A System to Dynamically Modulate Stiffness in a 3D Biopolymer Gel

A Major Qualifying Project Report:
Submitted to the Faculty
Of the
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the
Degree of Bachelor of Science

By
Elisabeth Boulanger
Megan Dempsey
Tara Jaroski
Emily Lurier

May 1, 2014

Approved:

1. Mechanobiology
2. Boundary stiffness
3. Tension

Prof. Kristen Billiar, Advisor
Table of Contents

Chapter 1: Introduction .............................................................................................................. 1

Chapter 2: Background .............................................................................................................. 2
  Current Research ..................................................................................................................... 4
  Current Devices ....................................................................................................................... 6

Chapter 3: Project Strategy ....................................................................................................... 8
  Initial Client Statement ........................................................................................................... 8
  Project Objectives and Constraints ...................................................................................... 8
  Revised Client Statement ....................................................................................................... 9
  Project Approach .................................................................................................................. 10
  Project Management ............................................................................................................ 10
  Financial Considerations ...................................................................................................... 11

Chapter 4: Design Alternatives and Verification .................................................................... 12
  Project Challenges and Preliminary Designs ....................................................................... 12
  Design Evaluation ................................................................................................................ 14
  Prototypes and Verification Experiments .............................................................................. 14

Chapter 5: Design Verification ............................................................................................... 26
  Stiffness Experiments ........................................................................................................... 26
  Stainless Steel Ball Coatings ............................................................................................... 29

Chapter 6: Discussion .............................................................................................................. 33
  Project Impacts ..................................................................................................................... 33

Chapter 7: Final Design and Validation .................................................................................. 37
  PDMS Curing Conditions ..................................................................................................... 37
  PDMS Post Stiffness Experiments ....................................................................................... 37
  Lid Design ............................................................................................................................ 38
  Imaging Techniques ............................................................................................................. 39
  Cell Gel Production ............................................................................................................. 39
  Feasibility of System ........................................................................................................... 39

Chapter 8: Conclusion .............................................................................................................. 42
  Future Recommendations ..................................................................................................... 42
  Future Opportunities ........................................................................................................... 43

References ............................................................................................................................... 44

Appendix A: Current Devices .................................................................................................. 46

Appendix B: Pairwise Comparison Chart .............................................................................. 50

Appendix C: Objectives and Functions/Means ..................................................................... 51
  Ranked Objectives ................................................................................................................ 51
  Functions-Means Tree ......................................................................................................... 51

Appendix D: Organizational Tools .......................................................................................... 52
  Gantt Chart ........................................................................................................................... 52
  Work Breakdown Structure ................................................................................................. 52
Table of Figures

Figure 1 Stem cell differentiation based on substrate stiffness (Engler et al., 2006) ......................... 3
Figure 2 Effects of tension on fibroblast phenotype (Eckes et al., 2006) ........................................... 4
Figure 3 System developed by John et al. using stainless steel cantilevered beams to modulate mechanical environment ..................................................................................................................... 6
Figure 4 System designed by Legant et al. to modulate mechanical environment on a micro-scale (2009).................................................................................................................................................. 7
Figure 5 24-well plate mold stamp ................................................................................................... 16
Figure 6 Beam clamp assembly, computer-aided design model ........................................................... 17
Figure 7 Constructed prototype with one clamp and stainless steel beams with Vyon ® pads ...... 19
Figure 8 Initial beam condition (left); beam with weight (right) ........................................................... 19
Figure 9 Three different positions in which the clamp was placed along the beam ......................... 20
Figure 10 Change in active length of beams results in modulation of stiffness ................................. 20
Figure 11 Image of deviation of beams as clamp height is decreased (left to right) ......................... 21
Figure 12 Graph of deviation of beams as active length changed by clamp ..................................... 22
Figure 13 (a) Three-part mold for PDMS post fabrication: Teflon base, stainless steel cast, and Teflon top (b) Stainless steel cast from both sides (c) Vacuum casting chamber ........................................... 23
Figure 14 Computer-aided design model of lid (left); aluminum prototype (right) ......................... 24
Figure 15 Schematic of PDMS post and magnet system ................................................................. 25
Figure 16 Knife edge and micrometer experimental setup ................................................................. 26
Figure 17 Schematic of measuring force vs. displacement values to obtain stiffness ...................... 27
Figure 18 Maximum stiffness values with stainless steel balls or magnets on end of posts; stiffness changes induced by forces of different magnets ................................................................. 28
Figure 19 Representative images of stainless steel balls with no coating (left), optical adhesive coating (middle), and a magnet with rust visible on Day 9 of incubation ............................................. 31
Figure 20 Posts placed on knife edge to determine magnetic interference ....................................... 31
Figure 21 Varying stiffness conditions depending on distance between stainless steel ball and magnet ................................................................................................................................. 38
Figure 22 Modified lid to allow for gas exchange and sterility ......................................................... 39
Figure 23 Example of analyzed image (left); table of measured values obtained by ImageJ analysis (right) ................................................................................................................................. 39
Figure 24 Image of cell culture using 1 mm magnet spacing (right); table of force per cell calculated for least and most stiff conditions (left) ........................................................................... 40

All figures from outside sources received copyright permissions.
**Table of Tables**

Table 1 Design constraints ................................................................................................................. 9
Table 2 Theoretical calculations for cell density for experiments.......................................................... 15
Table 3 Calculated and expected stiffness and force values for the scale device designed .............. 16
Table 4 Stiffness values calculated from beam bending experiment ................................................... 20
Table 5 Distances of magnets with no effect on stiffness ................................................................. 28
Table 6 Average stiffness values for posts with stainless steel balls at four magnet distance ........ 29
Table 7 Representative image of PDMS posts on knife edge with two magnets to determine interference ................................................................................................................. 32
Chapter 1: Introduction

According to the World Health Organization, cardiac disease will lead to 20 million deaths in 2015 [1]. Cardiac fibrosis is commonly found in those who have hypertensive cardiac disease, but there are a limited number of therapies to treat cardiac fibrosis [2]. Therefore, it is important to understand the pathology of cardiac fibrosis to improve existing drugs or lead to new therapies.

