2018

Probing the Effects of Substrate Stiffness on Astrocytes Mechanics

Ariege Bizanti

PROBING THE EFFECTS OF SUBSTRATE STIFFNESS ON ASTROCYTES MECHANICS

by

ARIEGE BIZANTI
B.S. University of Central Florida, 2016

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Mechanical and Aerospace Engineering
in the College of Engineering and Computer Science
at the University of Central Florida
Orlando, Florida

Fall Term
2018

Major Professor: Robert Steward Jr.

ABSTRACT

Astrocytes are among the most functionally diverse population of cells in the central nervous system (CNS) as they are essential to many important neurological functions including maintaining brain homeostasis, regulating the blood brain barrier, and preventing build-up of toxic substances within the brain, for example. Astrocyte importance to brain physiology and pathology has inspired a host of studies focused on understanding astrocyte behavior primarily from a biological and chemical perspective. However, a clear understanding of astrocyte dysfunction and their link to disease has been hampered by a lack of knowledge of astrocyte behavior from a biomechanical perspective. Furthermore, astrocytes (and all cells) can sense and respond to their external biomechanical environment via the extracellular matrix and various other biomechanical cues.

One such biomechanical cue, substrate stiffness changes within the brain under certain pathologies, which subsequently leads to changes in the biomechanical behavior of the cell. For example, increased tissue stiffness is a hallmark of brain tumors that subsequently alters astrocyte biomechanical behavior. Therefore, to gain a better understanding of this process we cultured astrocytes on stiffnesses that mimicked that of the normal brain, meningioma, and glioma and investigated astrocyte biomechanical behavior by measuring cell-substrate tractions and cell-cell intercellular stresses utilizing traction force microscopy and monolayer stress microscopy, respectively. Our findings showed an increase in traction forces, average normal intercellular stress, maximum shear intercellular stress, and strain energy proportional to increased substrate stiffness. A substrate stiffness of 4 kPa showed 2.1 fold increase in rms tractions, 1.8 fold increase

in maximum shear stress, 2.6 fold increase in average normal stress, and 1.6 fold increase in strain energy. While 11 kPa showed a 4.6 fold increase in rms tractions, 6.6 fold increase in maximum shear stress, 5.2 fold increase in average normal stress, and 2.3 fold increase in strain energy. Cell velocity, on the other hand, showed a decreasing trend with increasing stiffness. This study demonstrates for the first time that astrocytes can bear intercellular stresses and that astrocyte intercellular stresses and traction can be modified using substrate stiffness. We believe this study will be of great importance to brain pathology, specifically as it relates to treatment methods for brain tumors.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Robert Steward Jr., for the immense knowledge, patience, motivation and support he gave me throughout this research. His contributions helped me in every part of this study and I was indeed privileged to have him as my mentor for this study. He allowed this paper to be my own work and steered me in the right direction whenever I needed it.

I would also like to thank my lab partner Md. Mydul Islam for his continuous assistance and advice and to my other lab collogues for their insightful comment, as and encouragement while writing this research. I also thank the management of Burnett School of Biomedical Sciences laboratory and the laboratory personnel for being very accommodating and for providing a well enabling environment for the crux of this study.

Last but not the least, I would like to thank my family for their continuous and unparalleled love and support. I am immeasurably grateful and forever indebted: to my parents for their moral and financial support and believing it would (one day) come to an end, and to my siblings, especially my brother Anas, for the endless love, support and strength to reach for the stars. This journey would have not been possible if not for them. I dedicate this milestone for them.

Thank you, Allah, for always being there for me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Significance:

Brain pathologies like brain tumors have devastating effects as it threatens the control center for vital functions of the body. Astrocytes are key players in the CNS response to tumor growth and its characteristic response to injury in the process of reactive astrogliosis seen in numerous neuropathologies, including brain tumors. The increased stiffness of tissue is a hallmark of brain tumors that stimulate astrocytes to respond to the biomechanical change in the environment of extracellular matrix. The exact mechanism governing the tumor cell development and metastasis is unclear. Therefore, this study aimed to provide unprecedented knowledge of quantifying and visualizing astrocytes mechanical behavior in response to variation of substrate stiffness. Additionally, it shed light on a potential cell-cell junction that could possibly be responsible of transmitting the forces and as a result might contribute to tumor metastasis.

Astrocyte Cells and Their Importance in the Central Nervous System

Astrocytes are glial cells that resides in the brain and spinal cords performing critical functions. They tile the whole Central Nervous System (CNS) and are 5 times the number of neurons in the entire CNS (1). They were once only recognized as simple functional support cells of the CNS but they play an active role in neuronal processing and higher cerebral functions (2). Astrocyte derives its nomenclature from two Greek words; astron and kytos meaning star vessel reflecting the star-shaped morphology as can be seen in figure 1 below that shows stained astrocytic cell from rat brain that was performed by GerryShaw own work.

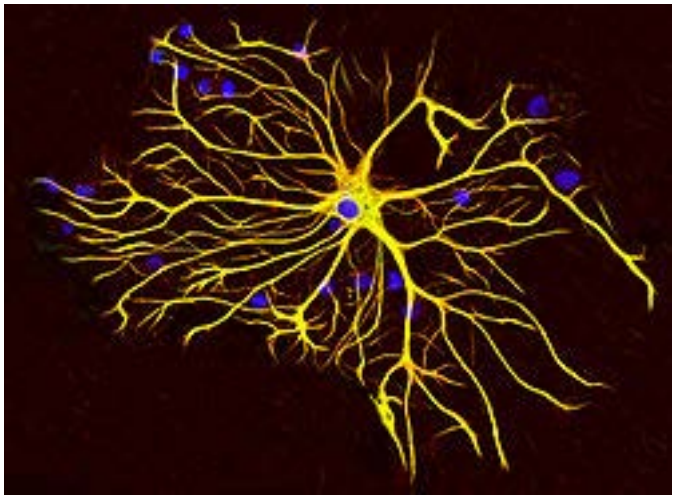

Figure 1: Astrocyte
Source: GerryShaw, "Astrocyte", 2013
https://commons.wikimedia.org/w/index.php?curid=29369565

Functions of astrocytes are outlined below (2-3):

1. They Guide Neuronal Migration during CNS growth and development
2. Astrocytes are the main supply of adhesion molecules in the CNS and extracellular matrix (ECM) proteins. They have receptors for the matrix proteins. The adhesion molecules are crucial for maintenance and development of structural relationships between cells in the CNS. These adhesion molecules also aid regeneration and repair of the CNS after an insult. Some of these molecules are laminin, cytotactin, fibronectin and neural cell adhesion molecule.
3. Astrocytes express some neurite-promoting and neurotrophic factors which are required for neuronal survival and neurite formation.
4. They are key elements in the process of angiogenesis or formation of new blood vessels in the CNS. Their angiogenetic property is important in the repair of the CNS.
5. They induce the blood brain barrier (BBB) and maintain its integrity via their astrocytic end feet. They maintain very tight junctions between endothelial cells in the CNS

6. They are involved in neurotransmission as they can store and breakdown neurotransmitter molecules. This is especially important for termination of transmission. In its control of glutamate transmission, astrocytes take it up from the synaptic cleft and break it down into products for reuse in the neurons.
7. They are important in preventing the build-up of toxic substances in the CNS
8. They regulate ion concentrations, pH and osmolarity in the CNS and Cerebrospinal fluid (CSF)
9. They perform immune functions because they serve as a liaison between the CNS and the immune system by functioning as phagocytes and Antigen Presenting Cells (APC).

All that is stated above represent key functions the astrocytes play in the CNS. Among the most important functions of astrocyte is a process known as reactive astrogliosis, which is the universal response of astrocytes to brain injuries, including neurodegenerative diseases, trauma, and infection. In this process, a biomechanical change in astrocyte cell respond to diverse insults to the CNS hence they play a vital role in any disease of the CNS. In response to an injury to CNS, hypertrophic astrocytes form a glial scar to isolate the injured tissue from normal tissue. Another important function of astrocyte is their ability to sense and integrate external signals in their microenvironment. The 2017 study by Universidad de Barcelona illustrate one mechanism of how cells sense their surrounding environment. In this study, it was concluded that cells apply force through ligand joining that enable them to detect changes in the cell environment. A descriptive statement for that mechanism was stated by Roca-Cusachs “In some way, this would be the equivalent to recognizing someone’s face in the dark by touching the face with your hand, instead of seeing the person.” (2017). Cells usually sense both chemical and mechanical cues from their microenvironment that can impact the fate of cells in vivo. Among the most investigated

mechanical properties is the substrate elasticity. In this research, the focus will be on investigating the effect of substrate elasticity on the biomechanics of astrocytes.

Extracellular Matrix (ECM)

Tissues are made of cells and extracellular space that surround the cells and occupy a substantial volume of tissues. ECM provides a physical scaffolding and mechanical and biochemical cues to cells, additionally, it constitutes of web of fibrous proteins and proteoglycans that is secreted from cells to support surrounding cells (Frantz, Stewart, & Weaver, 2010). The characteristics of ECM generates the mechanical and physical properties of the cells they surround. The biochemical and mechanical cues that ECM provides is essential for various biological process, including morphogenesis and cells homeostasis. Even though ECM components are similar in all tissues, each tissue has a unique composition that contribute to their specialized function. ECM undergoes continuous remodeling that contribute to its dynamic structure. Any dysregulation of ECM modeling or change in composition can lead to pathological conditions. For instance, cancer and fibrosis is characterized with abnormal ECM composition and stiffness (Bonnans, Chou, & Werb, 2014).

