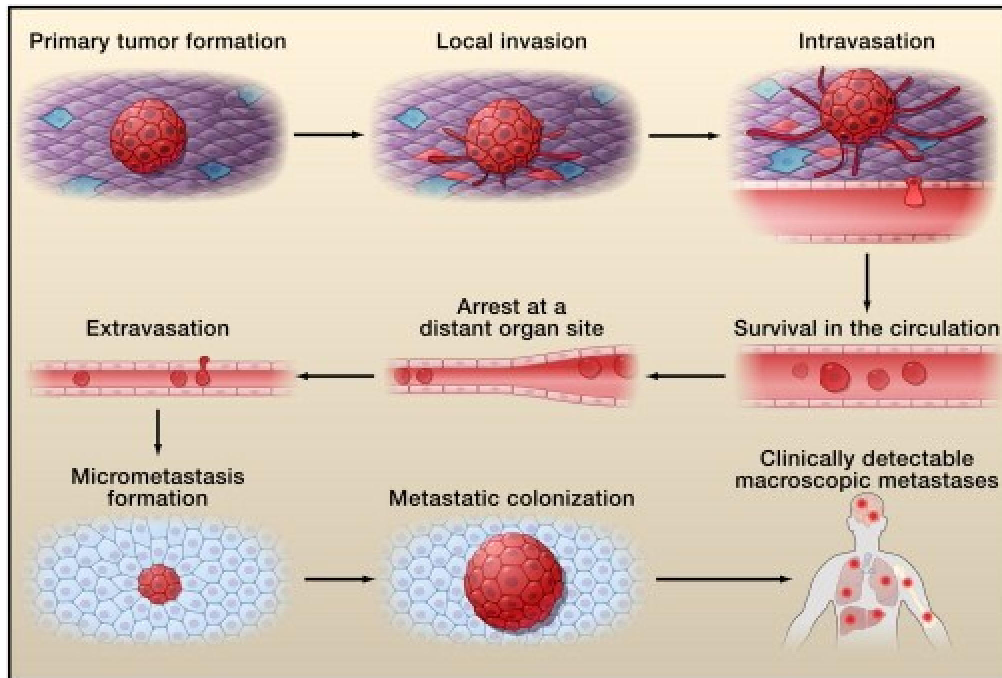

Figure 4: The Invasion-Metastasis Cascade[7]
During metastasis, tumor cells spread out from their primary sites, travel through circulation, arrest at a distant organ site different from the primary site and form another tumor there. Carcinoma cells are in red.

2.2 Tumorigenesis

Tumorigenesis, which is the formation of tumors, is characterized by extracellular matrix (ECM) remodeling and stiffening. While the characteristics regarding cancer formation are still largely unknown, the physiological state of the tumor microenvironment (TME) is closely connected to every step of tumorigenesis[9]. Reduction of cell tension has been shown to normalize the behavior of breast cancer cells in culture, and ECM crosslinking and stiffness have been shown to cooperate with an oncogene to promote breast cell invasion[10]. Tumor microenvironments have also been found to display much higher stiffness than normal tissue. Regarding breast cancer, while normal breast tissues are around 150Pa, tumor tissues have been found to range from 3 to 160 kPa[11].

2.2.1 Extracellular matrix rigidity and metastasis

The extracellular matrix (ECM) is pivotal for the transmission of signals in and out of cancer cells through integrins[12]. Desmoplasia, which is characterized by the formation of dense ECM with increased levels of collagen and fibronectin, is associated with increased production and secretion of tumorigenic growth factors[13]. The mechanical properties of the ECM are therefore important factors in regulating the differentiation and proliferation of cells. It has been shown that the stiffness of the ECM and the cell environment effects cell morphology, motility, protein expression, and force generation[14]. Additionally, an increase in expression of collagen is associated with elevated incidence of metastasis (Fig. 5)[15]. ECM rigidity has been shown to upregulate cancer invasion, cell contractility, and focal adhesion formation, and the interactions between single cancer cells and their microenvironment have also been evaluated to be directly correlated with the cells' invasiveness and metastatic potential: an increase in ECM stiffness is proven to enhance cell growth and survival and promote migration[16].

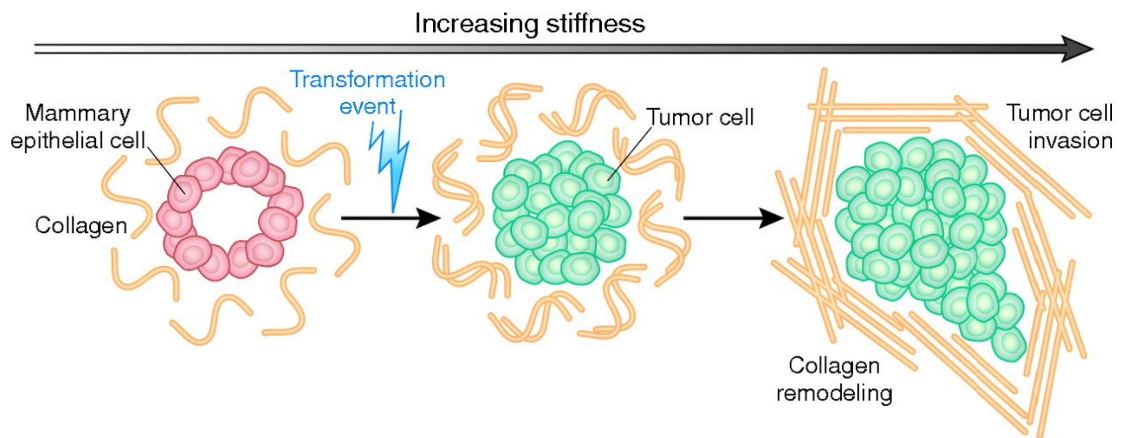

Figure 5: Modeling mammary epithelial cells in vitro[15]. Increased invasive ability correlates with the development of disorganized and branching collagen structures around the tumor, which increases the stiffness of the surrounding microenvironment

