Capstone Project – Learning Cell Mechanobiology Using a Stretch Chamber

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Abstract

An undergraduate Capstone Design project was structured to enhance students' understanding of some aspects of cell biomechanics by building a low-cost and portable stretch chamber. Cells apply tensile forces to their surrounding media that are necessary for realization of different phenotypes. In addition to the contractile forces generated internally, cells are also subjected to the external forces applied by their extracellular matrix. A group of undergraduate students from Mercer University designed and tested a device to observe the effect of substrate deformation on the geometry and shape of adherent cells. The team was formed by engineering students with different majors to work on a research project. Students benefited from the cross-disciplinary nature of this research by learning about the functions of cells as a complex biological system, learning how cellular functions are regulated mechanically, and understanding how life science and engineering can be brought together to design biomedical devices.

Keywords

Capstone Design; Scholarship of Teaching & Learning; Cell adhesion; Stretch chamber.

Introduction

Cell mechanics is an emerging field that deals with the mechanical properties of cells and how they impact cellular functions.[1] An essential part of cell mechanics is mechanotransduction, the process through which mechanical cues are transduced into internal chemical signals.[2] A wide variety of tools have been developed to measure the forces applied to the cell and respective response to those forces.[1] Concurrent with technological advancements, cell mechanics has become more relevant in understanding the pathological functions of cells.[3] This emerging and unsaturated field of science leaves a lot of room for engineering students to make contribution in biomedical science. Pedagogical lab modules have proven to be effective tools to help students implement their knowledge of mechanics to understand the behavior of cells.[4] These lab modules can be designed by undergraduate students as a part of their Capstone Design and subsequently used for advanced research or teaching graduate courses. An example of such modules is a stretch chamber that is designed in our lab to study the effect of substrate deformation on cell phenotype. This contribution outlines the design process of this chamber as a Capstone project.

Background

Engineering students are not familiar with cell internal structure and the role of different compartments and membrane proteins to secure cell adhesion. Therefore, the students were instructed

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to conduct a literature review on cell adhesion prior to the conceptual design stage. Tissue cells are able to generate contractile forces due to the activity of the myosin motors.[5] The generated force is transduced via the adhesion sites between the cell and the substrate. These sites consist of clusters of non-covalent bonds between transmembrane mobile receptors and the ligand components of the substrate. The heterodimeric receptors mediating cell adhesion are members of the integrin superfamily (with α and β subunits), which selectively interact with substrate ligands, such as collagen, laminin, fibronectin, vitronectin, etc. The adhesion sites act as anchorage points of cells to secure their position on the substrate and provide the driving force for cell migration. The mature adhesion sites between the cell and substrate are called focal adhesions (FAs), which can laterally grow up to a few microns (Figure 1).[6] While integrins interact with substrate proteins at their extracellular domains, they bind to a dense sub-membrane plaque via their cytoplasmic tails. The plaque is comprised from more than fifty different types of scaffolding and adaptor proteins.[7] FAs are associated with actin fibers (F-actins) and transmit the stress generated by cell's contractile machinery to the substrate.

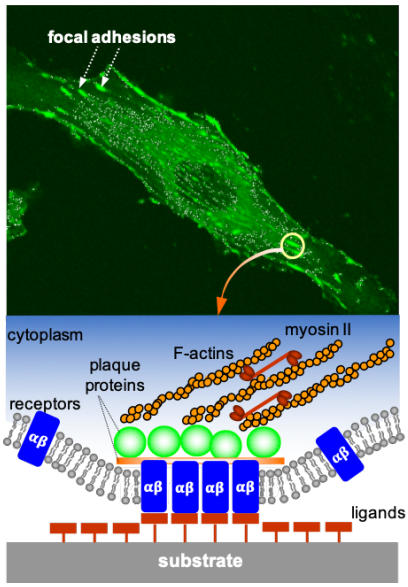

Figure 1: Molecular interactions in cell focal adhesions.