Substrate Stiffness of Extracellular Matrix

Fundamental biological processes, including cell differentiation, proliferation and migration are regulated by matrix elasticity [Hadjipanayi et al, 2009]. The importance of substrate stiffness of the extracellular environment of brain cells in directing fate of neuronal cells have been illustrated in many research studies (Engler et al, 2006). Science is looking at how to cure diseases by purely focusing on interfering with these mechanical forces. An example of how the importance of this

is exhibited can be easily illustrated with the effect of interaction of astrocytes with the extracellular cellular matrix in different diseases which affects its movement, migration and morphogenesis. Changes in stiffness in the ECM can be detected by integrins transmembrane proteins that leads to activation of certain protein effectors and amplifiers and eventually induce expression of ECM modifying genes as can be seen in figure 2 below. Normal physiological processes like wound healing undergoes the mentioned mechanosignaling cascade that is eventually resolved, however, in diseased conditions like cancer this cascade is not controlled and remains active (Barnes, Przbyla, and Weaver, 2017).

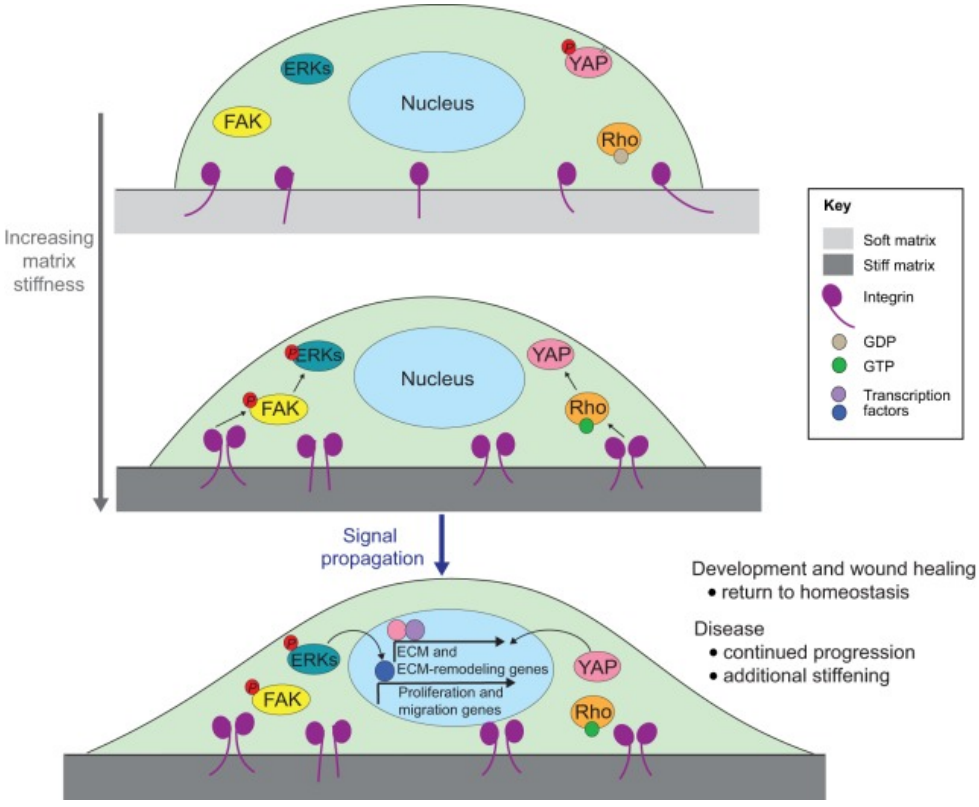

Figure 2: Mechanosignaling in response to increased stiffness (Source: Barnes, Przbyla, and Weaver, 2017)

The healthy human brain stiffness varies with age and sex but averages between 0.1-1kpa and the diseased human brain can have a stiffness as high as 12kpa (Engler et al.-5). Less stiffness can also be a pointer to a diseased brain (6). A study that quantified mechanical properties of freshly isolated human brain showed that meningiomas brain tumor demonstrated a stiffness around 4kpa (Stewrt. Et al. 2017). Another study that was done by Chauvet et al, found that elastic modulus of glioma brain tumor was around 11kpa. In other words, both stiffness extremes can lead to a pathology, therefore, an investigation of how astrocytes behave in soft and stiff substrate can direct us to the etiology of common pathologies. Changes in stiffness influence the biomechanics of astrocytes and their function (6). In Alzheimer's Disease (AD) for example, this neurodegenerative disease is characterized by reduced substrate stiffness throughout the CNS. Using Magnetic Resonance Elastography (6-7), it was shown that there is reduced stiffness especially in the frontal, parietal and temporal lobes. In Alzheimer's, oligomeric Amyloid Beta Peptide (Aβ) alters the mechanical properties of membrane fluidity and molecular order leading to change in substrate stiffness and this has been shown to have effect on astrocytes (8). It is known that cancerous cells embedded within ECM causes elevation in ECM stiffness (Paszek et al,). Glioblastoma is an aggressive brain tumor that is characterized with significant increase in stiffness.

The effect of substrate stiffness on many other cell types in the body have been studied but there is paucity of data on its effect on the biomechanics of astrocytes. Current evidence concerning AD and other neurodegenerative diseases submit that atrophy of astrocytes occurs in the initial stages of such disease. It was shown that astrocytes contribute to the inflammatory component of the neurodegeneration. This is likely majorly influenced by a change in biomechanics of the astrocyte (9). Substrate stiffness has a marked effect on dendritic cells (DCs) which are also APCs (Antigen

Presenting Cells) as astrocytes are. Different degrees of substrate stiffness changed the phenotype and function of DCs drastically such that they could not internalize certain antigens. Looking at these results achieved on DCs, it suggests that substrate stiffness would play a key role in the immunity function of astrocytes as an APC. It is also certain that biomechanics are a key factor in the optimal immune function of astrocytes as a phagocyte and an APC (10).

In a study that was done by (Wang, Tong & Yang), researchers examined varying matrix stiffness on brain tumor. Their study results showed decreased cell proliferation in stiff hydrogels that was assumed due to increased physical constraints and higher retractive forces applied on the cells from the surrounding matrix.

The Polyacrylamide gels (PA) substrate was used in the study of Englar et al. to model brain stiffness in rats on a range of 200 Pa (healthy) to 8000 Pa (diseased). It was observed that astrocytes grown on less stiff substrate demonstrated a close to normal phenotype while those on highly stiffer PA showed varied degrees of astriogliosis. The study proposed that therapeutic strategies targeting the brains microenvironment and astrocytes signaling pathways is a possibility for a closer step to treat neurodegenerative diseases (11).

In this research, I utilize thin polyacrylamide gels (PA) with different ratios of acrylamide to bis-acrylamide to obtain various substrate stiffness while controlling the chemical properties.

Traction Forces

Traction force is the local force that a cell exerts on the underlying substance or ECM which provide the means for many biological processes, including migration, morphogenesis and maintaining cell homeostasis (15). Traction forces are transmitted to ECM via focal adhesions and an illustration for cell traction forces involved in migration can be seen in figure 3 below (Wang & Li). Asides the importance of biochemical interaction between cells, mechanical forces such as traction forces and intercellular stress reactions between cells are important for normal physiological activity (14). The first step to understand how tractions generated by each cell is to localize the traction forces at the leading edge and assess it. Assessing the force at the lead cell and comparing it to other cells would show that large tractions are exhibited by most other cells independent of the lead cell. Thus, all cells can generate traction individually to other signals in the ECM in varying degrees.

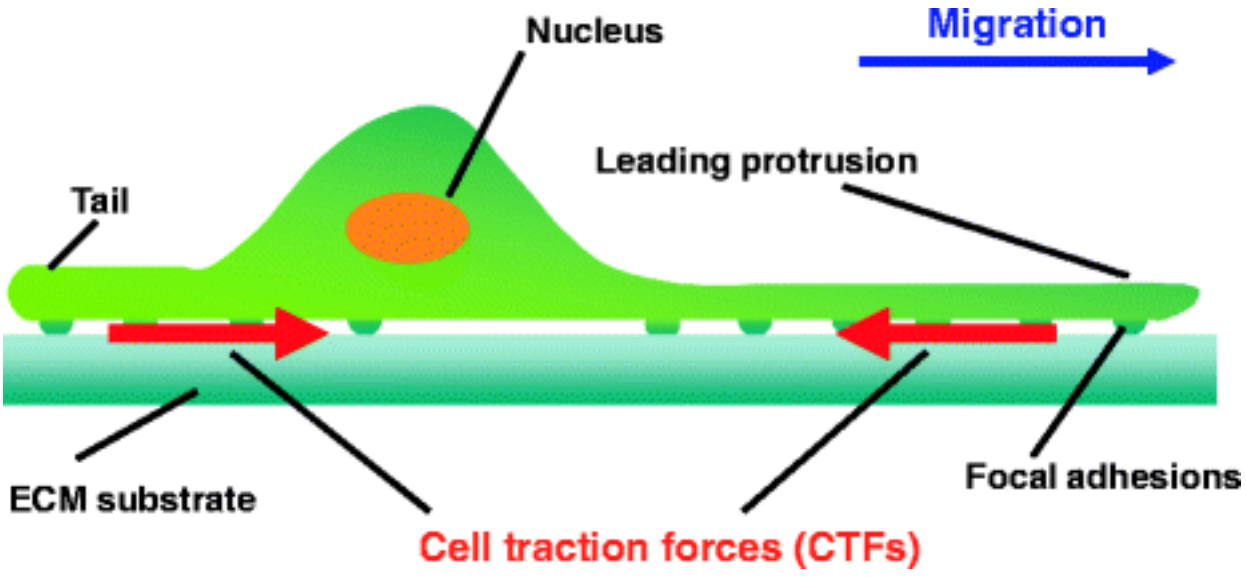

Figure 3: Cell Traction Forces
(Source: R.H. Gavin, 2009)

One of most efficient reliable methods in quantifying traction forces is using Fourier transform force microscopy (FTTC), which will be used in this study. Cell migration is an important function of cells to perform appropriate physiological outcomes, such as wound healing and morphogenesis.

