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BENG 353 Biomechanics Final Project
Professor Murrell
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PROJECT 1: TRACTION FORCES

BACKGROUND QUESTIONS

1. Can cells move without integrins and focal adhesions? If so, how?

While focal adhesions have long been thought of as essential for cell motility, cells may actually move without them, according to a 2016 summary by Sixt et. al.[1] While Abercrombie's 3-step mechanism for cell motility holds fairly well for cells moving along 2D substrates, in which case substrate anchoring through integrin-specific focal adhesion is necessary to counteract Brownian motion and ensure contact, scientists are finding that 3D is a different story.

Through 3D confinement of the cell and its substrate of interest, Brownian motion may be counteracted in the absence of focal adhesions for sustained contact. Interestingly, one study[2] found that interfering with integrin function on endothelial cells did not entirely inhibit the leukocyte extravasation cascade but rather slowed it down. Malawista & de Boisfleury Chevance[3] found that neutrophil granulocytes continued to migrate despite integrin inactivation when they were confined between two glass plates; however, motion ceased on a planar surface. Several other studies substantiated these findings. While the extent to which a cell can move independently of integrins is still under research, it is clear that cells may move without integrins when confined. Cells that typically display an amoeboid migration mode (fast moving and does not degrade the extracellular matrix) either 1. lack integrins and have high actomyosin contractility or 2. are under confinement and their substrates do not favor cell adhesion.

One proposed mechanism of integrin-independent motion is cell migration by swimming, whereby cells create propelling forces by coupling shape deformations to the surrounding fluid via hydrodynamic interactions. Since cells operate at a considerably small Reynold's Number, they must continuously propel themselves to continue moving without being stalled by the more prominent viscous forces. To complicate this mechanism further, due to low inertial forces, one step forward would equal one step backwards, so cells would need to move in a corkscrew fashion or asymmetrically like flagella (scallop theorem).

A more commonly suggested mechanism is for force transmission through cell-substrate intercalation. By this method, seen in Schwann cells during peripheral nerve repair, form multiple lateral bleb-like protrusions from the cell body, which form interdigitations with gaps and discontinuities of the substrate/matrix and exert forces in these "footholds". Now, while this model would explain traction forces, it does not explain cell propulsion forward in as much detail.

In the absence of substrate footholds, the chimneying mechanism is suggested, by which the cell pushes off of its confining walls to propel itself forward. The fourth and final suggested mechanism of integrin-independent motility is flow-friction-driven force transmission in which nonspecific friction between the cell and its substrate are responsible for adhesion-independent migration; however, the origin of said friction has not been investigated thoroughly at this point.

2. Do cancer cells generate higher traction forces than non-cancer cells?

The Reinhart-King group[4] understood that metastasis of solid tumors would likely require large forces to reorganize and navigate throughout the body and spread, so they sought to investigate the differences in traction forces exerted by metastatic (cancerous) versus non-metastatic (non-cancerous) cells. They concluded that metastatic cells generate higher traction forces than non-metastatic cells across varied stiffness and collagen density, two matrix properties that increase traction forces independent of cell type. These results support traction force generation as a potential biophysical marker of metastatic cells.

3. Can cells move without the F-actin cytoskeleton? If so, how?

Similar to the review by Sixt et. al.[1], Konstantopoulous et. al.[5] conclude that cells are capable of moving in 3D environments without the aid of substrate adhesions. The confinement of the cell by the three dimensions induce cytoskeletal alterations that decrease dependence of cells on adhesion-contraction force coupling by actomyosin-associated focal adhesions. When confined in 3D space, cells secrete proteases to locally remodel the ECM or switch to the amoeboid mode of movement described in question 1.

The group also shows that confined movement continues even after F-actin is interfered with. Cell motility in 3D depends more so on microtubule dynamics than F-actin; disrupting MT does in fact reduce cell migration by at least 80%. Through experimentation, they saw that 3D confinement suppresses the formation of focal adhesions and a cell's dependence on tractive pulling forces through substrate adhesion.