Valvular interstitial cells (VICs) are the fibroblast-like cells which work to repair and remodel small injuries to the heart valves caused by normal blood flow. Depending on the mechanical environment and stiffness of the surrounding tissue, the cells will differentiate into their activated phenotype to produce collagen or will remain in a quiescent state. In a healthy heart valve, these cells will produce regulated amounts of collagen to repair the damage to the valvular tissue and then return to their natural quiescent state. However, when this process becomes dysregulated, the myofibroblasts can over-produce collagen leading to a buildup of scar tissue in the valve known as cardiac fibrosis.

Therefore, it is important to understand the mechanism by which the proliferation and differentiation of valvular interstitial cells is regulated. It is more commonly understood that the biochemical environment surrounding cells affects their behavior, such as adding growth factors to induce the differentiation of stem cells. The mechanical environment of the cells, however, is equally important in regulating cell behavior. For example, the stiffness of the valvular tissue directly correlates to the amount of tension produced by VICs which determines the phenotype of the cell.

Several devices have been designed to study the effects of the mechanical environment on cell tension and proliferation. Both two-dimensional (2D) and three-dimensional (3D) models have been used to study these mechanisms, and each has advantages and disadvantages. 2D cell and ECM interactions do not accurately mimic in vivo conditions, and thus 3D gels were developed to overcome this limitation. However, current 3D research models are only able to achieve a single change in stiffness and are unable to accurately quantify the cell-generated tension. Therefore, the goal of this project was to fill this gap in knowledge by designing, creating, and validating a system to uniaxially induce varying levels of VIC-generated tension in real time in a 3D gel matrix.

This report consists of eight chapters outlining the project approach and process. Following this introduction, the second chapter contains a literature review that provides background information on the significance of this project both clinically and scientifically. It describes current research methods and their shortcomings, which this device sought to remedy. In the third chapter, the client statement will be outlined along with the initial approach taken to solve it, which includes the design objectives, functions, constraints and means. Initial designs and proof-of-concept testing will be described in Chapter 4. Chapter 5 then discusses the creation and validation of several of these designs alternatives, and Chapter 6 provides an analysis of the results of these tests. In Chapter 7, the final design is presented along with its validation and subsequent results. Finally, Chapter 8 concludes the findings of the project and outlines recommendations for the future of the system.
Chapter 2: Background

Cardiac valves are connective tissue structures that are critical for maintenance of proper hemodynamic flow through the heart [3]. The most prominent cell type in heart valves is the valvular interstitial cell (VIC), or the fibroblastic cell of the heart. VICs change phenotype and function in response to changes in their mechanical environment in order to sustain the valves’ structural integrity [3, 4]. In the presence of transforming growth factor-β (TGF-β) due to cardiac injury, VICs will differentiate into myofibroblasts which produce collagen to help with tissue remodeling and wound healing [3]. TGF-β also promotes contractile behavior in the fibers formed by VICs [5]. Activated VICs produce tension forces, which further encourages proliferation of the myofibroblasts to heal the cardiac injuries. Cardiac valves are also composed of extracellular (ECM) proteins such as collagen, elastin, and glycosaminoglycans, making the valves flexible yet strong enough to withstand hemodynamic stresses. As blood circulates through the heart, the valves undergo varying mechanical stresses and are constantly experiencing mild injuries during physiological function. As a result, VICs continuously synthesize ECM and express ECM-degrading enzymes to remodel and repair the valvular tissue.

Dysregulation of this mechanism can be causative of diseases such as cardiac fibrosis [6]. A better understanding of how these cells react to abrupt changes in their mechanical environment, such as in cases of severe injury or disease, would aid in the development of more effective treatment methods [4]. When VICs become activated to their myofibroblastic phenotype, they have higher levels of ECM production, express more alpha smooth muscle actin, and have increased contraction compared to their precursor cell type [3]. In healthy tissues, once remodeling occurs by myofibroblasts, they are removed by apoptosis. However, when the life cycle of myofibroblasts is not regulated correctly, they continue to generate forces and produce collagen, which results in fibrosis, scar buildup and a stiffer tissue [3]. More specifically, in cardiac fibrosis, myofibroblasts hyperproliferate and continue to produce ECM even when wounds in the heart valves are healed. As a result, fibrotic scar tissue forms. Normal heart muscle has an elastic modulus of about 10 kPa while fibrotic scar tissue has an elastic modulus of 20 to 100 kPa [7]. Thus, the myocardium stiffens, and impedes ventricular contraction and relaxation. The heart’s architecture then becomes distorted, and its functionality is negatively impacted [5]. Researchers do not fully understand all of the biochemical and mechanical cues necessary for the myofibroblast phenotype to become activated or the role of dysregulated myofibroblasts in valvular cardiac disease [3].