How Traction Forces Are Obtained

In this research, the cell-substrate forces were calculated using Fourier transform force microscopy (FTTC), which is a computational method that measure traction field given the displacement. The traction force is represented by deformation of substrate due to cell-generated stresses. The displacement field is mapped by tracking beads embedded near the surface of a substrate (15-16).

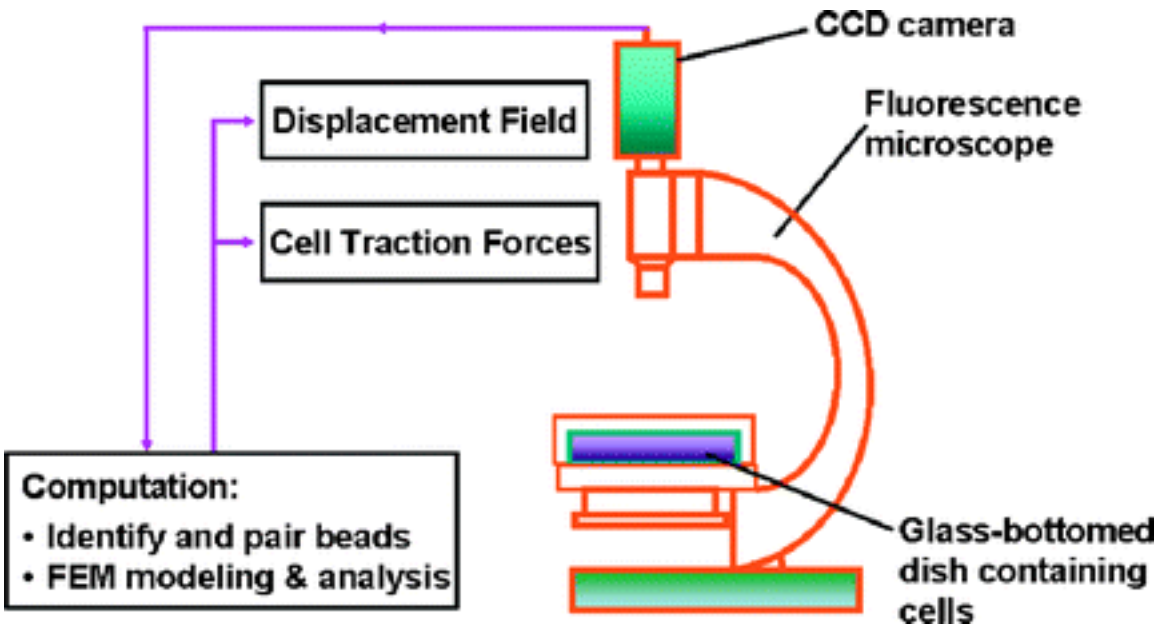

Figure 4: Scheme of Cell Traction Force Microscopy
(Source: R.H. Gavin, 2009)

There are two subcases of the Fourier Transform Traction Cytometry (FTTC), which are constrained and unconstrained. In this research, unconstrained FTTC to calculate the displacement field and then taking the inverse Fourier transform of the result to obtain the tractions. Advantages of using unconstrained FTTC is that cell boundary need not to be identified so investigator judgment is not need it to detect the boundary. Moreover, errors in the recovered tractions exterior to the real cell boundary will have zero mean. A disadvantage of this method is introducing artifactual tractions at the boundary of the field as the measured displacement are not strictly periodic. This disadvantage is overcome in this research by cropping a section in the middle of the field to eliminate the boundary artifacts.

With the complexity of mechanics amongst the cells, the FTTC represents the best means of measurement.

Compute the spatial averages of T1 and T2

Traction Forces and Substrate Stiffness

For most tissue cell types, high extracellular stiffness correlates with large traction forces and large cell–matrix adhesion contacts. These large contacts are thought to not only ensure higher mechanical stability, but also to reflect increased signaling activity. This leads to a stiffness-sensitive response of cells, e.g. during cell spreading and migration (12, 13).

In a previous study done by (Lo CM, et al. 2000) it was observed that substrate stiffness can influence generation of cell tractions forces. Particularly, they cultured 3T3 fibroblasts on flexible polyacrylamide sheets and introduced a transition of rigidity. It was observed that cells on the soft

side migrate easily with a concurrent increase in traction forces, while cells on the stiff side retracted.

Intercellular Stress and How it is Obtained

Force exists between a cell and a neighboring cell which is referred to as intercellular stress for every unit area of contact (14). The stress can be extrapolated from the traction force by balancing the traction forces across the monolayer as required by Newton's third law. In most times, all the cells tend to move together because of intercellular adhesions and communications (15). Despite signals from throughout the ECM that tend to pull the cells apart, the intercellular stress keeps them together (2).

Intercellular stress is very important on different substrates physiologically because it is the force that enables other cells follow the lead cell as it pulls in a particular direction, as shown in figure 1.

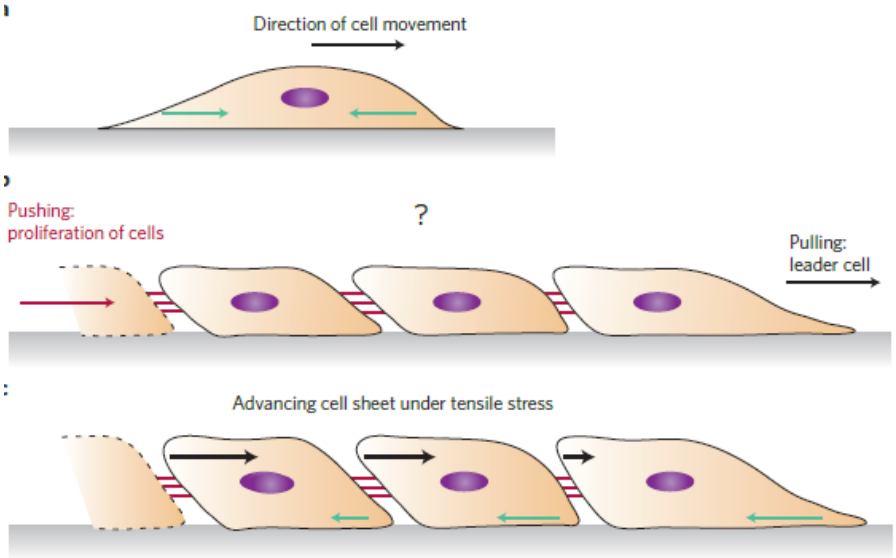

Figure 5: Force distribution during cell movement
Source: Ladoux, Benoit. Cell Guided on Their Journey. Digital image. Nature physics, n.d. Web

Adequate intercellular stress is important for all cells to be carried along in an immune response, in growth or in wound healing. The local intercellular stress comprises of the normal stress which acts in lines perpendicular to the cell junction as shown in figure 2 (red lines) and the shear stress which acts in parallel to the cell-cell junction (blue line).

Newton's third law can be applied in obtaining the intercellular stress at various distances within a sheet of cells. Balance all the forces as is required by Newton's law using the formula below

$$\langle \sigma_{xx}(x) \rangle = \frac{1}{h_z h_y} \int_0^x \int_0^{h_y} T_x(x', y')\, dx'\, dy'$$

"σxx(x)" indicates stress within a cell sheet that is parallel to the edge and perpendicular to the substrate. "T" is the cell-substrate traction. "hy" is the length of the field view. "Hz" is the cell height.

The intercellular stress increases the further the cells are from the edge.