2.2.2 Extracellular matrix rigidity and cell morphology

Cell morphology depend strongly on substrate stiffness. Previous studies have shown that cells generate more traction force and develop a flatter morphology on stiff substrates. Cells are observed to be rounder and smaller in area on soft substrates, while appearing well-spread and elongated on stiff substrates[17]. Low invasion cells are also less effected morphologically than high invasion cells: while there is a 77% increase in cell area for MCF7 cells seeded on stiff substrate compared to moderate substrate, there is a 92% increase for MDA-MB-231 cells (Fig. 6)[17]. MDA-MB-231 cells were also more elongated and had more noticeable lammelopodia, which is an indication of higher invasion ability[18]. Additionally, while stress fibers are absent from single cells on soft substrates, once cells made cell-cell contact they become elongated and develop stress fibers[18].

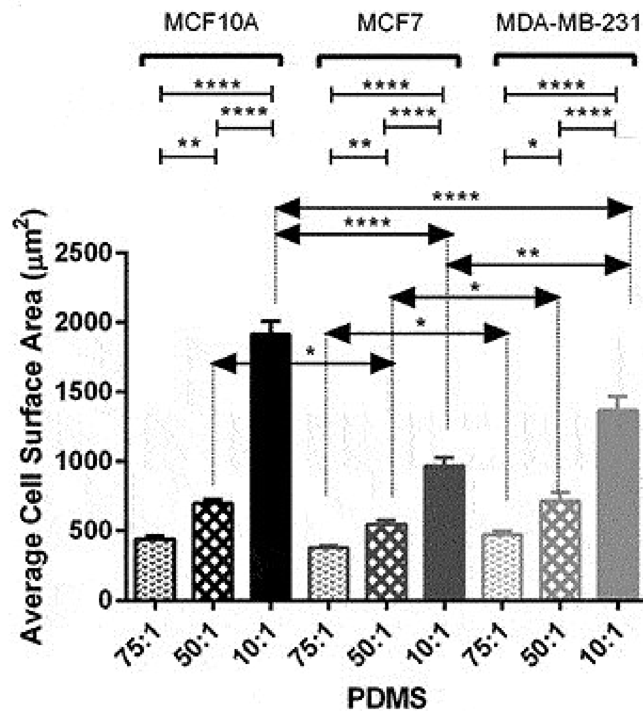

Figure 6: Morphological analysis of MC10A, MCF7 and MDA-MB-231 on substrates with different elastic moduli[17]. Average cellular surface area of different lines after 48 hours.

2.3 Cell Morphology

Cell morphology is the observation of the form and structure of cells. As the ability of cells to change shape and modify their internal structure is directly correlated to their ability to invade surrounding tissue, a change in morphology could imply changes in cell phenotype. While morphological change is not a guarantee that there are genomic changes, it is an efficient and cost-effective way to detect characteristic changes from environmental causes. Quantification of gene expression through cDNA synthesis and real-time PCR is complicated, expensive, and requires many cells. The RNA extraction step alone requires a sample of 10^6 cells, which makes working with a wide variety of samples improbable. However, physical changes to cell shape can be easily observed using high throughput cell array phenotyping (htCP) imaging.

2.3.1 High Throughput Cell Array Phenotyping (htCP) imaging

HtCP imaging is a way to compare and observe a large sample size of heterogenous cell populations. Cell nuclei and F-actin are stained with fluorescent stains, allowing morphology to be observed using fluorescent microscopy. By utilizing custom MATLAB algorithms, the microscopy images can then be analyzed digitally where cells are segmented from the image background based on intensity. This allows a further understanding of cell area, shape, and proliferation rate. Additionally, htCP imaging only requires 2000 cells per well to obtain enough range of data.

2.4 Cell culture substrate

While *in vitro* cells are typically cultured on plastic or glass culture plates, they are not ideal for experiments regarding stiffness. Glass has an elastic modulus of 50-90GPa, while polystyrene is around 3-3.5GPa[19]. In comparison, neural, lung, breast, and endothelial tissues are all under 1500Pa. Therefore, special plates are required to replicate the ECM we want to observe. For this project, we chose to observe elastic moduli of 0.5 kPa, 25 kPa, and polystyrene to represent soft tissue (brain, lung, endothelial), breast cancer tumor, and bone, which are all locations that breast cancer metastasize to[15].

2.5 Breast cancer cell lines

Breast cancer is highly heterogeneous and composed of many distinct cell lines. The cell lines can be conventionally subtyped into 3 categories based on their receptors: estrogen receptor (ER), progesterone receptor (PR), and human epithelial receptor (HER2). Depending on their receptor status, cell lines can be further classified as luminal A, luminal B, HER2 positive, and triple negative. While luminal tumors have better prognosis and are less aggressive, triple negative tumors and cell lines are highly invasive[20]. To cover a wide range of cell morphologies, we have chosen cells from various categories and tumor sources (Table 1).

Table 1: Categorization and clinical features of tumors of breast cancer cell lines[20]

	ER	PR	HER2	Subtype	Tumor
HCC1954	-	-	+	Her2+	DC
T47D	+	+	-	Luminal A	IDC
MCF-7	+	+	-	Luminal A	IDC
ZR-75-1	+	+/-	-	Luminal A	IDC
MDA-MB-468	-	-	-	Basal A	AC
SUM149	-	-	-	Basal B	InfDC
BT-549	-	-	-	Basal B	IDC
MDA-MB-231	-	-	-	Basal B	AC
SUM159	-	-	-	Basal B	AnC

The status on ER, PR and HER2 show the receptor status. The 'Tumor' column shows the clinical property of the tumor where the cell lines are derived: AC is 'adenocarcinoma', AnC is 'anaplastic carcinoma', DC is 'ductal carcinoma', IDC is 'invasive ductal carcinoma', InfDC is 'inflammatory ductal carcinoma'.