Over the past several decades, a large number of studies have been and continue to be devoted to understanding cellular behavior in response to biochemical cues. For example, the addition of growth factors and other chemicals has been shown to drive stem cell differentiation. However, cells are not only subjected to a biochemical environment but also to a mechanical environment. Research has demonstrated the profound influence of mechanoregulation on cellular differentiation, shape, migration, and proliferation. Namely, substrate stiffness has been shown to play a role in directing stem cell differentiation as shown in Figure 1.
Cells generate certain amounts of tension in response to the stiffness of the substrate upon which they grow [9]. Tension production drives reorganization of the extracellular matrix which consequently affects cell motility and orientation as well as structural integrity of whole tissues [10, 11]. The level of tension produced by cells is dictated by the stiffness of the tissues to which they attach, namely other cells or the ECM, which have elastic moduli on the order of 10 to 10,000 Pa [12]. Normal cells will respond to stiff substrates by applying contractile forces produced by the cross-bridging of actin and myosin filaments in the cytoskeleton [13]. When these cell-generated forces transmit to the substrate, they are called traction forces, which affect migration speed and cell shape. Figure 2 illustrates how the presence of tension causes resting fibroblasts to differentiate into an activated phenotype.
One of the primary roles of fibroblasts is maintenance of tissue homeostasis. When activated under tension, fibroblasts will differentiate into myofibroblasts, proliferate, and synthesize collagen, proteoglycan, and fibronectin. A lack of tension will cause fibroblasts to secrete proteases capable of degrading the ECM and proliferation will be inhibited [6]. Myofibroblasts exhibit higher expressions of α-smooth muscle actin (α-SMA) and contractile forces, harkening their features to that of smooth muscle. As part of the granulation tissue formed during the wound healing process, these cells are responsible for contracting and closing the wound through focal adhesions with the ECM [6, 15].

In summary, a greater knowledge of the mechanoregulation of cells has the potential to advance research in the fields of cardiac fibrosis, wound healing, stem cell differentiation, and more. An understanding of cellular response to various mechanical stimuli could be used in future applications to enhance, inhibit, or regain certain cellular processes. Thus, in order to better understand the mechanisms that control these responses, researchers have developed several methods for modulating substrate stiffness and cell tension.

Current Research

Current research devoted to understanding cell-generated tension has been done in two-dimensional (2D) or three-dimensional (3D) cell culture systems. The former has predominantly served as the means for studying how cells transduce and generate tension. Studies with 2D culture systems have shed light on the role of cytoskeletal tension, mechano-specific signaling pathways, and the importance of specific proteins in cell-cell and cell-ECM adhesions [11]. In 3D culture systems, such phenomena as “dynamic reciprocity” have been extensively studied. Each system has its own advantages and disadvantages so both have continued to be employed by researchers. However, cell morphology, cytoskeletal organization, and cell focal adhesion differ between 2D and 3D systems. This suggests dimensionality alone may substantially influence cell behavior [16].
In two-dimensional culture systems, cells are cultured on top of a protein-laminated substrate. The protein coating, typically collagen or another ECM protein, allows cells to adhere to the substrate. The role that stiffness, or Young’s modulus ($E$), has on cell behavior has primarily been investigated on 2D polymer substrates including polyacrylamide (PA), polyethylene glycol (PEG), and polydimethyl siloxane (PDMS) [15]. By changing polymer chemistry, researchers are able to manipulate the stiffness of the substrate making the mechanical properties easily tunable. Additionally, the transparency of the polymer substrates enables the use of time lapse microscopy to study and quantify cell-generated forces [15]. Cells have been cultured on surfaces with stiffness ranging from 2 to 55,000Pa [17]. These studies have shown that cells, including fibroblasts, generate more traction on stiff substrates than on soft substrates, even when the stiff and soft substrates have equally adhesive surfaces. Therefore, the stiffness of fibroblasts has been found to relate to the stiffness of the substrate they are cultured on; stiffer substrates lead to stiffer cells. Moreover, fibroblasts develop a broader, flatter morphology on stiffer substrates and will preferentially migrate from soft to hard areas when cultured in 2D [17].

However, 2D systems have a multitude of drawbacks. First, the synthetic polymers used as 2D substrates fail to recapitulate the natural biochemical and mechanical environment of tissue. This inaccurate representation of interstitial conditions may cause the cells to exhibit behavior inconsistent with that of cells in vivo. Second, fibroblasts cultured on 2D substrates tend to be “pancake” shaped, whereas fibroblasts cultured in 3D matrices tend to be spindle or stellate shaped [15]. A study conducted by Chen et al. suggests that cell shape governs a cell’s ability to proliferate [18]. Thus, the difference in cell shape between cells cultured on 2D substrates and 3D matrices is important to note. Cell migration also differs greatly between the two systems, as the difference in tension produced by the cells directly affects their migration rate. Furthermore, flat 2D substrates induce an artificial polarity between the upper and lower surfaces of fibroblasts, which are normally nonpolar cells [19]. Finally, cells cultured on 2D surfaces are under the influence of fixed adhesion sites which are asymmetrically distributed over the cell surface [20]. The physical forces at these adhesion sites play a critical role in cell growth. Thus, the homogeneity of the protein coating of 2D substrates can play a part in cell behavior.

To overcome some of the shortcomings of 2D culture systems, many researchers turn to three-dimensional cell matrices. In 3D, the cells are cultured in a more complex physical environment than in 2D, exposing them to conditions more closely resembling those found in vivo [19]. The most widely utilized systems for the study of mechanobiology in 3D are single-protein matrices ordinarily made of collagen or fibrin [11]. Type I collagen, the primary constituent in connective tissue, is the most commonly used biopolymer gel [15].