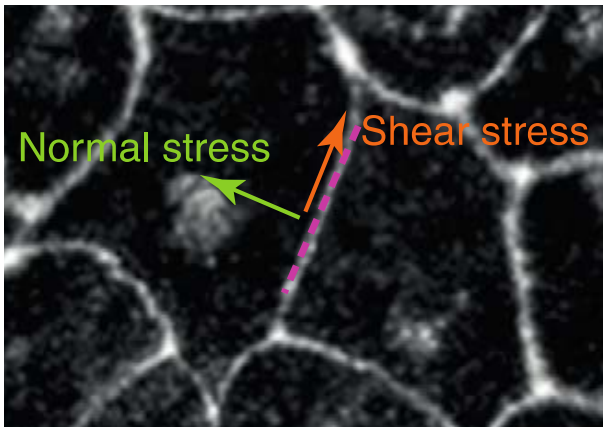

Figure 6: Intercellular stresses showing shear stress and normal stress
Source: Tambe DT, Hardin CC, Angelini TE, Rajendran K, Park CY, Serra-Picamal X, Zhou EH, Zaman MH, Butler JP, Weitz DA, Fredberg JJ. Collective cell guidance by cooperative intercellular forces. Nature materials. 2011 Jun;10(6):469

If the whole system of cells and substrate is handled as a 2-dimensional plane, the intercellular stress can be extrapolated mathematically as shown below:

The internal stress tensor σ(ij) is assumed as the plane stress in an x, y plane where i and j run over the coordinates of x and y and all stress components associated with the x direction vanish. The force balance can be represented by this equation:

$$\sigma(ij,j)= T(i)$$

Additionally, rotating the coordinate system allows to compute the maximum and minimum principal stresses with their respective orientation. Furthermore, the average normal stresses can be obtained by averaging the maximum and minimum principal stresses.

Substrate Stiffness and the Relevance of these Forces

The relationship between traction force, intercellular stress and substrate stiffness is important because cells generate traction against their substrate during adhesion, during growth and during migration. Cells also use traction to sense their substrate. Additionally, cell area and substrate stiffness are predictors of traction force and intercellular stress, where the force and stress increase with substrate stiffness. Traction forces are important for extracellular matrix (substrate) reorganization and assembly. Another important mechanical parameter is strain energy, which is the energy stored in an elastic body undergoing deformation. In aspect of cellular level, it is the energy transferred from the cells to the elastic distortion of the substrate and can represent the contractile strength (butler).

All these above relationships bear relevance to normal and diseased states. Example of disease states where higher stiffness has been shown to bear relevance includes in wound healing, cancer progression and atherosclerosis. ECM (Extracellular Matrix) stiffness has been shown to increase cell area and cell area bears relevance to calculation of traction force and intercellular stresses. Increased substrate stiffness has been shown to disrupt cell to cell contact.

This research is borne out of the need for a clear understanding of the effect of differed substrate stiffness in different disease states on the molecular biomechanics of astrocytes. This research can help us understand how differed substrate stiffness affects the ability of astrocytes to carry out their well-established functions. We can also see how this can be targeted pharmacologically and otherwise towards the end of optimal treatment of CNS diseases.

CHAPTER 2: METHODOLOGY

Cell Culturing and Micropatterning

Cell culture: Human astrocytes (HA) were purchased from ScienCell and cultured in astrocytes medium supplemented with on a % Poly-D-lysine at 37 C and 5% Co_2. Passage 4 and passage 5 were used for all experiments.

Polyacrylamide gel fabrication: PA gels of stiffness 1 kPa, 4 kPa, and 11 kPa were prepared by first treating 35mm petri dishes with bind saline for 1 hour and then air-dried the. The alteration of stiffness was done by mixing the components in table 1 below that was used as a reference protocol in several previous studies in their appropriate proportion and then de-gassed the solution for 45 minutes. After that, 10% Ammonium persulfate and TEMED were added to polymerize the gel on the treated petri dishes followed by flattening the gels to a height of 100 um by using 18mm circular cover slips.

Table 1: PA gels stiffness components

Total solution (15 m)	1 kPa	4 kPa	11 kPa
Ultra-pure water	12.78 mL	12.225 mL	10.63 mL
40% Acrylamide	1.875 uL	750 uL	525 uL
2% BIS	750 uL	750 uL	525 uL
Pink beads 0.5 uL	80 uL	80 uL	80 uL

Micropattern preparation: A thin layer of PDMS was cured in 10mm petri dish by mixing silicone and a curing agent with a ratio of 20:1, respectively. The cured PDMS was left overnight at room temperature, then a circular PDMS sections were extracted a hole puncher and then 1.25mm diameter biopsy punch was used to punch holes. The removed circular section was then put on previously made PA gels and treated with SANPAH (sulfosuccinimidyl-6-(4-azido-2-nitrophenylamino) hexonate diluted with 0.1 M HEPES (Fisher Scientific). Then a SANPAH burning was performed by putting the PA gels under UV lamp for 8-10 minutes followed by rinsing the gels with HEPES and PBS to remove any SANPAH remainders. After that, the patterned gels were treated with collagen 1 (advanced Biomatrix) overnight at 4 C. Excess collagen was removed from the gel the next day and HA were seeded and allowed to attach for an hour. After attachment, micropatterns were removed and human astrocyte cells were allowed to form confluent monolayer for 8-12 hours prior to experimentation.

Experiment Description

Time lapse microscopy: phase contrast and fluorescent images were obtained using Zeiss inverted microscope with a 5X objective and Hamamatsu camera for 3 hours with intervals of 5 minutes. After 3 hours, 10x trypsin was added and incubated for 10 minutes to remove cells from the gel surface. The trypsin provides a stress-free image of the gel top surface to use in traction forces calculations.

Traction force microscopy and monolayer stress microscopy: forces that cells apply on surrounding substrate were calculated using Fourier transform traction force microscopy as described in the introduction above. The intercellular stresses were calculated using monolayer stress microscopy that applied force balance to obtain 2D stress tensor as explained above.