2.5.1 Single clone cell lines

Cancer cell lines have a mixed cell population, and it is likely that the changes we observe are merely from a selection of the cell population. We want to see whether the previously seen results are because of a selection of the cell population, or if they are intrinsic. Therefore, in addition to the breast cancer cell lines in the table above, we also chose a few MDA-MB-231 single cell clones: SCC6, SCC304, and SCC308. Single cell clones are homogenous in theory, and therefore any morphological changes are more likely to be intrinsic.

Materials and Methods

3.1 Cell Culture

MDA-MB-231, MDA-MB-468, SCC6, SCC304, SCC308, SUM149, SUM159, HCC1954, ZR-75-1, T47D, MCF7, and BT549 cells were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich, St. Louis, MO) supplemented with 10% (v/v) fetal bovine serum (FBS, Corning, Corning, NT) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA). Cells were maintained in a humidified environment at 37% and 5% CO_2 during culture. Whenever passaging is required, cells are passaged with 0.25% trypsin in 0.1% EDTA in HBSS without calcium, magnesium and sodium bicarbonate (Corning, Corning, NY).

3.2 Stiffness Plates

Stiffness plates were purchased from Matrigen. We purchased the Softwell® collagen coated 96-well glass bottom high-throughput screening plates as well as Softwell® collagen coated 6-well plastic plates in 0.5 and 25 kPa (Matrigen, Brea, CA). Collagen coated plastic plates were 6-well cell culture dishes (Corning, Corning, NY) coated with 50µg/mL of type 1 rat tail collagen (Corning, Bedford, MA) in PBS at room temperature for 30 minutes to allow the gelation of collagen. Although we can make our own plates out of PDMS or Matrigel, we chose to purchase commercially available plates coated with polyacrylamide hydrogels for efficiency and better quality control.

3.3 Cell staining

Culture media was removed from the assay plate. 50μL of 4% paraformaldehyde in PBS was added to each well for 15 minutes, then each well was washed with 50μL of PBS 3 times. The cells were permeabilized for 10 minutes in 0.5% Triton-X in PBS at room temperature, then washed with PBS for 3 times again. Each well was then blocked with 50μL of 1% bovine serum albumin (Corning, Bedford, MA) for 30 minutes in the dark. Lastly, 50μL of staining solution of 1% Hoechst 33342 and 1% Phalloidin 488 in PBS (Invitrogen, Carlsbad, CA) was added to each well for 40 minutes, then washed with PBS 3 times.

3.4 Imaging

To monitor cell proliferation, images were collected every day using a Nikon TE2000 microscope equipped with a x2 objective (Nikon, Melville, NY) and a Cascade 1K CCD camera (Roper Scientific, Tucson, AZ). For htCP imaging, a x10 objective (Nikon, Melville, NY) was used; each well was sectioned into 16 images, which was then stitched together using MATLAB and ImageJ. Using a custom MATLAB module, we segmented the cell and nucleus from the image background to automatically calculate cell area and count based on fluorescence intensity.

3.5 Short term culture of breast cancer cells on HTS for repeatability

Cells were seeded at a concentration of 2000 cells/well on to a Matrigen Softwell® collagen coated 96-well glass bottom high-throughput screening plate for 24 or 48 hours. Since

each column is a different hydrogel stiffness, each row was a different cell line. They were then fixed, stained, and imaged.

3.6 Long term culture of breast cancer cells

MDA-MB-231, SUM159 were seeded at a density of 15,000 cells on substrates of 25 kPa, collagen coated plastic, and plastic 6-well plates, and a density of 30,000 cells on substrate of 0.5 kPa. HCC1995 was seeded at a density of 40,000 cells on substrates of 25 kPa, collagen coated, plastic, and plastic 6-well plates, and a density of 60,000 cells on substrate of 0.5 kPa. Each condition had 2 repeats. Cells were cultured for a week, with media changes ever 3 days. Cells from each condition were then seeded to a 96-well plastic bottom black assay plate at a confluency of 2000 cells/well, with 4 repeats for each condition. The assay plate was incubated for 24 hours before being fixed and stained. Remaining cells were seeded back into their respective 6-well plates for another week then repeated. This is shown in Figure 7:

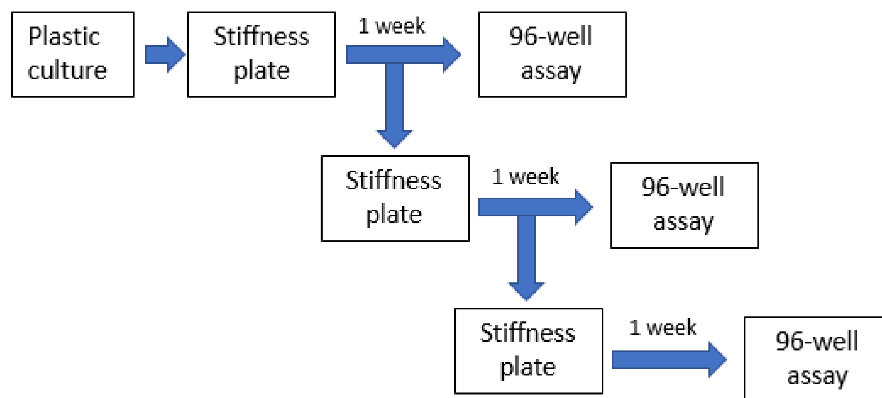

Figure 7: Flow chart for long term culture of breast cancer cells

3.7 Long term culture of single cell clones

SCC6, SCC304, and SCC308 cells were seeded at a concentration of 15,000 cells/ml on plastic, collagen coated plastic, and 25 kPa 6-well cell culture plates, and at a concentration of 30,000 cells/ml on 0.5 kPa 6-well cell culture plates. Each plate had 2 repeats of each cell line. Media was changed every 3 days, and cells were passed on to new plates at the same concentration whenever confluency is reached. At 4 weeks and 8 weeks, samples of cells from each condition was plated to a 96-well black assay plate at a concentration of 2000 cells/well for 24 hours and then fixed, stained, and imaged.