4. What determines the total mechanical work performed by the cell through traction forces?

According to Gardel et. al.[6], cell spread area alone determines the total mechanical work performed by the cell through traction forces. Furthermore, given a particular spread area, the local curvature along the cell exterior determines the distribution and magnitude of traction stresses. Even varying geometry (affects aspect ratio and number of focal adhesions), cells with the same spread area performed the same amount of work, reiterating the fact that mechanical work depends solely on cell spread area.

5. How does substrate stiffness alter the production of traction forces?

Gardel et. al.[6] found that substrate stiffness did in fact affect the average substrate strain and the average traction stress magnitude, but in different directions. Cells on stiffer substrates exhibited small strains with large stresses, whereas cells on softer substrates exhibited large strains and small stresses. These opposing effects end up balancing each other out when calculating the strain energy (or the energy stored following deformation), such that the cell ends up performing approximately the same amount of work in either case. This leads one to conclude that despite substrate stiffness affecting the traction force magnitude (stiffer substrates experience larger traction forces), the average amount of work by the cell on the substrate remains effectively constant.

PART 1

1. You see that liposomes can generate traction forces. What causes the contraction?

Liposome adhesion to substrate builds up membrane tension, generating internal and hydrostatic pressures that affect large traction forces on compliant materials. These traction stresses cause the substrate (the deformable gel in this experiment) to contract after initial spreading on the gel substrate. Interestingly, hydrostatic pressure can be manipulated to cause cells to mechanically work in a specific way.

In the 2014 study by Murrell et. al[7], the group found that "adhesion of bare liposomes generates large stresses on compliant substrates," and "these stresses qualitatively alter the dynamics of liposome spreading on soft matrices, as they cause contraction of the contact area". They propose that the substrate contraction we see is a result of the minimization of the total energy of the system (elastic and adhesion contributions), which a system will tend towards (second law of thermodynamics). Essentially, "contraction comes at the cost of elastic energy stored in the substrate".

The group reports that their PIV results indicate that the liposome vesicle formation induces contraction of the underlying substrate (PAA gel) that increases over time with strain energy as the liposomal contact area decreases. They conclude that there is a direct relationship between reduction in contact area and gel contraction[7].

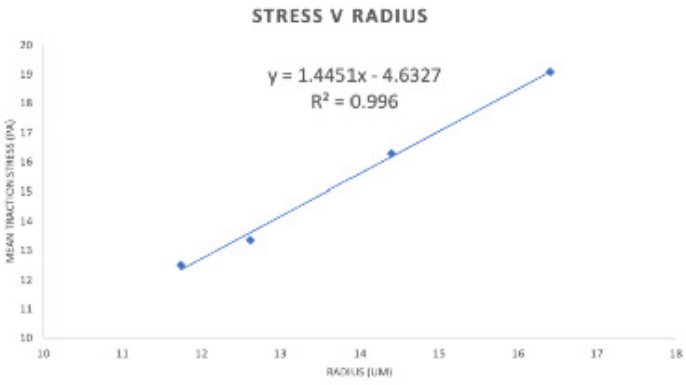
2. What causes the indentation into the gel?

The liposome adhesion increases membrane tension, and the resulting vertical pressure on the elastic gel substrate causes indentation through applied forces. More specifically, when the floppy liposome makes contact with the gel, it first begins to spread to

reduce increases in membrane tension and minimize free energy from adhesion. After a particular adhesion energy (Ea0) is reached, further spreading causes membrane tension to increase with the surface. The elevated membrane tension subsequently elevates the Laplace pressure, inducing a positive outward pressure difference seen by the indentation in the elastic gel substrate[7].

3. Plot the mean traction stress along the radius by calculating mean traction stress of rings of different radii.

Radius (um)	Stress (Pa)
16.409	19.066
14.4	16.288
12.623	13.349
11.746	12.505



4. What law describes the generation of these stresses?

The Laplace Pressure law,

$$P_i - P_o = \frac{2\tau}{R_c}$$

where Po is the pressure outside the liposome (Po << Pi), Rc is related to the radius of adhesion, and tao is the surface tension, describes the generation of these stresses. For a 10 um cell at a tension close to lysis tension, Pi is found to be ~60Pa[7].