CHAPTER 3: RESULTS

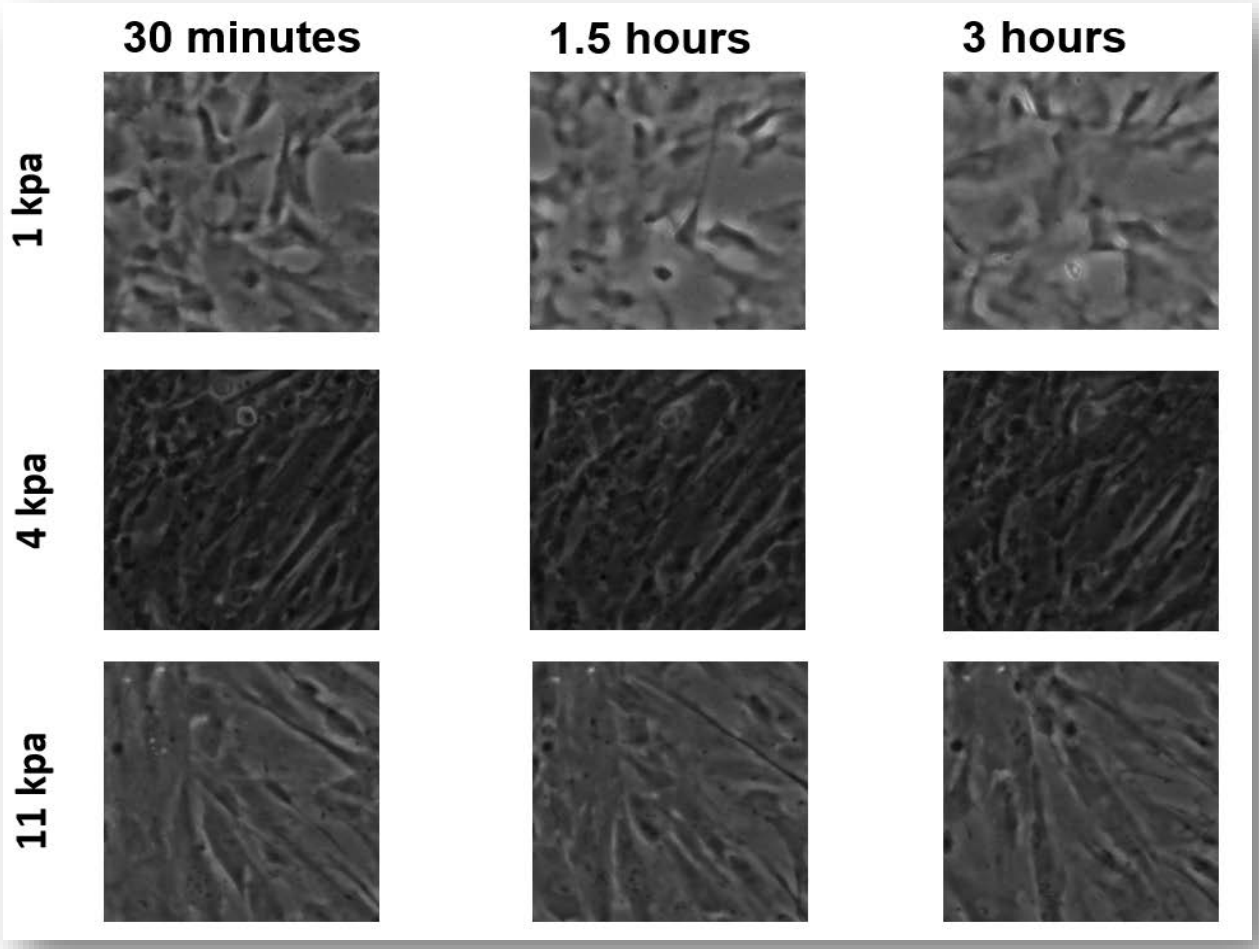

Figure 7: Phase Images Astrocytes

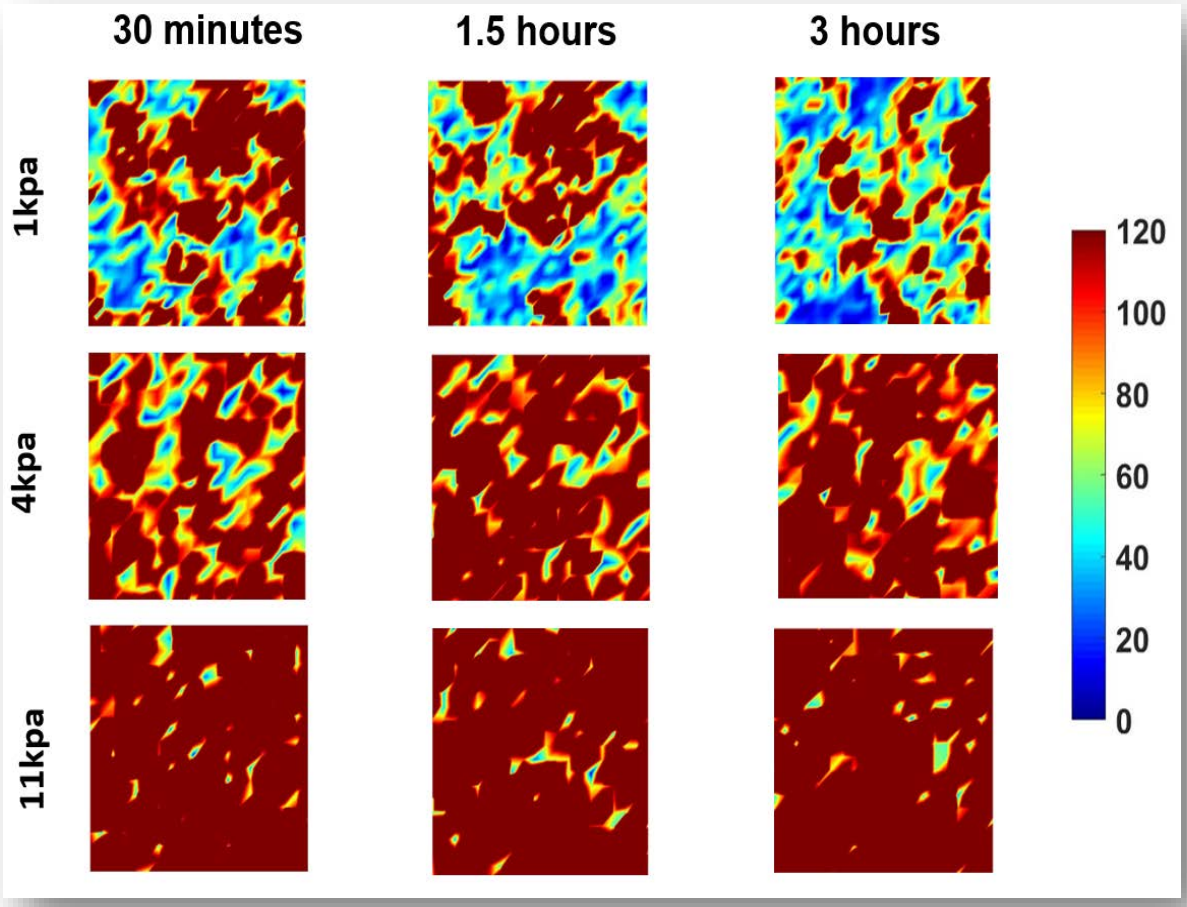

Figure 8: RMS Tractions

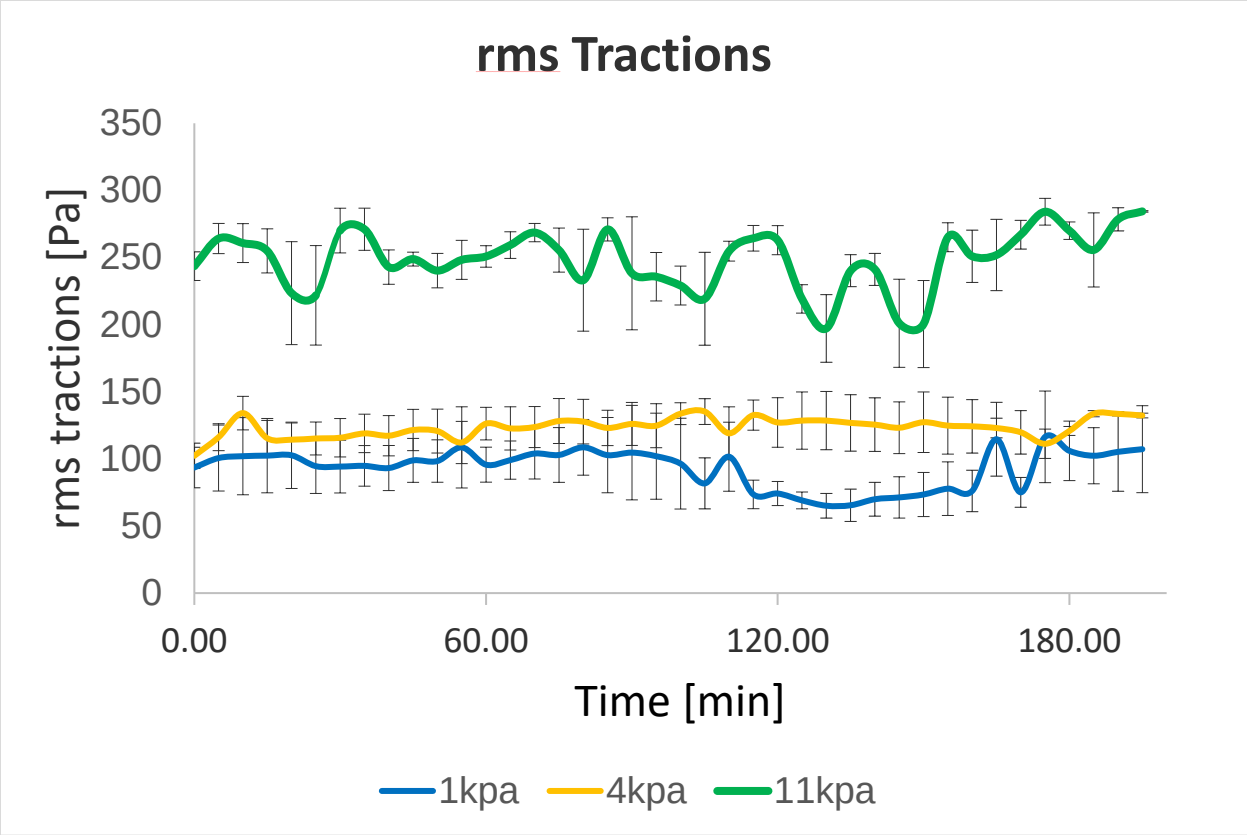

Figure 9: RMS Traction of averaged 4 islands from each stiffness vs time

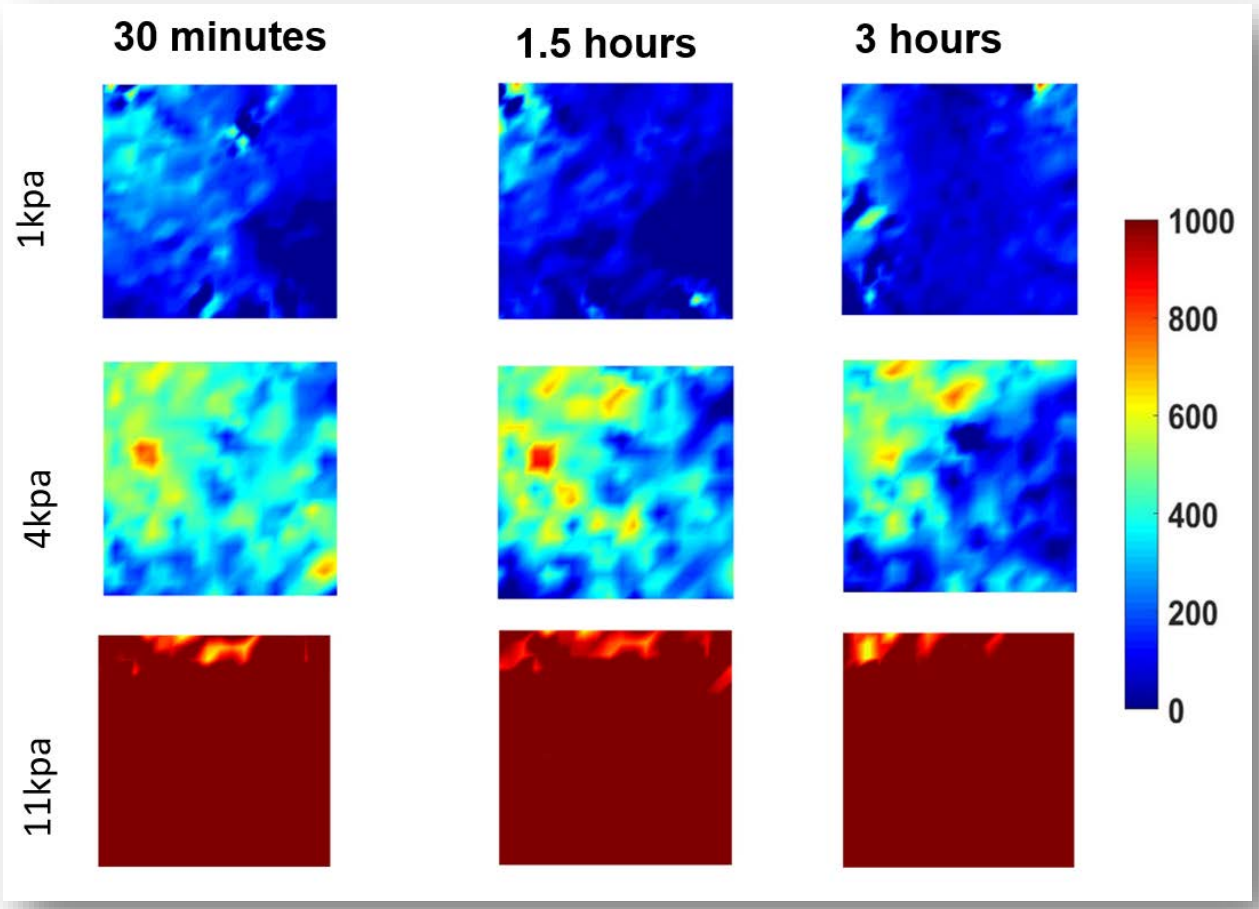

Figure 10: Max Shear Stress

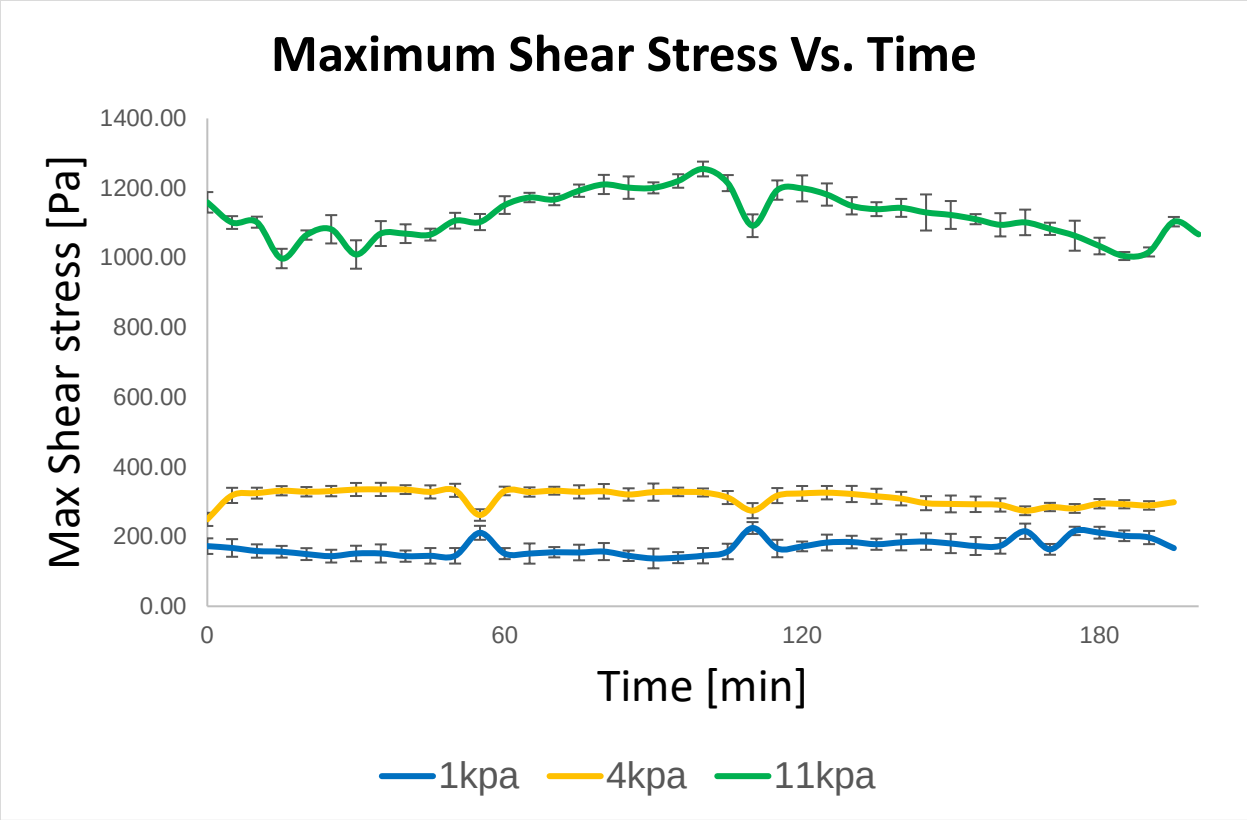

Figure 11: Max Shear Stress of averaged 4 islands from each stiffness vs time

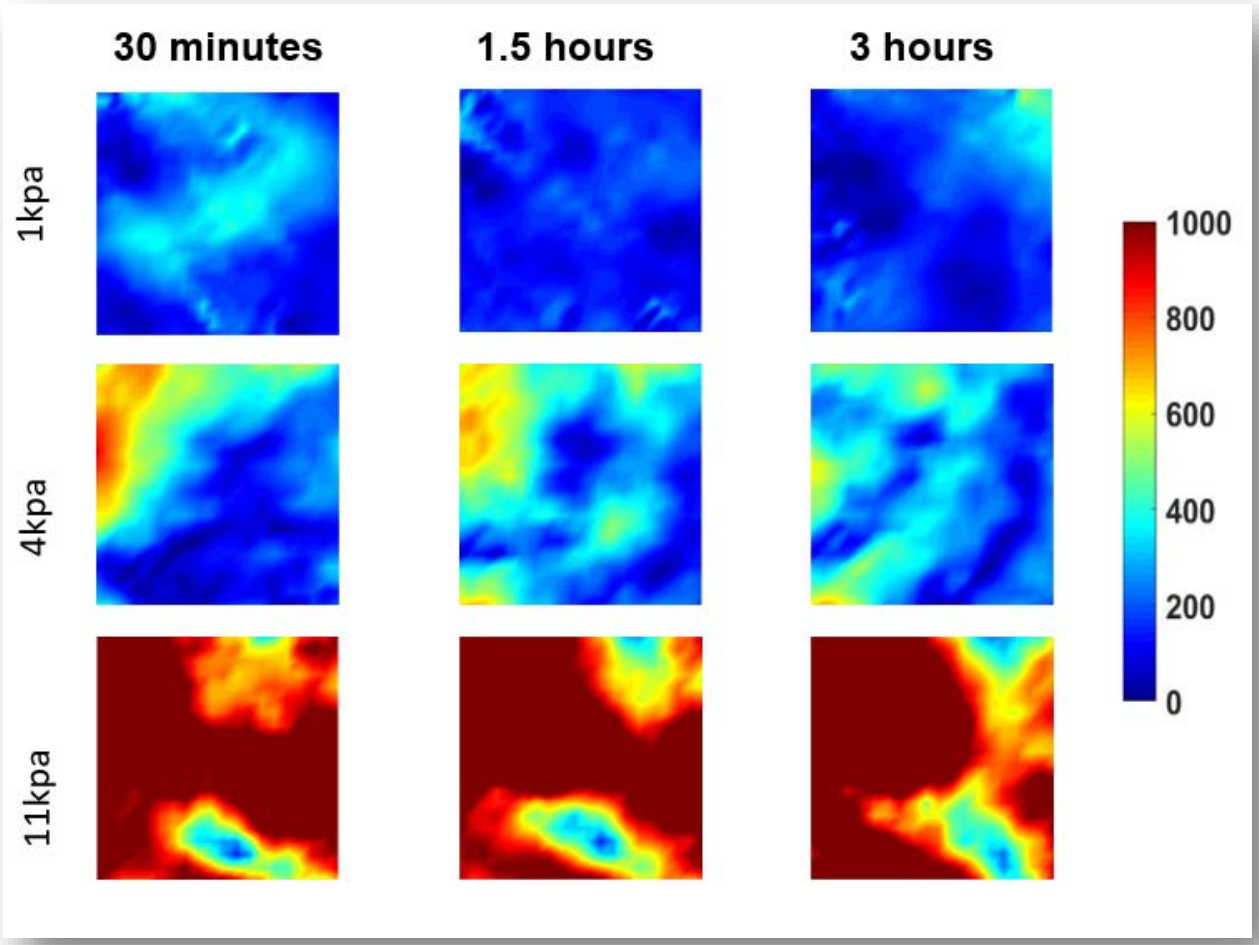

Figure 12: Average Normal Stress

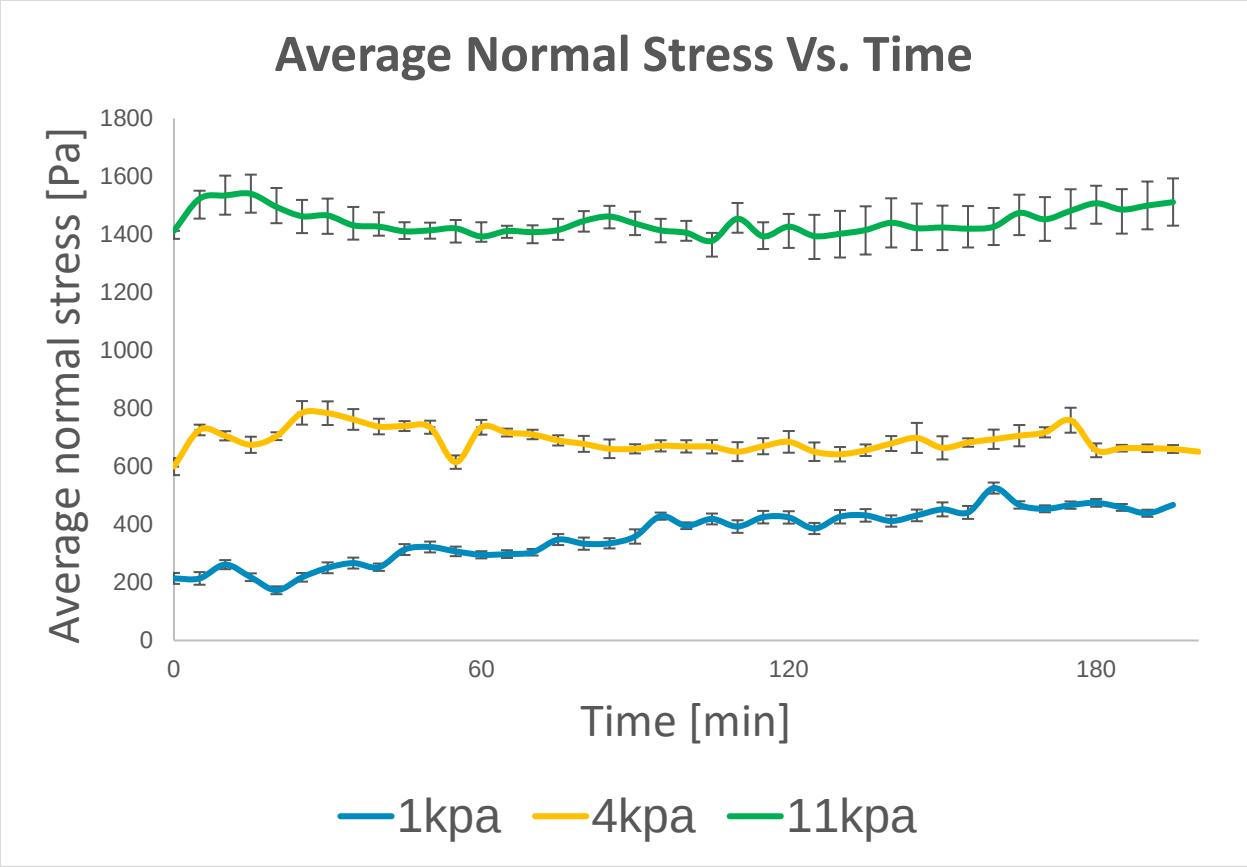

Figure 13: Average Normal Stress of averaged 4 islands from each stiffness vs time