Similar to 2D, the properties of 3D cell matrices can be manipulated intrinsically. For example, protein concentration levels can be varied to control gel stiffness, porosity, and the number of available adhesion sites. For collagen gels, an increase in collagen concentration increases stiffness and has been shown to increase cell proliferation, cell spreading, as well as more organized actin stress fibers [11]. The mechanical properties of fibrin gels can be tuned over larger ranges since fibrinogen is cross-linked with thrombin and calcium which are not cytotoxic even at relatively high concentrations [11]. Increased fibrinogen concentration causes an almost linear
increase in initial gel stiffness, storage modulus, and fiber density, yet it decreases cell proliferation and spreading. Polymerization conditions, such as temperature and pH, can also be varied to influence fiber density, fiber diameter, and pore size. Cross-linking can be adjusted to create a range of stiffnesses 1 to 100 kPa [13]. These techniques for manipulating stiffness, however, alter not only the biochemistry but the fibrous structure of the 3D gel. This may inadvertently affect cell behavior and make it difficult to accurately isolate and assess the effects of the mechanical environment [15]. In response to this concern, researchers developed an alternative way of changing the mechanical properties of the 3D gel matrices. This method involves altering the mechanical conditions of the boundary attachments of cell-seeded gels [11].

Boundary conditions have a profound effect on cell behavior as demonstrated by the differences observed in fixed and free gels. Fixed gel matrices are ones in which the boundaries are rigidly anchored. For this reason, fixed gels are also referred to as “anchored”, “loaded”, or “stressed” gels. In contrast, free gel matrices unrestrictedly float in media. Therefore, they are also termed “unloaded” and “unstressed” [11]. Though the tension in free gels is not directly measurable, it is believed to be negligible in the center. Providing little resistance to cellular force, matrix remodeling can occur without any kind of orientation of collagen fibrils [20]. In fixed matrices, however, mechanical resistance is present. Over 24 hours, cell-generated tension rises rapidly to reach a homeostatic tension level. Cells generate enough tension against the fixed boundary of the matrix to differentiate [11]. As mechanical tension develops, collagen fibrils will also orient themselves along the same plane as the restraint force. Furthermore, fibroblasts cultured in fixed gel matrices develop fibronexus junctions. These junctions can be stimulated by TGF-β to encourage the differentiation of fibroblasts into myofibroblasts.

**Current Devices**

After extensive research of current approaches, a summary of these devices can be found in Appendix A. It became apparent that two main approaches are currently taken to studying the effects of changing boundary stiffness on cells in a 3D environment. The first method utilizes thin, stainless steel cantilevered beams suspended in a 3D biopolymer gel (Figure 3).
Changing the diameter of these beams changes the boundary stiffness of the gel, as governed by the following equation:

\[ F = k \times \frac{\Delta x}{2} \quad (1) \]

However, this change cannot occur in real time because beams are integrated into the gel and therefore cannot be replaced by beams of another diameter. A different experiment must be conducted in order to observe the effects of each stiffness condition. The second method utilizes PDMS posts, around which 3D gels will form and contract (Figure 4).

![Figure 4 System designed by Legant et al. to modulate mechanical environment on a micro-scale (2009)](image)

Altering the curing conditions of the PDMS alters the stiffness of the posts. As a result, though, this method cannot modulate stiffness in real time. Again, separate experiments must be conducted.

Both methods have their advantages and disadvantages. The stainless steel beam method is done on a relatively large scale, allowing for ease of handling. The PDMS post method is done on a much smaller scale (on the order of micrometers). This allows for a higher throughput. Both methods employ a 3D model system, and introduce a boundary stiffness of known value. Multiple experiments can be performed where boundary stiffness is changed, to observe the effects different mechanical environments have on cells in 3D. However, neither of these methods, nor any other currently practiced, can dynamically modulate stiffness experienced by cells in 3D.
Chapter 3: Project Strategy

The unknown effects of stiffness modulation in 3D cell culture models leads to the focus of this project. The client of this project, Professor Kristen Billiar, approached the design team with an initial client statement. The design team addressed this problem statement by creating an objectives tree which led to the generation of a function means tree. A pairwise comparison chart was developed to rank these objectives. The newly-ranked objectives allowed for the creation of a revised client statement, which led to the generation of device designs to accomplish the client’s needs.

Initial Client Statement
The client tasked this design team with the following statement:

*Design, construct, and validate a meso (mm-scale) system to dynamically alter stiffness and stretch of cells in 3D tissue models and to assess the effects of the mechanical environment on tissue development and contraction.*

After conducting an initial interview with the client, this team was able to begin conducting background research. Upon gaining a more in-depth knowledge of the project topic, several more interviews were conducted with both Professor Billiar, Ph.D., and Mehmet Kural, M.S., to better clarify the needs of the client. This led to the development of objectives, functions and constraints that will govern the design of the resulting device.

Project Objectives and Constraints
The requirements for the design, provided in the client statement, were broken down into objectives, functions, and constraints. The objectives dictate what the design should accomplish, the functions will determine what the device will do and the constraints will dictate the design boundaries.

The device objectives are as follows:

1. *User-friendly*
   a. Portable
   b. Easy to Assembly
   c. Easy to Clean
   d. Easy to Use

2. *Safe*
   a. Aseptic
   b. Autoclavable

3. *Reliable*
   a. Consistent
   b. Durable
4. **Versatile**
   a. High throughput
   b. Multifunctional

5. **Cost Effective**

A pair-wise comparison chart was created to rank the objectives, which can be found in Appendix B, and required input from both the project team and client. The device must be safe for both the user and most importantly, the cells. This was ranked as the most significant objective as no data will be generated if the cells are not functional. The reliability of the device will affect the consistency of the results, which is important during research; thus, the device must also be durable so that the device does not experience any deformation, which would alter the recorded data. The design must allow for versatile function because the device must modulate cell-generated tension, which requires the device to be multifunctional. To generate a significant amount of data for analysis, the goal is to design for high throughput of the device. User friendliness of the device is not as significant as the previously mentioned objective but can be achieved upon proof of concept. Likewise, the device does not necessarily need to be cost effective, as it is not going to be designed for commercial purposes.