Cells are constantly exposed to external mechanical forces *in vivo*. These include static, dynamic, and hydrodynamic forces generated externally via cell interactions with extracellular matrix, adjacent cells, or fluid movement across cell surfaces. These mechanical stimuli affect different aspects of cellular behavior such as adhesion, migration, and proliferation.[8] Mechanical loads stimulate inter- and intracellular communications. Once cells receive these mechanical cues, they transduce them into internal chemical signals.[9] In this way, cells communicate with their environment and respond to the mechanical signals. For example, the blood pulsing through veins cyclically deform the vessel walls. Endothelial cell (EC) monolayers form the lining layer of blood-contacting surfaces of the vascular system. Due to their unique position, on one side they are in direct contact with the circulating blood flow and on the other side adhered to the vascular and connective tissues of the vessel wall. Continual blood flow exerts continuous hemodynamic pressure and shear stress on the vessel wall. It is known that the pressure-induced circumferential stretch of the arterial wall during the cardiac cycle regulates EC morphology and function.[10] The stretching of ECs is a major contributor to vessel permeability and atherosclerosis. The deformation of extracellular matrix can be simulated in the laboratory environment *in vitro* using a stretch chamber, a device that applies uniaxial (or biaxial) forces to living cells by stretching their substrate (Figure 2).

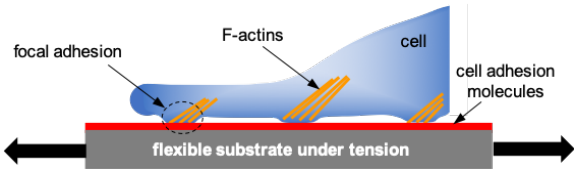

Figure 2: Schematic of cell lamellipodia adhered to a flexible substrate under tension.

Project Objectives and Feasibility Criteria

The objective of this project was to design and assemble a device that can be used to study the effect of substrate deformation on morphology and structure of vascular ECs and their function.

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The device will be capable of applying static and dynamic stretch to the cells cultured on an elastic substrate. The feasibility criteria were listed as the following: (1) the main frame of the device must fit atop of an Olympus CX31 microscope stage and be functional, (2) the main frame and motor must be able to hold up to incubator conditions (37 ºC with 98% humidity), (3) the main frame of the device must be able to withstand sterilization treatment, (4) the device must be able to stretch a polydimethylsiloxane (PDMS) film by 20%, and (5) the device must be able to stretch and relax digitally, at the user's command.

Design Process

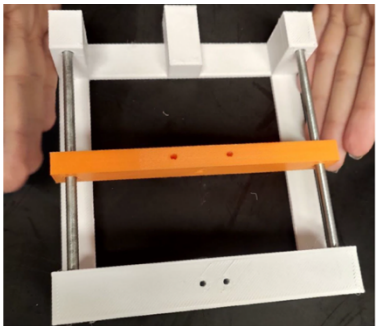

Figure 3: 3D printed main frame with rods and moving pieces.

The key function of the stretch chamber is to apply an external tensile force on a deformable substrate. Different alternatives were considered to drive the mobile piece of the mainframe design to stretch the substrate. This included a lead screw, belt and pulley system, and a linear actuator. Among all other possibilities, the linear actuator design was considered the best choice from alternative designs. The linear actuator worked much like the lead screw; however, it did not extend the screw over the substrate and obstruct the microscope visibility. The linear actuator had much less room for error and was a much simpler design compared to the belt and pulley system.

Main Frame Design

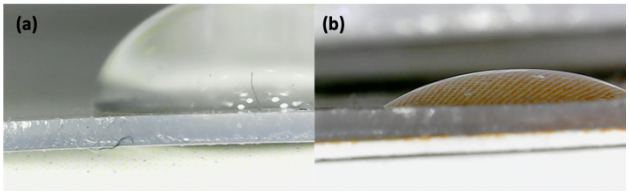

Figure 4: Contact angel of water (a) before and (b) after oxidation of PDMS surface.