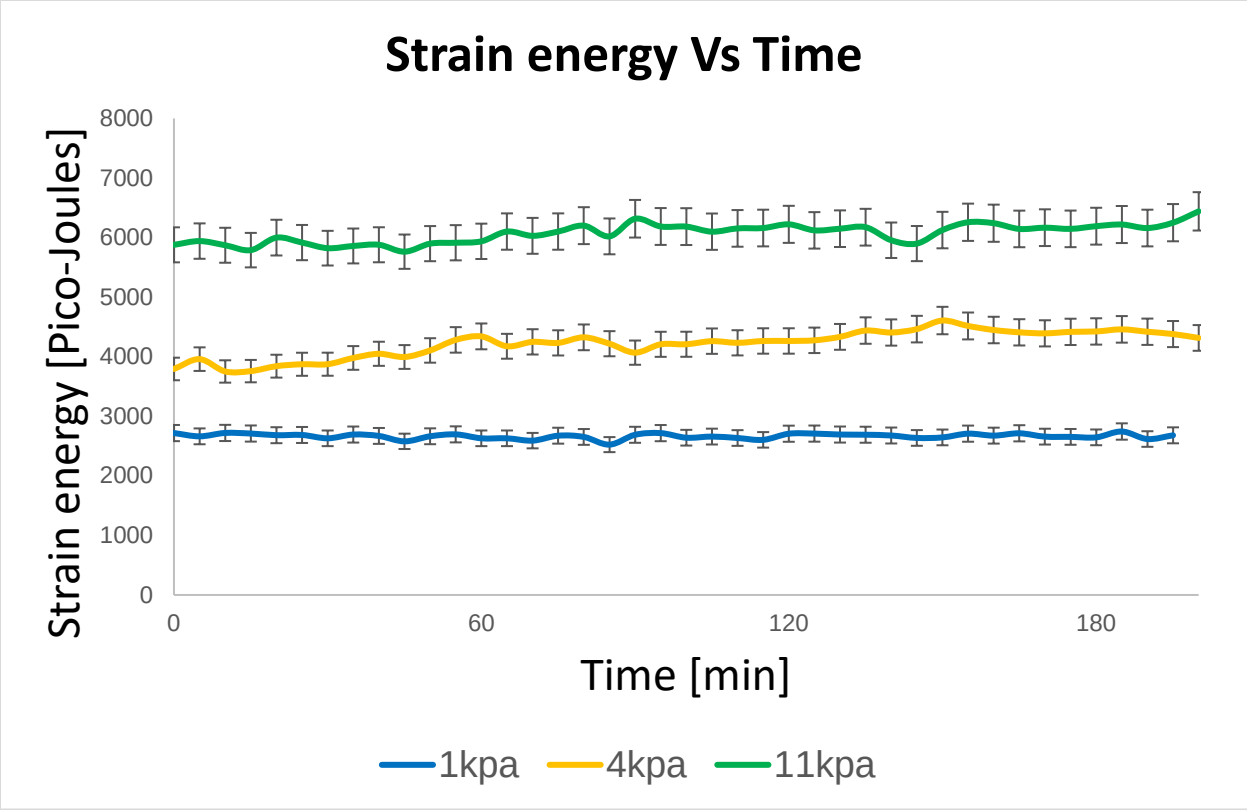

Figure 14: Strain Energy of averaged 4 islands from each stiffness vs time

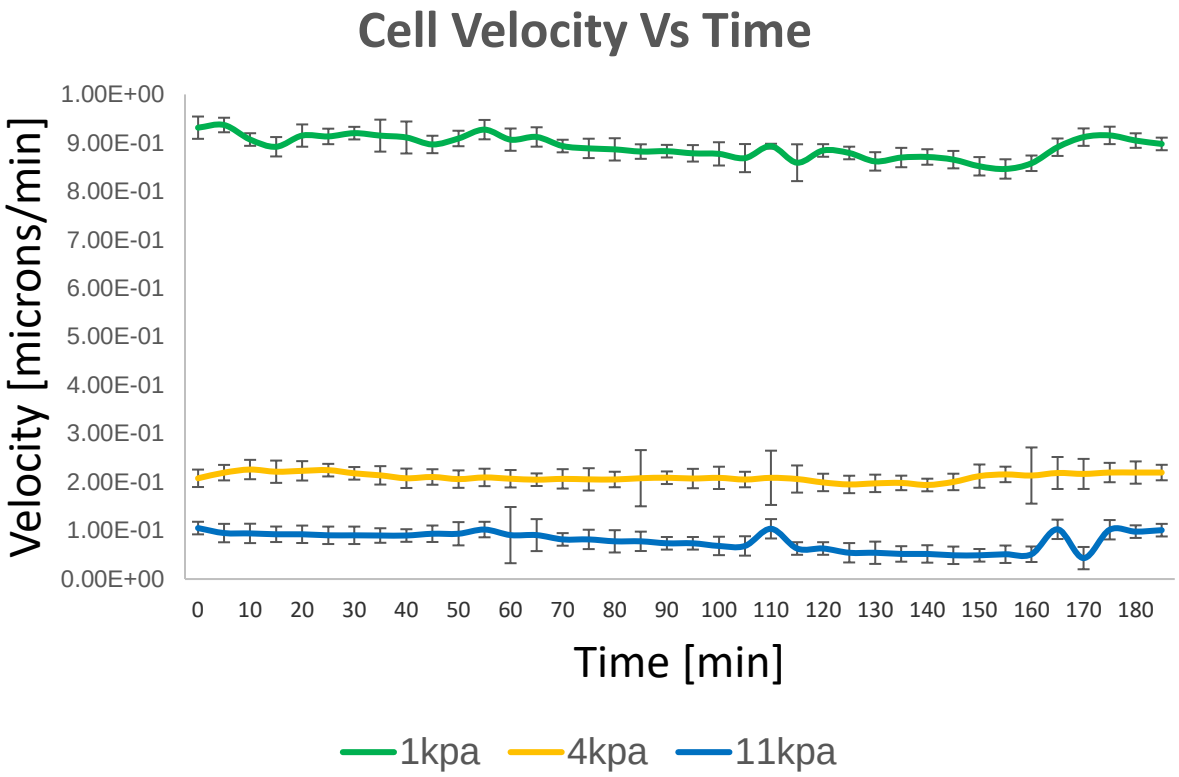

Figure 15: Velocity vs Time

Discussion

It is a well-known fact that mechanics play a key role in the spatial organization of cells and tissues. Traction forces, adhesion, intercellular stresses and migration at the cellular level are essential for organization of tissues and maintenance of normal physiology. Cells are subject to mechanical influence from the extracellular matrix (ECM) and this study shows that a research on human astrocytes done with good methodological design can be a good extrapolation of what happens physiologically. More research is still needed to integrate the effect of chemical signals to see the influence it has on these mechanical forces. The root mean square (RMS) traction is shown to generally increase with stiffness. This means that a slower migration of cells (i.e. astrocytes) results in stiffer substrates. This is in consonance with the study by Mennens et al (10) which showed similar results in rat astrocytes. At the various stiffness ranges used (1kPa, 4kPa and 11Pa), distinct RMS values were produced which shows that the physical properties of a substrate matrix mechanically influence cell response.