From these ranked objectives, a functions means tree was generated to brainstorm the possible design functions, which can be found in Appendix C. The design team created four primary functions that the device must perform which include holding a well plate, dynamically modulating cell-generated tension, measuring the cell tension and maintaining sterility. These functions led to the development of design constraints, which focus primarily on maintaining sterility of the device and the success rate of the experiments. These constraints are displayed in Table 1.

<table>
<thead>
<tr>
<th>Incubator Size</th>
<th>Autoclave Size</th>
<th>Well Plate Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 cm x 49 cm x 12.5 cm</td>
<td>17 cm x 41 cm</td>
<td>12.8 cm x 8.5 cm x 2.3 cm diameter= 16.85 cm</td>
</tr>
<tr>
<td>Durability</td>
<td>Success Rate (aseptic methods)</td>
<td>Amount of Cells per Experiment</td>
</tr>
<tr>
<td>Material must last for a minimum of 100 experiments</td>
<td>90% success = 22 out of 24 wells need to be successful</td>
<td>1 million cells per 24 samples</td>
</tr>
</tbody>
</table>

**Revised Client Statement**

Upon completion of a secondary meeting with the client, the following revised client statement was developed based on the previously defined objectives, functions and constraints:

*The goal of this project is to design, create and validate a device to dynamically modulate the level of tension generated by fibroblast cells in a 3D tissue model. The design should allow for*
Chapter 3: Project Strategy

*a minimum of three levels of tension generation, and modulate between them. The device must be easy to assemble, easy to operate, and must elicit reliable and reproducible levels of tension in the cells. The device also needs to be reusable, portable, and compatible with aseptic techniques.*

This revised client statement provides a more specific and detailed understanding of the designs objectives and functions.

**Project Approach**

This section will describe the design process and development of the device, which will elicit VIC-generated tension by modulating stiffness using mechanical means. The consistent analysis of data relies on several assumptions, which include the following:

1. The valvular interstitial cells will be evenly distributed throughout the fibrin or collagen 3D gel
2. The 3D gel will be uniform in area, concentration of fibrin or collagen, and have initial flat surfaces
3. Each cell will generate equal values of tension in a uniform direction

It is important to have an even distribution of VICs throughout the gel so that the method of measuring the cell-generated tension is consistent. The tissue should grow evenly inside the well, which requires this even distribution. The same theory applies to the distribution of fibrin or collagen in the gel. The gel should also have smooth surfaces so that the dimensional area of the gel can be accurately measured to compare to the eventual tissue.

The cell-generated tension will be found using the tissue that will grow during these experiments. It is important to assume that each cell is generating an even amount of tension so that accurate analysis can be conducted.

To create preliminary designs, three main device tasks were developed. The device should hold the well plate in a fixed position in order to assure the measured deflection is not skewed. The device should then control the level of stiffness provided by mechanical means and measure the applied stiffness. Measuring the applied stiffness in real time will verify that the data collected correlates with the assumed stiffness.

These assumptions and tasks will assure the device will accurately modulate stiffness to elicit varying levels of cell-generated tension. From these objectives, functions, constraints, assumptions and device tasks, the stiffness applied to valvular interstitial cells can be controlled to elicit the generation of tension.

**Project Management**

The project goal was to be accomplished in 8 months. With this time constraint, it was important to create a Work Breakdown Structure and Gantt Chart to facilitate task organization and time management. The Work Breakdown Structure breaks the overall tasks into subtasks so each objective can be clearly and fully completed. This hierarchy of tasks can be compiled into a Gantt chart which provides information on when certain parts of the project must be completed and allows for project progress to be tracked. These charts can be found in Appendix D.
Financial Considerations

The cost per experiment was estimated based on experiments currently being conducted in the Billiar lab. The cost analysis can be found in Appendix E. The estimation for prototype and manufacturing costs of the device, as well as the resulting costs of the experiment can be found in Appendix E.
Chapter 4: Design Alternatives and Verification

Based on the design objectives, functions, and constraints, design alternatives were developed to meet the needs of the client. A design evaluation matrix was used to compare and contrast each alternative in order to choose the two highest-ranking designs (Appendix F). These designs were then prototyped and underwent design verification experiments to determine the designs’ viability.

Project Challenges and Preliminary Designs

To create preliminary designs, three main device challenges were discussed. First, the device must hold the well plate in a fixed position in order to assure the measured deflection is not skewed. The device must then control the level of stiffness provided by mechanical means and, finally, allow the user to easily quantify the amount of tension produced. Measuring the applied stiffness in real time will verify that the data collected correlates with the theoretical stiffness.

Four methods of overcoming these challenges and modulating beam stiffness were designed based on the design specifications, assumptions, and challenges stated previously. These methods include using a clamp to modulate the active length of the beam, altering the applied stiffness of the cells by altering the beam material (using water, air, polymers, metals, etc.), using piezoelectric actuators, and using magnets in combination with PDMS beams to alter material stiffness.

Beam Clamp Method

A framework will be built around the well plate. This framework will hold beams at a designated length so that they are held within the gel. The beams will pass through a clamp which will attach to the frame. The clamp will be able to adjust the active length of the beam which will alter the stiffness of the beam. The stiffness can be calculated with the following equation:

\[ Stiffness = k = \frac{3EI}{L^3} \]  

(2)

where \( E \) is the Young’s modulus of the material, \( I \) is the moment of inertia, and \( L \) is the active length of the beam.

This method has many advantages as well as disadvantages. The stiffness of the beam can accurately be controlled and determined as well as be easily modulated, as the clamp will be able to shift easily. The deflection of the beam will be easy to view and image. The tissue growth will also be easy to image as the framework will have a clear bottom so the gel can be imaged from below. A disadvantage would be sterility because there is a lot of material suspended above the gel, including the top plate and clamp. Another issue that could arise is the consistent position of the beams in the gels.