3.8 Recovery of breast cancer cells from culture on soft substrate

SUM159, SUM149, HCC1954, MDA-MB-231, T47D and MCF7 cells were cultured on stiffness 6-well plates of 0.5 kPa, 25 kPa, collagen coated plastic, and plastic for 1 week. 15,000 cells were seeded per cell line for stiff plates, and 30,000 cells were seeded per cell line for 0.5 kPa plates. After 1 week, each condition was plated to a 96-well black assay plate at a concentration of 2000 cells/well for 24 hours and then fixed, stained, and imaged. The remaining cells were plated on to plastic 6-well plates for another week before being plated to an assay plate. This flow is shown in Figure 8:

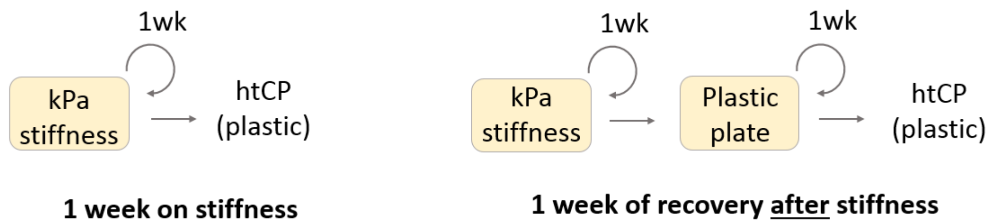

Figure 8: Flow chart for recovery of breast cancer cells from culture on soft substrate

Results and Discussion

4.1 Cell area and proliferation increases with substrate rigidity after 24 and 48 hours

To test the repeatability of prior literature, we utilized the Softwell high-throughput screening plates to observe morphological changes over a range of stiffnesses for 24 to 48 hours. As described in the methods, we seeded 2000 cells per well then fixed and stained one plate after 24 hours and one after 48 hours. Afterwards, we used MATLAB and ImageJ to segment each image and obtain numerical values for cell area and count.

We see that MDA-MB-231 becomes less clustered and more elongated with increase of stiffness and culture time. This is especially prominent between cells on the 8 kPa well at 24 hours and 48 hours, where the cells change from being mostly round to more similar in shape to on those on glass (Fig. 9).

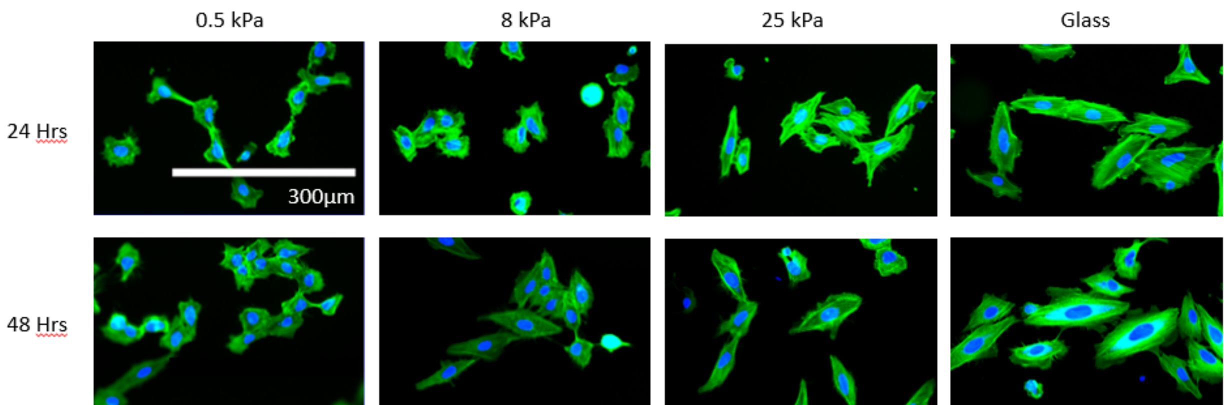

Figure 9: MDA-MB-231 after 24 and 48 hours on various stiffnesses

We observe an increase in cell area with stiffness. Additionally, cells are more elongated after 48 hours compared to 24 hours. Cells on 0.5 kPa are also more clustered than cells on stiffer wells.

We plot the average cell area for each cell line over the range of stiffnesses and observe that all the cells have a positive correlation between area and substrate rigidity (Fig. 10). While the increase is less drastic in non-invasive cell lines (T47D, MCF7) there is still a slight increase. This is in accordance to what is shown in existing literature.