The main frame was 3D printed using polylactic acid (PLA), as shown in Figure 3. 3D printing the device allowed the team to do several iterations of the device to ensure the dimensions were aligned properly. A block was added to the main frame as a stage for the motor. The 3D printed moving piece was supported by two aluminum rods on each side. Originally, it was planned to secure the mobile piece onto the screw with a nut on each side. However, the nuts would not hold the screw in place, causing it to rotate in place instead of moving the mobile piece. The moving piece was able to move on the rods, along the main axis of the chamber. After several 3D print iterations, small adjustments were made to the initial conceptual design including a slit in the motor stage for the zip tie that secures the motor, and changes to hole sizes for the rods, screws, and clamps. A PDMS film was used as the deformable substrate. The film had to be coated by cell adhesive molecules to make it adhesive for the cells. Fibronectin was used as the ligand molecule. PDMS, however, is hydrophobic and therefore prevented the functionalization of the film surface. To overcome this problem, the surface of PDMS was oxidized by prolonged UV radiation. Contact angle goniometry showed that the surface of PDMS was oxidized and turned hydrophilic after UV radiation

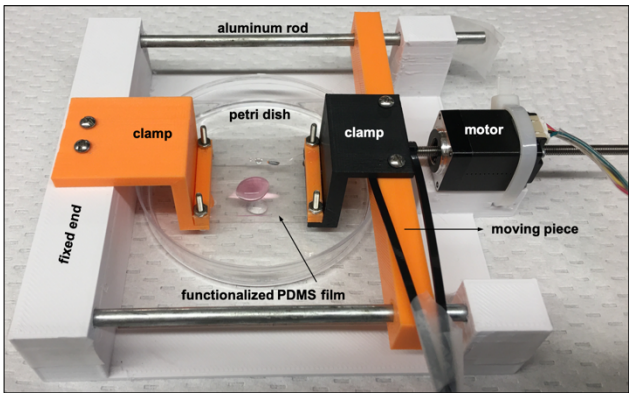

Figure 5: Final assembly of the main frame with motor.

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(Figure 4). The Fibronectin solution was subsequently added to the film and left overnight at 4 °C. The final design of the frame with the actuating motor is shown in Figure 5.

Electrical Design

The electrical components consisted of a proto-board, wires, drivers, LCD screen, and linear actuator. The motor was screwed to the main frame. The wires that connect the motor to the electrical box were chosen to be thin jump wires, allowing the incubator door to be closed without affecting the incubation process. The driver was chosen to be a stepper motor driver designed for low voltage and an Arduino kit's breadboard power supply. The power outlet could feed 9 V to the Arduino Mega and 9 V to the breadboard power supply. The breadboard power supply was used to feed 3.3 V to the motor. The last addition was a fan to cool all the components due to power dissipation by the motor and voltage regulator. The schematic of the electric circuit is shown in Figure 6.

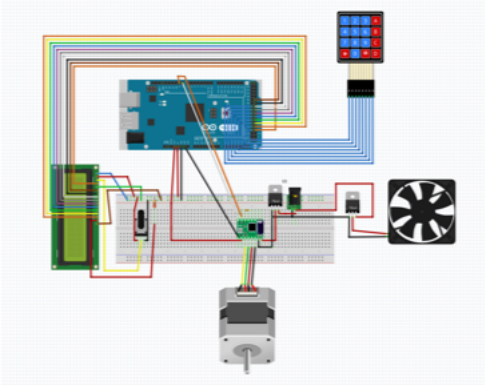

Figure 6: Schematic of breadboard

Test Results and Discussion

Individual Component Test Plan

Since the device had multiple small parts, dimensions needed to be precise or the whole device would be susceptible to failure. Dimension testing ensured the team that the following were correct: the main frame would fit on the device, the hole sizes for the screws, the size of the stand for the motor, the holes for the horizontal bars, and the depth of the clamps in a petri dish that has a height of 15 mm. The clamps need to sit low enough to submerge the substrate and cells in culture media.

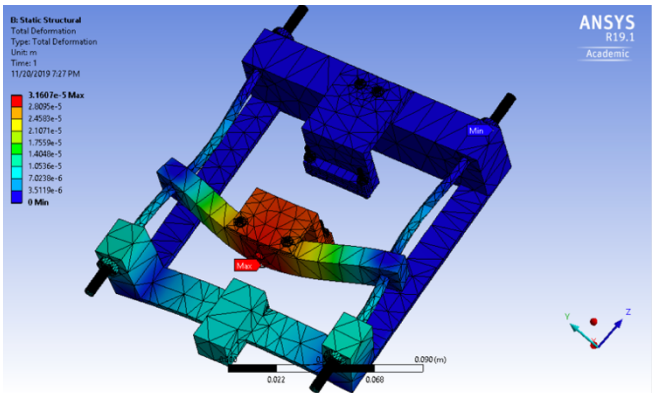

Figure 7: Finite element analysis results for deformation. The maximum deformation occurs at the middle of mobile piece.