5. How does the magnitude of the stress compare to the magnitude of traction stresses caused by contractility of the cell cytoskeleton?

The magnitude of traction stresses due to contractility of the cytoskeleton is larger than the magnitude of stresses caused by membrane tension. The former is around a 100 Pa (99 Pa for mouse embryonic fibroblasts and 96 Pa for bone osteosarcoma)[7], while the latter is approximately 60 Pa[8].

6. How would thickness of polyacrylamide gel alter the mechanical responses for traction force microscopy experiments?

Lin et. al[10] found that the cells on the thinner substrate have a larger projected area and exert larger traction forces than the cells on the thicker substrate. They also discovered that as the polyacrylamide gel increases beyond the length scale of lateral dimension of cells (~100 μm here), the average projected cell area and traction forces decreased to a steady state. Essentially, substrate thickness affects the stiffness experienced by the cell. Mullen et. al.[11] propose that the increased projected area when using a thinner substrate may be due to an increase in effective shear stiffness– the stiffness experienced by the cell.

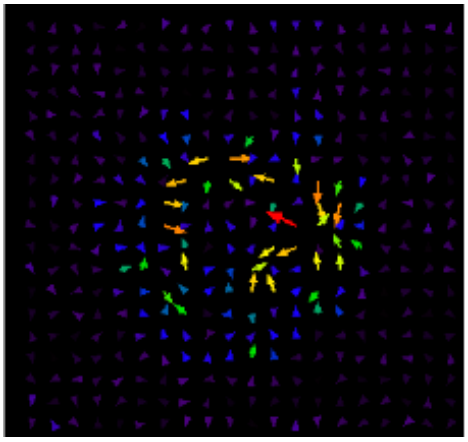
PART 2

1. Show the traction plot for 25, 50, and 100 um diameter colonies (the vector version). Within each colony, where are the mechanical stresses localized? Do cells throughout the colony generate mechanical stresses, or only a subpopulation?

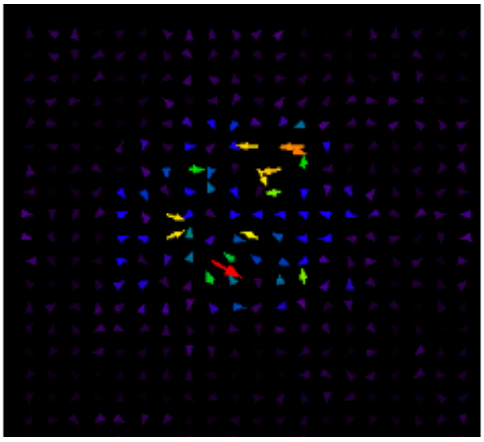
Throughout all of the colonies and samples, mechanical stresses are localized to the outer edge of the colony. Cells in the center do not display strong mechanical stresses. Also, all of the vectors point towards the center of the colony or along the edge of the colony but not directly outwards. Only a subpopulation of the cells generate mechanical stresses. This group of cells consistently appears to be positioned around the outer edge of the colony but are not in the same locations in each sample. They also do not constitute the entire outer edge of the colony but rather they are sparsely distributed around the edge.