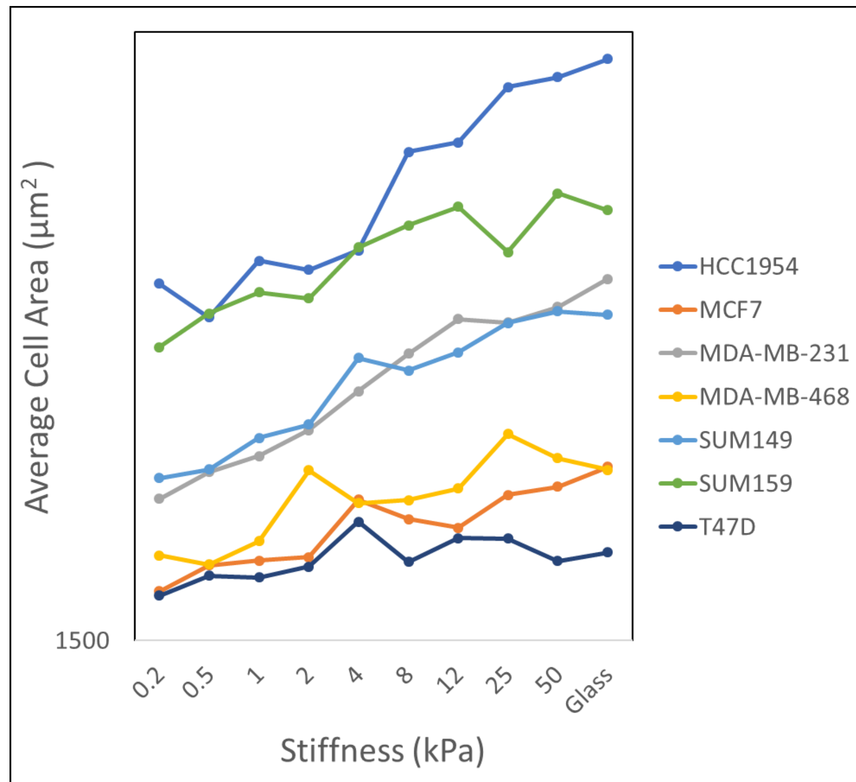

Figure 10: Average cell area of breast cancer cells over substrate stiffness at 24 hours

An increase in cell area is observed as stiffness increases

4.2 Results of long-term culture on stiffness substrate is cell line dependent

Substrate rigidity induced morphological changes on breast cancer cells are time dependent and unique to cell lines. HCC1954, SUM159, and MDA-MB-231 all show an increase in cell area as well as elongation as stiffness and culture time increased, but at varying degrees.

HCC1954 has drastic morphological changes depending on substrate rigidity and culture time (Fig. 11). On 0.5 kPa, cells appear to be small, round and clustered. But on 25 kPa, cells are observed to have very elongated and thin "arms" that branch out; clusters are still observed but there are no round cells present. On collagen coated plastic and plastic plates, cells are in a single layer and shapes range from round to slightly elongated but none of the cells were as drastically elongated as the ones on 25 kPa. After a week, cells on all stiffnesses become even more elongated. By the third week, even cells on 0.5 kPa have begin exhibiting branching although clusters are still observed.

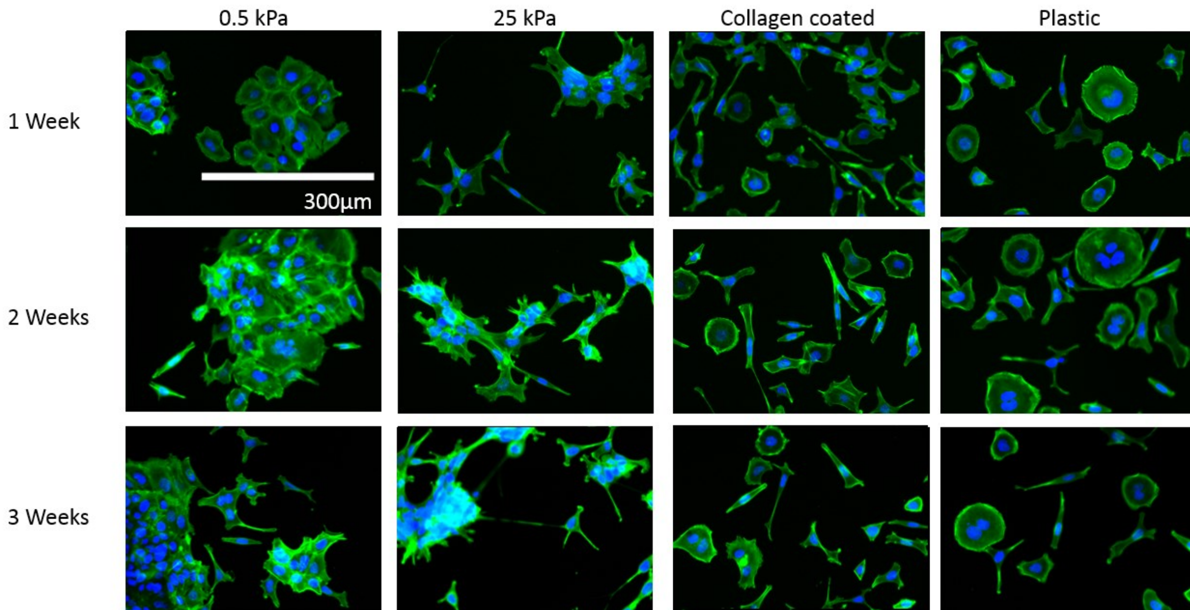

Figure 11: HCC1954 over a range of stiffnesses and time, stained for DAPI and TRITC. As seen in the images, HCC1954's morphology is highly dependent on the substrate stiffness as well as culturing time.

SUM159, on the other hand, does not appear to change much morphologically whether over stiffness or time (Fig. 12). Based on the observed images, the cell shape and area remain

constant throughout the 3 weeks and over the 4 levels of stiffnesses. Cells tend to group more on 0.5 kPa and 25 kPa, but there are no large clusters of cells observed.