Altering the Structural material

This method would use a shell material, similar to a thin rubber, which will have extremely
low structural properties. The shell will touch the surface of the 3D gel and a material with a specified stiffness will be put inside the shell. The stiffness of the material will then be applied to the tissue. To modulate the applied stiffness, the material will simply be removed and replaced with a material with another specified stiffness.

The advantage of this method would be that the modulation of the applied stiffness would rely on the material placed inside the shell. In this way, the inner material could easily be replaced with another material with a higher or lower stiffness and thus, the applied stiffness would be modulated. During this changing process, depending on the sharpness of the tip of the thin material, the shell could be pierced or the tissue could slide off of the shell.

**Pneumatic/Hydraulic Method**

Using the sample principle, a pneumatic or hydraulic method could be applied as well where a certain water or air pressure will be applied inside the shell. The pressure will be modulated thus altering the stiffness applied to the tissue. The same advantages apply but many disadvantages are created. The system would have to be leak-proof in order to assure the correct pressure. This method would also require a power source and a water or air source. The device could be very expensive and would not be as portable as specified in the design objectives.

The equation relating pressure and stiffness is as follows:

\[
PWV^2 = \frac{E_{in\times h}}{pr_i} \quad (3)
\]

where \(PWV\) is the ratio between strain and the change in pressure inside the shell, \(E_{in}\) is the slope of the stress strain curve of the material, \(h\) is the wall thickness, \(p\) is the density of the fluid, and \(r_i\) is the radius of the material (or shell).

**Piezoelectric Actuators**

Piezoelectric actuators are commonly used to prevent beams from vibrating by inducing a voltage to the beam. The beam will cease to vibrate as the material becomes stiffer. Modulating the applied voltage to the beam could alter the beam stiffness for this application upon demand. The advantage of this application is that the stiffness could be accurately controlled. However, an insulating material must separate the beam from the tissue as an electric current would alter the actions of the cells.

The equations, which relate voltage to the bending stiffness and membrane stiffness of the beam, is as follows:

\[
(EL)_{eq} = (EL)_{mec} + \frac{e_{31}^2}{e_{33}}S_s\left(z_s^2 - \frac{h_s^2}{12}\right) \quad (4) \text{Bending Stiffness}
\]

\[
(EL)_{mec} = E_cI_c + c_{11}(I_s + I_a) + c_{11}(S_s z_s^2 + S_a z_a^2) \quad (5) \text{Beam Stiffness}
\]
**PDMS Beams and Magnets**

Similar to experiments performed by John et al. and Kural et al., two PDMS posts will be mounted next to one another with a specified distance in between. The distance separating the posts will be governed by the outer diameter of one well in a 24-well plate in which the posts will be mounted. This is a bottom-up method in which the cell gel will be formed over the posts. Magnetic forces will be used to modulate the stiffness of the posts using two different methods.

The first method includes attaching a magnetic sphere or sphere made of a ferrous material to the top of the post. As the cells begin to produce tension and cause deflection in the PDMS posts, a cylindrical magnet will be placed over the disk to re-align the post perpendicular to the ground. The spheres will also act as an anchor for the cells to grow around and generate tension.

The alternative to using these spheres is to place a wire (magnetic or ferrous) in the center of the PDMS post. This method will work similarly to using the spheres, in which a magnet will be placed over the beam to realign it perpendicular to the ground once cells begin to apply tension and cause deflection.

The advantages of using PDMS posts are that they are easy and inexpensive to make. A mold will be used to create the posts (with or without the metal wire at its center) which, while time-consuming, is very simple as the PDMS only needs to be poured and placed in the oven for a specified amount of time. The mold can be used more than once which is cost effective, although the PDMS itself is slightly more expensive. The stiffness of the post is easy to modulate as well, as the intrinsic stiffness is based on the ratio of base to catalyst and curing time.

Using medical grade glue or another layer of PDMS to cement the sphere to the top surface of the post will be very simple. This will allow for better sterilization (as nothing will be placed over the cells) and will also provide an anchor for the cells to grow around. Gluing the disc to the top of the PDMS post is simpler than gluing the pads to the metal beams (in the beam-clamp method) as the diameter of the PDMS post is larger than that of the metal beam.

**Design Evaluation**

A design matrix was created to weigh the advantages and disadvantages of each design method. The success of each design approach was also measured based on the amount of objectives and constraints that were met. This matrix can be found in Appendix C.

**Prototypes and Verification Experiments**

Based on the results from the design matrix, the beam-clamp method and PDMS post method using magnets had an equal number of advantages and disadvantages. Thus, both designs were investigated simultaneously. The following section will discuss the design, manufacturing, and costs of each prototype.

**Cell Density and Gel Size**

Before prototypes were designed, it was necessary to calculate the approximate cell density, gel volume, and number of cells per gel as well as the range of stiffness and forces that the device aims to induce.
To estimate these values, two different studies were utilized and compared: a study by John et al. and one by Kural et al. Both of these experiments used devices with beams of varying stiffness to induce changes in cell-generated forces [11, 15]. The device designed by the MQP team will combine different characteristics of these two devices on the meso-scale. John et al. used a cell density of 500,000 cells/mL in a gel of 6 mL for a total of 3 million cells [15]. Kural et al. used a cell density of ~1.5 million cells/mL in a gel of 0.08 µL for a total of 100-180 cells [11]. The following calculations for hypothetical MQP Device Options 1, 2, and 3 in Table 2 below were completed using a cell density of 500,000 cells/mL to use the least amount of materials and cells necessary to obtain the desired results.