See next page for plots

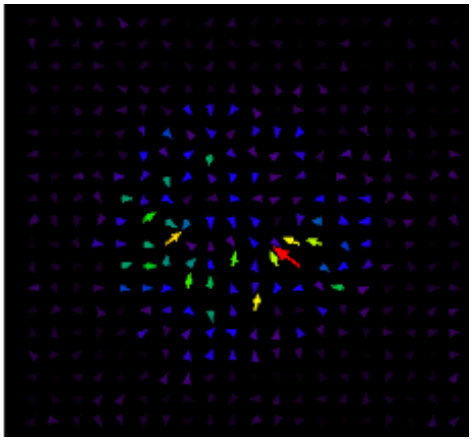
Traction plots for 25, 50, and 100 um diameter colonies



25um Sample 1



25um Sample 2



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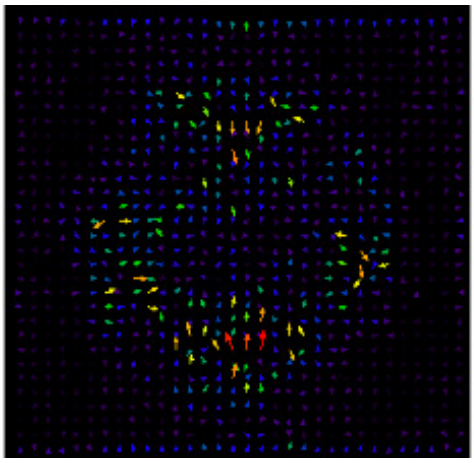
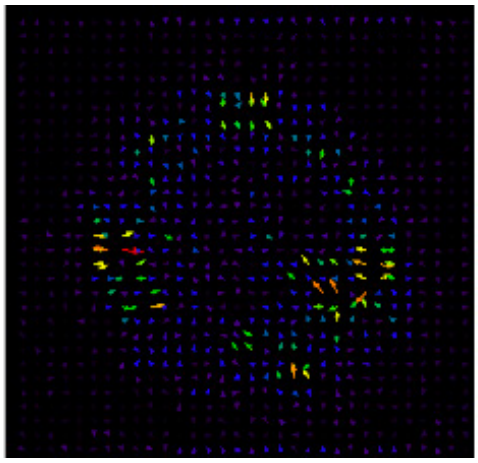
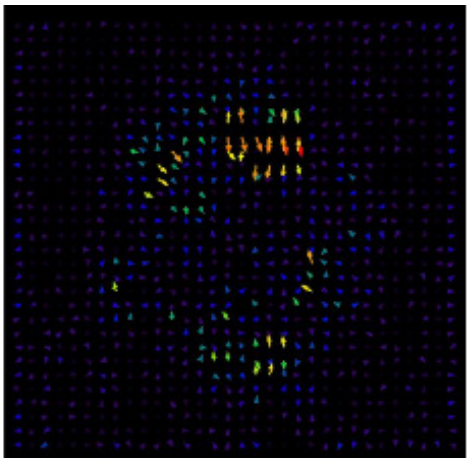