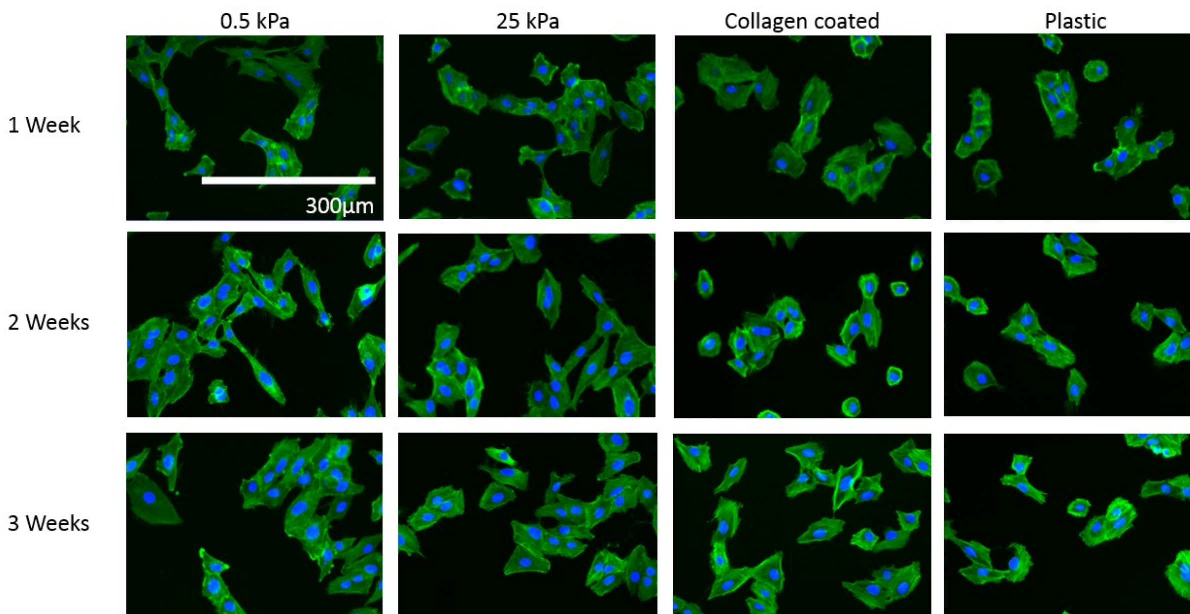

Figure 12: SUM159 over a range of stiffnesses and time, stained for DAPI and TRITC. As seen in the images, SUM159 does not appear to change morphologically over stiffness nor culture time.

MDA-MB-231, like in existing literature, is influenced by both substrate rigidity and culture time (Fig. 13). Cells become more elongated as stiffness and culture time increased. However, MDA-MB-231 cells seem to reach maximum cell area at 25 kPa then decreases in size afterwards, although cells on collagen coated plastic and plastic plates are still larger than ones on 0.5 kPa. Additionally, cells on all stiffnesses increased in area as culture time increased. The cells are not observed to form any sort of cluster, unlike HCC1954 and SUM159.

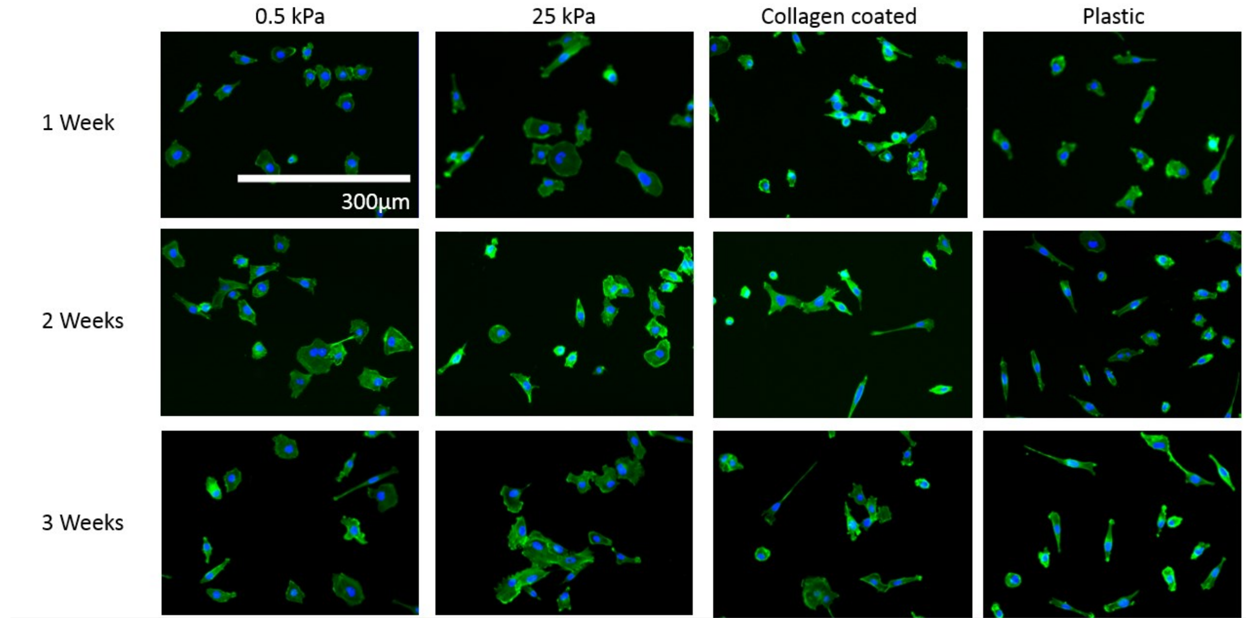

Figure 13: MDA-MB-231 over a range of stiffnesses and time, stained for DAPI and TRITC. As seen in the images, MDA-MB-231 has the largest cell area on 25 kPa then decreases in size. There is an increase in elongation and size as culture time increases, and no clusters are observed.