Table 2 Theoretical calculations for cell density for experiments

<table>
<thead>
<tr>
<th></th>
<th>Cell # per 1 Gel</th>
<th>Volume (mL)</th>
<th>Total Number of Cells for n=24 (per experiment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>John et al. device</td>
<td>3 million cells</td>
<td>6</td>
<td>72 million cells</td>
</tr>
<tr>
<td>MQP Device Option 1</td>
<td>125,000 cells</td>
<td>0.25 (250 μL)</td>
<td>3 million cells</td>
</tr>
<tr>
<td>MQP Device Option 2</td>
<td>83,333 cells</td>
<td>0.167 (167 μL)</td>
<td>2 million cells</td>
</tr>
<tr>
<td>MQP Device Option 3</td>
<td>41,667 cells</td>
<td>0.083 (83 μL)</td>
<td>1 million cells</td>
</tr>
<tr>
<td>Kural et al. device</td>
<td>100-180 cells</td>
<td>0.00008 (0.08 μL)</td>
<td>~4320 cells</td>
</tr>
</tbody>
</table>

There is an obvious difference in the amount of cells used between the John et al. device and Kural et al. device. For working on the meso scale, the cell number and density must be between these values while also using a 24-well plate in the most efficient manner possible. The recommended volume of a typical 24-well plate well is 0.6-1.6 mL. Because the well size in a 24-well plate is larger than the well size needed to accommodate the cell gel density (desired volume of well is 83 μL), PDMS will be used to create smaller rectangular shaped wells within the well plate. The rectangular molds for each well were constructed from laser-cut acrylic pieces. These pieces were then attached to a lid that was placed over the well plate to act as a stamp for the PDMS as pictured in Figure 5. PDMS was then injected through the lid into the well plate and cured for 12 hours at 60°C. The dimensions of the rectangle are larger than the required gel size of 83 μL to provide enough space for media or other future cell densities.
Based on these factors, different cell numbers could be used in future experiments, but to begin experimentation, a volume of 83 µL for each gel would be used. This means for an n=24 experiment, a total of 1 million cells would be needed. This volume/cell number was selected because it is closest to the client’s original requirement that 1 million cells be used per experiment of 24. This will use the least amount of cells and materials. However, depending on the relationship between stiffness and force, the cell number may need to be modified, which is why calculations were done for two other cell numbers (shown in Table 2 above).

Next, expected stiffness values and forces were interpolated using the desired values for cell number (see Table 3 below).

As explained in the Kural et al. paper, the forces generated are slightly higher possibly because of the contractility of the VICs used or a factor in the chemically defined media [11]. To calculate the stiffness range and force per cell range for each condition of cell number, linear interpolation was used. A sample calculation for Device 1 at low stiffness is shown below in equation 7, and this calculation was repeated for stiffness and force of each of the MQP Device

Table 3 Calculated and expected stiffness and force values for the scale device designed

<table>
<thead>
<tr>
<th></th>
<th>Low stiffness (N/m)</th>
<th>High stiffness (N/m)</th>
<th>Low force per cell (nN/cell)</th>
<th>High force per cell (nN/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>John et al. device</td>
<td>0.048</td>
<td>0.64</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>MQP Device Option 1</td>
<td>0.146</td>
<td>1.033</td>
<td>18.92</td>
<td>408.6</td>
</tr>
<tr>
<td>MQP Device Option 2</td>
<td>0.147</td>
<td>1.039</td>
<td>18.94</td>
<td>413.1</td>
</tr>
<tr>
<td>MQP Device Option 3</td>
<td>0.149</td>
<td>1.044</td>
<td>18.97</td>
<td>417.5</td>
</tr>
<tr>
<td>Kural and Billiar device</td>
<td>0.15</td>
<td>1.05</td>
<td>19</td>
<td>422</td>
</tr>
</tbody>
</table>
Options from Table 3.

\[
y - 0.048 \frac{N}{m} = \frac{0.15 \frac{N}{m} - 0.048 \frac{N}{m}}{125,000 \ cells - 3 \times 10^6 \ cells} = \frac{0.15 \frac{N}{m} - 0.048 \frac{N}{m}}{180 \ cells - 3 \times 10^6 \ cells}
\]

\[y = 0.146 \frac{N}{m}\]

If 125,000 cells are used per gel, the stiffness range is 0.146-1.033 N/m and the forces generated are expected to be 18.92-408.6 nN/cell. If 83,333 cells are used per gel, the stiffness range is 0.147-1.039 N/m and the force range is 18.94-413.1 nN/cell. If 41,667 cells are used per gel, the stiffness range is 0.149-1.044 N/m and the force is expected to be 18.97-417.5 nN/cell. These numbers calculated are guidelines for the device designed and can be further modified using the calculations shown above.

Prototype of Beam-Clamp Device

The beam-clamp device was designed to achieve three main goals which include modulating the applied stiffness by changing the active length of metal beams which touch in the 3D gel, mounting the well plate, and ease of use. This device is composed of the following parts (diagram shown Figure 6):

A. Base plate to mount well plate
B. Top plate to hold beams
C. Side tracks
D. Side track inserts
E. Clamp with extrusions
F. Clamp without extrusions
G. Spring Clamp

The top plate consists of small holes to hold the beams. These beams are 10 mm apart which accommodates the desired gel size. Each set of holes is 26 mm apart, which correlates with
the distance between each well in the 24-well plate. The purpose of the rectangular cutouts is to provide space for the user to adjust the metal beams or to further visualize the experiment from the top perspective. Four screw holes were placed in each corner, which will be attached to hex standoffs, providing a desired distance between the top and bottom plate as well as anchoring them together. The left and right side of the top plate also has anchoring holes for the side tracks.