The maximum shear stress placed on cells in different stiffness increases as stiffness increases. However, the average normal stress is similar across different stiffness for the first 1 hour. This suggests that the adaptive mechanisms of cells to withstand stress wears out over time.

Strain energy is also shown in this study to be directly proportional to substrate stiffness.

The phase contrast images, maps of displacement fields and traction fields illustrate what has been earlier discussed in the preceding paragraphs more graphically. The color scales indicate the traction forces (in micrometers) and the extent of displacement (in Pa).

Higher displacement or cell spreading is associated with higher root mean square (RMS) values. This means that cell stiffness is proportional to the tension within the cell which is generated by the traction of the cell. Cell stiffness is the ratio between shear stress and shear strain (1).

The phase image of the 11kPa medium shows more elongated cells and distorted cells probably by extension of lamellipodia in the direction of movement which would be associated with more substrate adhesions and cell strain. The phase images correspond to the displacement field which shows higher Pa values throughout the field. This means that higher traction occurs in stiffer substrate and is associated with higher cell strain. This is also reflected in the other substrate fields (1kPa, 4Kpa, and 11Pa) although it is subtler and less clear.

Additionally, as seen in the phase images, there seems to be faster cell growth and multiplication as substrate stiffness increases which is similar to results gotten in an experimental study by Saez et al who concluded that more growth of cells occurs in the stiffest part of substrate (2)

Our findings of the maximum shear stress tending to increase with increasing substrate stiffness suggested that astrocyte biomechanical response to increasing substrate stiffness involves a reinforcement of intercellular stresses. Hence, we propose that astrocytes that reside in the diseased brain could potentially impose higher intercellular stresses within the brain as well. Strain energy and average normal stress are also observed to follow a similar trend as the maximum shear stress as can be seen in figure 5,6 above. Additionally, the lower tractions associated with the highest stiffness could potentially explain the low efficiency of the diseased brain to perform important cell functions such as cell migration and cell proliferation since tractions are a quantitative metric of cell contractility

LIST OF REFERENCES

1. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta neuropathologica. 2010 Jan 1;119(1):7-35.
2. Montgomery DL. Astrocytes: form, functions, and roles in disease. Veterinary pathology. 1994 Mar;31(2):145-67.
3. Kimelberg HK, Nedergaard M. Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics. 2010 Oct 1;7(4):338-53.
4. Arani A, Murphy MC, Glaser KJ, Manduca A, Lake DS, Kruse SA, Jack Jr CR, Ehman RL, Huston 3rd J. Measuring the effects of aging and sex on regional brain stiffness with MR elastography in healthy older adults. Neuroimage. 2015 May 1;111:59-64.
5. Cha S. Experimental Measurement of Stiffness with Magnetic Resonance Elastography for in vivo Healthy Human Brains (Doctoral dissertation, The Ohio State University).
6. Murphy MC, Huston J, Jack CR, Glaser KJ, Manduca A, Felmlee JP, Ehman RL. Decreased brain stiffness in Alzheimer's disease determined by magnetic resonance elastography. Journal of magnetic resonance imaging. 2011 Sep 1;34(3):494-8.

   Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126:677–89
7. Murphy MC, Jones DT, Jack Jr CR, Glaser KJ, Senjem ML, Manduca A, Felmlee JP, Carter RE, Ehman RL, Huston III J. Regional brain stiffness changes across the Alzheimer's disease spectrum. NeuroImage: Clinical. 2016 Jan 1;10:283-90.
8. Yang X, Askarova S, Lee JC. Membrane biophysics and mechanics in Alzheimer's disease. Molecular neurobiology. 2010 Jun 1;41(2-3):138-48.

9. Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ. Astrocytes in Alzheimer's disease. Neurotherapeutics. 2010 Oct 1;7(4):399-412.
10. Mennens SF, Bolomini-Vittori M, Weiden J, Joosten B, Cambi A, van den Dries K. Substrate stiffness influences phenotype and function of human antigen-presenting dendritic cells. Scientific reports. 2017 Dec 13;7(1):17511.
11. Wilson CL, Hayward SL, Kidambi S. Astrogliosis in a dish: substrate stiffness induces astrogliosis in primary rat astrocytes. RSC Advances. 2016;6(41):34447-57.
12. Robert J. Pelham, Yu-li Wang.Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc. Natl. Acad. Sci., 94 (1997), pp. 13661-13665
13. Chun-Min Lo, Hong-Bei Wang, Micah Dembo, Yu-Li Wang. Cell movement is guided by the rigidity of the substrate. Biophys. J., 79 (2000), pp. 144-152
14. Ladoux B. Biophysics: cells guided on their journey. Nature Physics. 2009 Jun;5(6):377.
15. Trepat X, Wasserman MR, Angelini TE, Millet E, Weitz DA, Butler JP, Fredberg JJ. Physical forces during collective cell migration. Nature physics. 2009 Jun;5(6):426.
16. Butler JP, Tolic-Nørrelykke IM, Fabry B, Fredberg JJ. Traction fields, moments, and strain energy that cells exert on their surroundings. American Journal of Physiology-Cell Physiology. 2002 Mar 1;282(3):C595-605.
17. Tambe DT, Hardin CC, Angelini TE, Rajendran K, Park CY, Serra-Picamal X, Zhou EH, Zaman MH, Butler JP, Weitz DA, Fredberg JJ. Collective cell guidance by cooperative intercellular forces. Nature materials. 2011 Jun;10(6):469.
18. Califano JP, Reinhart-King CA. Substrate stiffness and cell area predict cellular traction stresses in single cells and cells in contact. Cellular and molecular bioengineering. 2010 Mar 1;3(1):68-75.

19. Zimmermann J, Hayes RL, Basan M, Onuchic JN, Rappel WJ, Levine H. Intercellular stress reconstitution from traction force data. Biophysical journal. 2014 Aug 5;107(3):548-54.
20. Sugimura K, Lenne PF, Graner F. Measuring forces and stresses in situ in living tissues. Development. 2016 Jan 15;143(2):186-96.
21. Pasqualini FS, Agarwal A, O'Connor BB, Liu Q, Sheehy SP, Parker KK. Traction force microscopy of engineered cardiac tissues. PloS one. 2018 Mar 28;13(3):e0194706.
22. Polacheck WJ, Chen CS. Measuring cell-generated forces: a guide to the available tools. Nature methods. 2016 May;13(5):415.
23. Discher, D. E., Mooney, D. J., and Zandstra, P. W. "Growth Factors, Matrices, and Forces Combine and Control Stem Cells." Science 324.5935 (2009): 1673-7. 7.
24. Engler, A. J., Griffin, M. A., Sen, S., Bönnetnann, C. G., Sweeney, H. L. and Discher, D. E. "Myotubes Differentiate Optimally on Substrates with Tissue-Like Stiffness: Pathological Implications for Soft Or Stiff Microenvironments." Journal of Cell Biology 166.6 (2004): 877-8
25. Hadjipanayi, E., V. Mudera, and R. A. Brown. "Guiding Cell Migration in 3D: A Collagen Matrix with Graded Directional Stiffness." Cell motility and the cytoskeleton 66.3 (2009): 121-
26. Paszek M.J., Zahir N., Johnson K.R., Lakins J.N., Rozenberg G.I. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005;8:241–254.
27. Bonnans, C., Chou, J., & Werb, Z. (2014). Remodelling the extracellular matrix in development and disease. Nature Reviews Molecular Cell Biology, 15(12), 786-801. doi:10.1038/nrm3904
28. Frantz, C., Stewart, K. M., & Weaver, V. M. (2010). The extracellular matrix at a glance. Journal of Cell Science, 123(24), 4195-4200. doi:10.1242/jcs.023820
29. R.H. Gavin (2nd ed), Cytoskeleton methods and protocols. Methods in Molecular biology, vol. 586)

30. Stewart, D. C., Rubiano, A., Dyson, K., & Simmons, C. S. (2017). Mechanical characterization of human brain tumors from patients and comparison to potential surgical phantoms. PLoS ONE, 12(6), e0177561. http://doi.org/10.1371/journal.pone.0177561

31. Chauvet D, Imbault M, Capelle L, Demene C, Mossad M, Karachi C, et al. In Vivo Measurement of Brain Tumor Elasticity Using Intraoperative Shear Wave Elastography. Ultraschall in Med. 2015;: 1–8.