4.3 Morphological changes of single cell clone on stiffness substrate continues after 4 weeks

SCC6 increases in cell area as substrate stiffness increases (Fig. 14). This is different from MDA-MB-231, which has the largest cell area at 25 kPa. At 4 weeks, cells on 0.5 kPa are small and round, like MDA-MB-231 on 0.5 kPa after 1 week. At 25 kPa after 4 weeks, cells are elongated and visibly smaller than MDA-MB-231 on 25 kPa. At 8 weeks, cells on 0.5 kPa became more elongated while decreasing in size, while cells on 25 kPa appear to be rounder. Neither observation matches what we previously observed with MDA-MB-231. However, SCC's on plastic are a lot larger and rounder than MDA-MB-231 on plastic; therefore, there is a possibility that the single cell clones did not resemble MDA-MB-231 to start with.

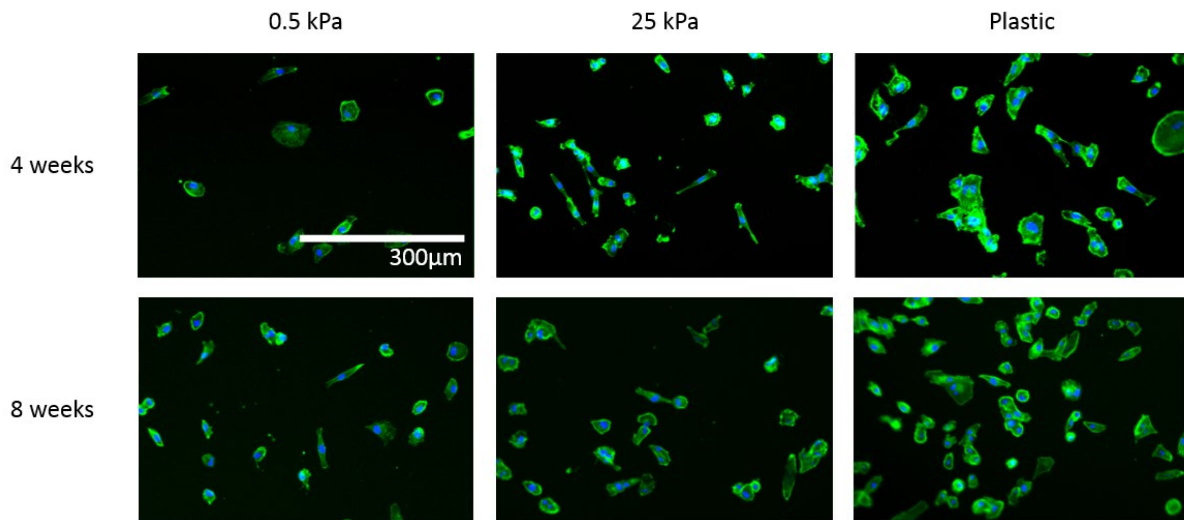

Figure 14: SCC6 over a range of stiffnesses and time, stained for DAPI and TRITC. As seen in the images, SCC6 increases in cell area as stiffness increases. Additionally, there are morphological changes between 4 weeks and 8 weeks, indicating that cell shape and area continues to change over time.

Although the relationship between SCC morphology and substrate rigidity does not mirror that of MDA-MB-231 and stiffness, based on our data it can be observed that even after 4 weeks the cells' morphology are still changing. Cells at 4 weeks and 8 weeks are visibly different, indicating that cells might not have a permanent shape or area. At the very least, it shows that 4 weeks is not long enough of a time to achieve cell shape stability.

4.4 Breast cancer cell lines revert to original morphology after recovery on plastic

For the previous experiments, breast cancer cells were cultured on soft plates then seeded on to plastic assay plates for 24 hours before being stained. To test recovery, we cultured the cells on soft plates for a week then on plastic for a week before seeding them on to an assay

plate. Although each cell line reacted to change in substrate stiffness differently, after a week of culture on plastic, all cells are observed to return to a morphology similar to that of cells only cultured on plastic (Fig. 15).