Stress-strain dependency of the PDMS film was measured separately to ensure it does not fail at a maximum uniaxial strain of 20%. The stress-strain curves of PDMS showed a typical nonlinear behavior of hyperelastic materials. The clamp design needed to be tested to ensure the it could securely hold the PDMS during the deformation. The test used two team members and the clamps, which were clamping down on a piece of PDMS. The PDMS clamp set up was placed on a table so that the film laid next to a ruler. One teammate held the stationary clamp still while another teammate uniaxially pulled the 4 cm PDMS to 20% of its initial length. In order to be considered a success, the film had to stay in clamps without sliding fully or partially out during and after the stretch. SolidWorks was used assemble the pieces of the main frame while ANSYS was used to analyze and simulate the deformations. The analysis was performed to ensure the PLA would not significantly deform during testing. Figure 7 shows the deformation of the frame with an applied force of 10 N much larger than the operating force at the strain of 20%. The results confirmed that the deflections would be sufficiently small.

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All proto-board connections were tested with no bad connections being found. A current limiting test was accomplished using the reference voltage on the driver. The amperage was tested by hooking the digital multimeter to one of the motor leads and measuring the amperage under load. The current flowing through the system was found to be 0.402 Amps, which is less than the maximum 5 Amps.

Comprehensive Tests

The stretch chamber conveniently fit the stage of Olympus CX31, as shown in Figure 8. Cells were seeded on the functionalized PDMS film and then the chamber was placed in the incubator. The chamber was wired to the control unit which was placed out of the incubator. Subsequent microscopy confirmed that cells were attached and proliferated on the PDMS surface over the course of incubation (Figure 9). All materials used for the device could withstand incubator conditions (37 °C with 98% humidity) as well as sterilization with 70% ethanol. PLA has a glass temperature of 60 °C, which is well above the incubation temperature of 37 °C, meaning the temperature of the incubator should not affect the PLA properties. The chamber was digitally operated inside and out of the incubator, confirming that humidity had no effect on the motor functions.

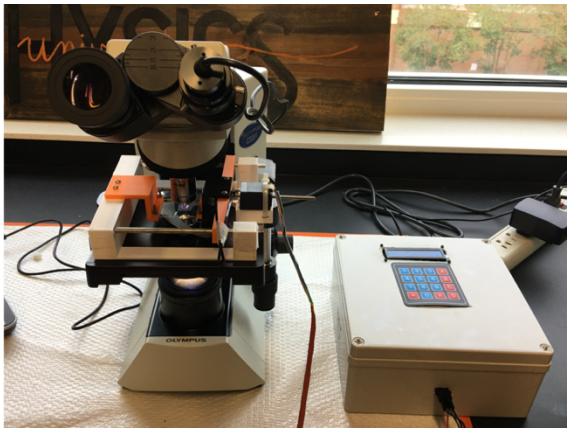

Figure 8: Stretch chamber on the microscope stage and connected to the control unit.

Motor accuracy test was performed by stretching the substrate digitally and measuring the distance between the clamps with calipers. The starting position was measured to the nearest tenth of a millimeter and then a distance to move with a specific direction was specified. Once the program ran and the motor was finished moving, the final position was measured with the calipers. This test was run 3 times in a forwarding motion and 3 times in the reverse motion. The motor was able to move the load to the desired distance with an error less than 3%. In total, the cost to build the chamber was $213.10, less than $300 allocated by the School of Engineering for each Capstone project. The parts list and their costs are listed in Table 1.