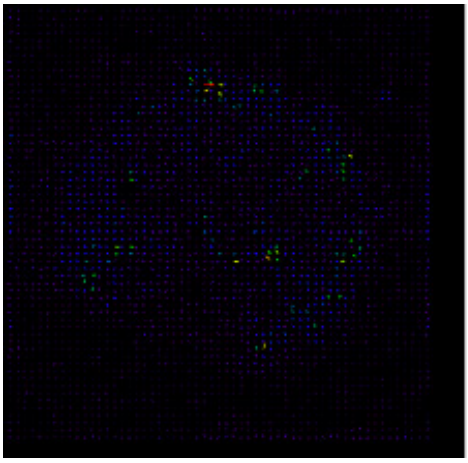
50um Sample 1



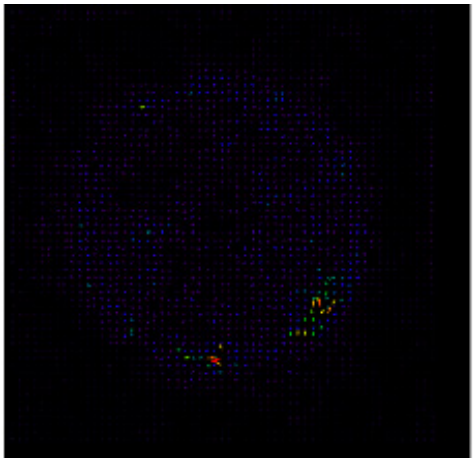
50um Sample 2



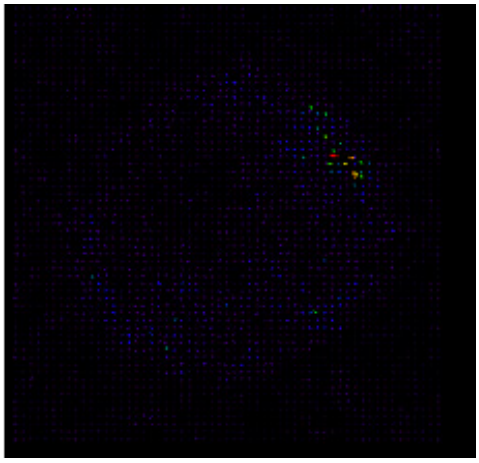
50um Sample 2



100um Sample 1



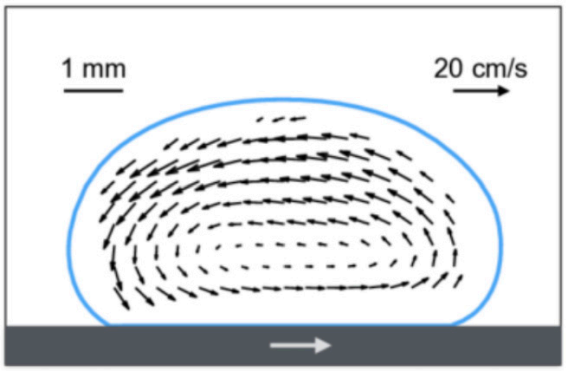
100um Sample 2



100um Sample 2

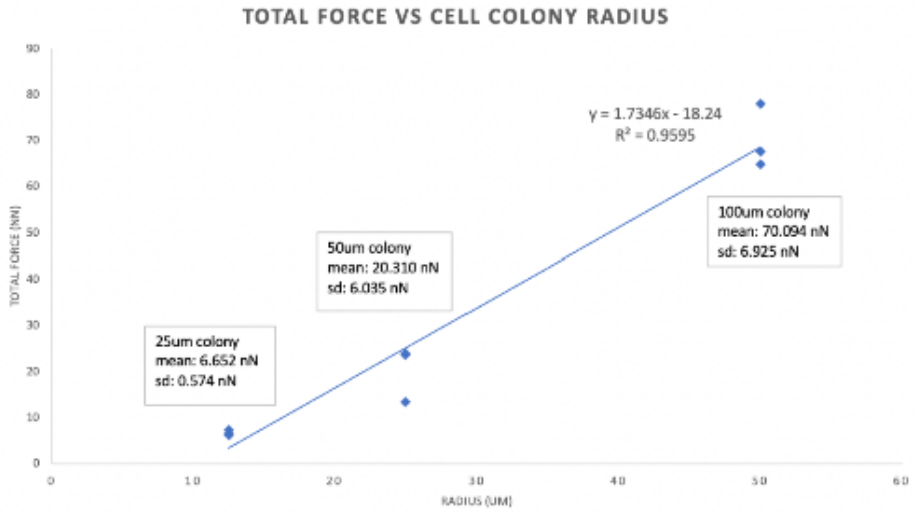
2. What is the pattern of stresses you would expect for a liquid droplet?

For a liquid droplet on a hydrophobic surface, we would expect its surface tension to produce a radial pattern of stresses that all point counter-clockwise or all clockwise with increasing magnitude with increasing distance from the droplet center. See Mouterde et. al.[12] for more information and documentation for the figure below.



3. Plot the total force of all the cell colonies as a function of the radius of the colony. Include mean and standard deviation. Fit the data with an appropriate curve.