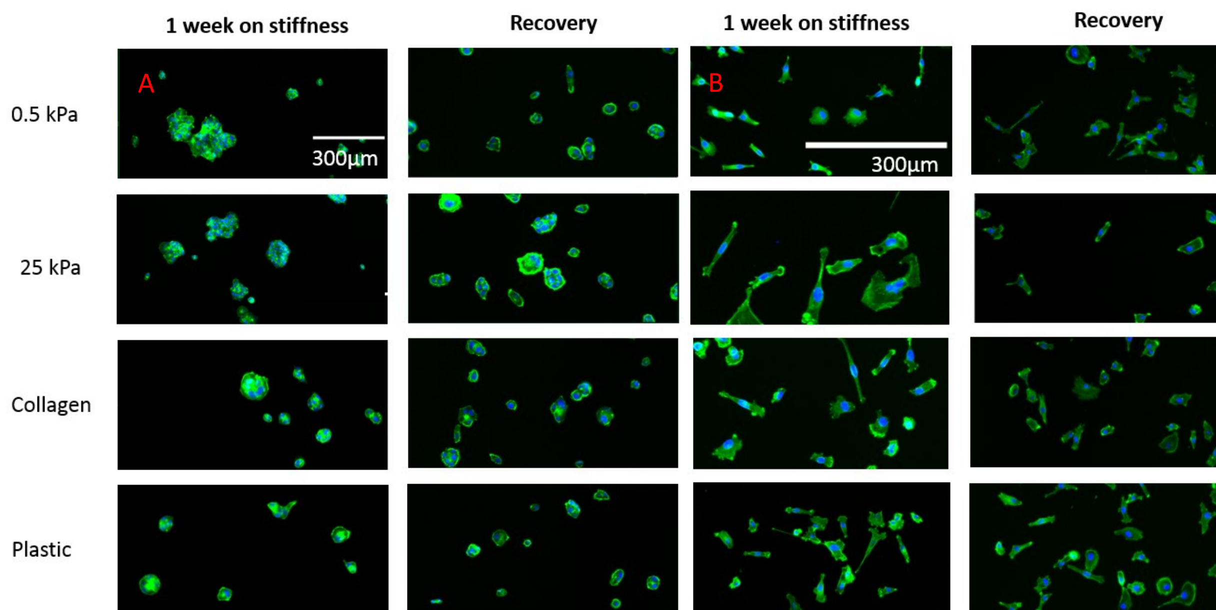

Figure 15: Morphology of cells after 1 week on stiffness substrate and 1 week on plastic. A) MCF7 form distinct clusters on 0.5 kPa and 25 kPa. However, after a week of culture on plastic, cells that were cultured on 0.5 kPa before no longer formed clumps. B)MDA-MB-231 that were cultured on 25 kPa decreased in size after a week of culture on plastic, to match the size of cells only grown on plastic.

MCF7 formed distinct clusters when cultured on soft substrates, with very few single cells. However, after a week of culture on plastic, cells that used to be cultured on 0.5 and 25 kPa are now single cells roughly the size of those only cultured on plastic. Although recovered cells that were cultured on 25 kPa before still appear to be slightly larger in area, they are also single cells and no clusters are observed. As for MDA-MB-231, cells are observed to decrease in size after being recovered on plastic for a week. This is especially apparent for cells that were cultured on 25 kPa previously; in fact, those cells seem to be smaller in area than cells that were only cultured on plastic.

Conclusion

The study described investigated the effects on cell morphology caused by long-term culturing on stiffness substrates. We utilized polyacrylamide coated soft plates with elastic moduli similar to that of tissues that breast cancer metastasize to, then used high throughput cell phenotype imaging to observe changes in cell shape and size.

Based on our results, the correlation between cell area, stiffness, and culturing time is mostly dependent on each cell line's unique intrinsic properties. While most cell lines increased in area as substrate stiffness increased, some remain mostly unchanged (SUM159), or have one specific stiffness where maximum area is observed (MDA-MB-231). Additionally, all cell lines are observed to elongate as culturing time increased.

Furthermore, our work shows that cell morphology is time dependent. This is shown by how despite MDA-MB-231 had a positive correlation between cell area and substrate rigidity when cultured for 24-48 hours, after 1 week we observe that 25 kPa has the largest cell area. This is further shown by the long-term culture of single clone cells, where the cell shape and area of SCC6 still varied after 4 weeks of culture. This is also shown by how breast cancer cell lines were observed to revert to the shape and size of cells cultured only on plastic, despite being cultured on soft substrate for a week beforehand. Our work suggests that the morphology of cells is elastic and constantly changing.

Future Work

Further research will be done to explore changes in cell nucleus area and count as an effect of substrate stiffness. Additional experiments with single cell clones, including recovery from soft substrate, will further demonstrate whether cell morphology is an intrinsic change. We hope to see a similar trend to what we currently see regarding relationship between cell area and rigidity. In the future, we can also stain for e-cadherin to further observe whether there are genomic changes relating to stiffness.

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EDUCATION

Johns Hopkins University — Baltimore, MD

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TECHNICAL SKILLS

Software: Excel, Microsoft Suite, MATLAB, Maple, Adobe Suite, ASPEN

Lab: Maintenance, storage, and treatment of cell lines, cell transfection, plasmid DNA isolation, RNA purification, cell assays (ELISA), protein assays (BCA), PCR, qPCR, Western Blot, microscopy (fluorescent, confocal), 3D cell culture, fix and stain

RESEARCH EXPERIENCE

Institute of NanoBioTechnology (Wirtz Lab) – Johns Hopkins University — Baltimore, MD

Research Assistant — 2015-Present

- Researched the role of substrate tension in regards to chromosome instability and motility within the context of cancer metastasis through long term culturing on varying substrate stiffnesses and fluorescent microscopy
- Documented and analyzed egression patterns of Toxoplasma Gondii in 3D gel matrices using confocal microscopy and Fluorescence Recovery After Photobleaching

Institute of NanoBioTechnology (Sun Lab) – Johns Hopkins University — Baltimore, MD

Research Assistant — 2015-2017

- Fabricated volume devices for growing and measuring cells, and monitored cell volume change using fluorescence exclusion
- Studied the effect of substrate stiffness on cell volume and proliferation controls by quantifying the nuclear localization of YAP/TAZ
    - Gonzalez, N.P; Tao, J; Rochman, N; Vig, D; **Chiu, E**; Wirtz, D; Sun. (2018) "Cell Tension and Mechanical Regulation of Cell Volume". *Molecular Biology of the Cell* 29, 2509-2601

Research Center for Dev. Biology- National Taiwan University Hospital — Taipei, Taiwan

Research Assistant — 2010-2014

- Analyzed the role of Endoribonuclease L (RNase L) regarding its role in inflammation within the context of obesity and adipocyte biology
- Researched the correlation between the increase of polyinosinic;polycytidylic acid and increase in secretion of RNase L in adipose cells using PCR and protein assays

STUDENT LEADERSHIP

Projects in ChemE Unit Operations with Experiments, Johns Hopkins University — Baltimore, MD

Teaching Assistant — Present

- Set up and supervise two 5-hour sections per week of senior ChemE lab, which includes large-scale equipment such as bioreactors, liquid distillers, and gas absorber systems

alpha Kappa Delta Phi International Sorority Inc., JHU Chapter Baltimore, MD
President 2015-2018

- Improved membership retention and funds raised for the Avon Breast Cancer Crusade by over 200% compared to past years
- Organized and hosted multiple community service and cultural awareness collaborations with other student organizations

MAPP (Mentoring Assistance Peer Program), Hop In Baltimore, MD
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