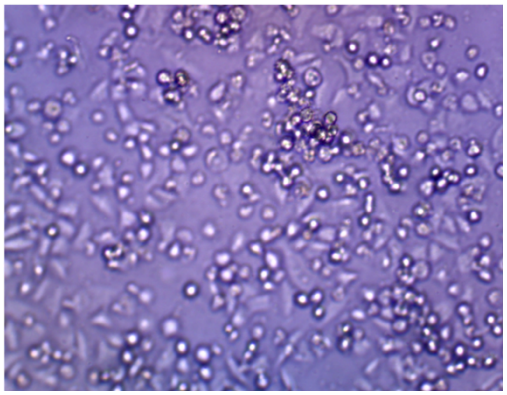

Figure 9: An image of the cells after incubation. The majority of cells were attached and spread on the functionalized PDMS.

Challenges and Benefit to Students

Cells are the fundamental units of living organisms. They are also microscopic machines whose function is governed by the laws of physics. Although understanding the molecular mechanisms underlying cell physiological functions has become an essential part of the undergraduate curricula in the biological sciences, mechanical aspects of cellular behavior is not a part of mainstream education in engineering programs. This Capstone project defined a team-oriented research project for undergraduate students in engineering. Because of the multidisciplinary nature of the project, close collaboration of all group members was essential for effective team performance.

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The successful completion of the project required knowledge from different disciplines, including design of electric circuits, solid mechanics, cellular biology, surface science, and chemistry (Figure 10).

Table 1: Cost of the project.

Item	Cost ($)
Petri dishes	7.95
Screws, clamps and nuts	20.11
PDMS film	13.99
Arduino Mega 2560 Ultimate Starter kit	59.99
DC power adaptor	13.90
Power splitter	5.49
DRV8834 low-voltage stepper motor driver carrier	11.13
Linear actuator	55.00
Wire disconnect	7.88
Rods	4.67
Project box	12.99
Total	213.10

Students experienced a number of challenges throughout the design and assembly of the chamber. To help them, additional information was provided to the students, including links to other learning materials and online information sources. In addition, technical advisors provided access to the equipment needed to make new observations and capture new data. Hydrophobicity of PDMS film, for example, posed a major challenge to experiments as it made it difficult to coat the substrate with cell adhesive molecules. To overcome the issue, students were encouraged to learn about surface science and understand how oxidation may change surface wettability and contact angles. Another challenging aspect of the project was integration of the undergraduate and more advanced topics in engineering. For instance, the force-displacement dependency is nonlinear for PDMS. Nonlinear elasticity, however, is not covered in undergraduate solid mechanics courses. The technical advisors encouraged the students to run simple uniaxial tests on PDMS to observe its nonlinear behavior during deformation. This helped student identify the maximum motor force needed for a given deformation of the substrate.

In conclusion, this Capstone project provided an opportunity for engineering students to understand how life sciences and engineering can be integrated to design and assemble a biomedical device. They learned about the functions of endothelium as an example of a complex biological system and understood how cells adhere to a substrate and how their functions can be regulated by mechanical forces. Biological and engineering requirements often impose restrictions on the design of biomedical devices.[11] In this project, incubator condition, sterilization, and functionalization of substrate were necessary to guaranty cell viability. In addition, compatibility with the microscope was essential for successful application of the chamber in future research studies. The design of the chamber (materials selection, electromechanical connections, size/geometry of the chamber, etc.) was restricted by these factors. The students efficiently collaborated and conducted the project to success by identifying the design restrictions, using their engineering skills, and gaining knowledge about cell mechanobiology from external resources.

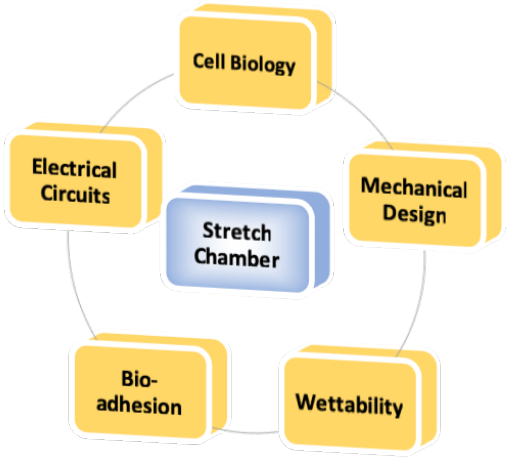

Figure 10: The multidisciplinary nature of the project helped student use their knowledge in cell biology, bio-adhesion, solid mechanics, electrical engineering, and surface science.

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