Radius (um)	Total Force (nN)
12.5	6.44370286
12.5	6.21089546
12.5	7.3008551
25	23.9371867
25	23.6494979
25	13.34424952
50	67.5239194
50	77.9368506
50	64.82154924



4. Does the stress increase or decrease with the size of the colony?

As the size of the colony increases, total force increases and stress increases locally at the outer perimeter. This is visualized in the plot of total force versus cell colony radius above as well as in the PIV pictures that display arrows of cooler color (smaller stress magnitude) closer to the colony center and arrows of warmer colors (larger stress magnitude) closer to the colony perimeter.

5. Is the line best fit by a linear fit, or a power law?

The data is best fit by a linear fit. The proposed equation is y = 0.0394x - 0.8945 with an R2-value of 0.8779, so highly correlated.

6. What is the surface tension for each of the colonies? Use peripheral and total surface force balance to calculate the surface tension gamma (N/m) for each colony. How do these values compare to the surface tension of water?

$$\text{Surface Tension} = F/(2\pi r)$$

Colony 25um: 4.235×10^{-5} N/m
Colony 50um: 1.293×10^{-4} N/m
Colony 100um: 2.231×10^{-4} N/m

Considering that water has a surface tension of 0.072 N/m[16], so the colonies have less surface tensions than water does by a magnitude of a hundred or so. Notably, as the colonies increase in size, the surface tension increases as well.

7. What is the expectation for how the total forces scale with that of a liquid?

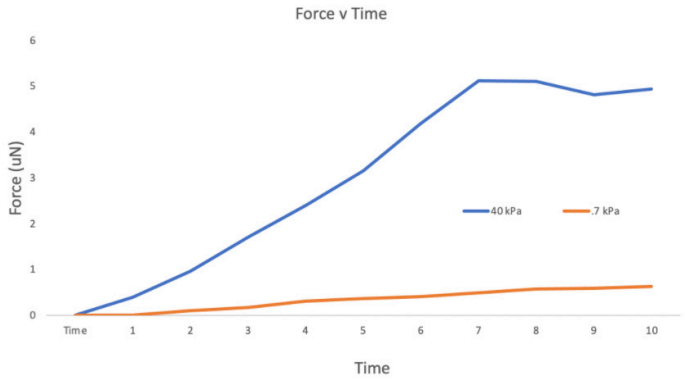
In a liquid, it is expected that the total forces are proportional to the size of the liquid droplet.

8. What does this comparison to liquids mean about how forces are distributed in a tissue?

Since the forces in both liquids and tissues are linearly related to size, tissues are comparable to liquids. Like liquids, the forces in a tissue are distributed around the periphery[1].

PART 3

1. Plot the total force of the aggregate over time for the 40kPa and the 0.7kPa gel. Since TFM uses an undeformed reference state to calculate stresses, use separate 2-slice stacks for each time point. Use 0.1um/pixel for the scale. For which substrate stiffness is the total force greater? Is this a large difference?



The stiffer 40kPA gel has higher forces at each time point. The difference is significant ($p = .0002775 << 0.05$).

2. For which substrate stiffness is the migration ("spreading") greater? Is this a large difference?

The stiffer 40kPA gel has greater spreading. The difference is significant ($p = 0.004931 < 0.05$). This is expected and explained in a paper by Adam Engler from the University of Pennsylvania[15].

3. Can you explain why forces are generated higher in one case than another? Can you provide a mechanism explained by the interaction between focal adhesions, the F-actin cytoskeleton and molecular motors?

Forces are generated higher on the stiffer substrate than on the softer substrate. This makes sense because tension is generated by the interactions of molecular myosin motors with the F-actin cytoskeleton and transmitted through integrin-based focal adhesions to the ECM. Notably, within the ECM, F-actin filaments are organized in a diverse assortment of interconnected networks.

Forces generated by actin polymerization or molecular motor activity are transduced across the cell eventually to the adhesion site. Since the way in which the actin cytoskeleton is organized affects the way force is transmitted across the cell, the stiffness of the substrate–and its adhesive geometry–is relevant to force generation. Cells placed on softer, more elastic substrates are unable to create as many focal adhesions and stress fibers because they do not need as many to generate enough tension to deform the matrix upon which they sit[14].

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