The base plate anchors the hex standoffs as well as the side tracks. The well plate slides onto the base plate and can be imaged from the underside, as the cutout of the baseplate correlates with the well-area of the 24-well plate. Three side tracks were mounted on each side of the bottom plate which hold the side track inserts used to adjust the height of the side clamp (Figure 6 above demonstrates only one side track; however, in the actual device there were three on each side).

The side track inserts fit within the side tracks. The side track insert height will be adjusted using the spring clamp, as shown in Figure 6 (above). The clamp without extrusions (CWOE) was mounted permanently to the side track inserts while the clamp with extrusions (CWE) was solely attached to the CWOE. A thin layer of PDMS was applied to each side of the clamp to provide a stronger fit around the beams. The clamp height was easy to adjust; thus, the active length of the beams was easy to modulate. Each of the three clamps can be set to a different height allowing for three different conditions for four of the wells in the well plate. Therefore, two devices will be required to achieve 6 different conditions with four samples.

Manufacturing

Acrylic was used to create the preliminary device. Acrylic is both easy to sterilize and easy to process, which was desired to fit the objectives as well as time constraints. The bottom plate, side tracks and both pieces of the clamp were machined by the machinists from Washburn labs at Worcester Polytechnic Institute. The top plate and temporary side tracks were laser cut, which were used to perform preliminary verification experiments. 304V stainless steel beams with a diameter of 0.005” (placed through the top plate) were purchased from Component Supply Company. Only one clamp was included in the constructed prototype to allow for easier verification experiments as shown in Figure 7 below.
Two sets of experiments were conducted to assess the stiffness and position of the stainless steel beams in the device. The stiffness at varying clamp positions was assessed using PDMS weights and the corresponding displacement of the beams. The beam positions were also evaluated at various clamp heights.

Stiffness of Stainless Steel Beams

One 0.0064” diameter stainless steel beam was connected to the top plate of the device using rubber cement. The plate was then oriented so that the beam was parallel to the bench top. An image was taken of this setup to provide the initial condition for the experiment (zero deflection). Three PDMS weights were used to generate a force vs. displacement curve, the slope of which is considered the measured $k$ value (N/m) for this experiment. These weights were then placed on the end of the beam to create an initial deflection as shown in Figure 8.

The clamp was machined out of acrylic following the CAD model seen previously. A thin layer of 10:1 PDMS was applied to each side of the clamp to ensure the beams would not slip through the clamp.
The distance between the clamp and top plate was varied in order to create a set of data points with which the stiffness of the beam could be calculated using ImageJ (NIH), as shown in Figure 9 below.

![Figure 9 Three different positions in which the clamp was placed along the beam](image)

The theoretical stiffness values obtained using Equation 2 are compiled in Table 4 below.

<table>
<thead>
<tr>
<th>Active Length (mm)</th>
<th>Deflections with specified weights (mm)</th>
<th>Theoretical k (N/m)</th>
<th>Actual k (N/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.2</td>
<td>0.03 g 30.9 0.05 g 43.1 0.08 g 53.4</td>
<td>0.014</td>
<td>0.013</td>
</tr>
<tr>
<td>55.3</td>
<td>0.03 g 15.3 0.05 g 16.9 0.08 g 25.0</td>
<td>0.046</td>
<td>0.046</td>
</tr>
<tr>
<td>26.2</td>
<td>0.03 g 2.17 0.05 g 4.80 0.08 g 5.27</td>
<td>0.43</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The graph in Figure 10 shows proof-of-concept that changing the active length of the beam using the acrylic clamp does make the beam stiffer. Though several of the values are within range of their calculated counterparts, the shortest active length had a large deviation between the calculated and measured stiffness value. Error in this experiment could be due to the beams not maintaining their original positions each time the clamp was moved and the beams may also be slipping through the two layers of PDMS within the clamp.
To prevent the beam from moving within the clamp, lanes were cut into the PDMS layer in hopes of aligning the beam while still clamping it to ensure the correct active length. However, due to the extremely small diameter of the beam, the lanes did not allow full clamping of the beams to occur. The modulus of the PDMS was then altered to create a stickier PDMS that would prevent the beams from shifting within the clamp. The following verification experiment determined the magnitude of deviation of the position of the beams as the clamp height was modulated.

Relative Position of Beams in Well Plate

The stainless steel beams must stay in the same position as they are clamped and unclamped throughout the experiment to assure that no other factors contribute to the cell-generated tension produced. To verify the position of the beams within the well plate as the clamp moves, six beams were placed inside the assembled device using rubber cement. A camera was placed underneath the device and beam position was captured for three different clamp heights. ImageJ was used to calculate the deviation of the beams from their initial position. Figure 11 displays an example of the deviation observed in the position of the beams as the clamp moved positions. The resulting data from ImageJ can be found in Figure 12.
On average, there was about a 2 mm deviation in position that occurred. This movement would cause issues in the final experiments due to the small well size and desire to observe beam movement solely based on cell-generated forces. It is not understood how the movement of the beams within the well would affect the cell gels, but a better method of securing the beams would be ideal to create a more controlled experiment.

**Prototype of PDMS Post and Magnet Method**

The PDMS post and magnet system was designed to use magnets to modulate stiffness. This is desirable over the previous clamped beam method because the posts’ positions will be fixed to the bottom of a well plate. This will also result in increased sterility and greater control in future experiments.

**Manufacturing**

The equipment and materials needed for PDMS post fabrication were donated by Myomics, Inc. The high quality of the donated items made the post production process much more reliable. Furthermore, the process reduced the time frame for producing PDMS posts to only a few hours. This new approach has, therefore, become the primary method for post fabrication.

The PDMS posts are made using a three part mold, consisting of a Teflon base, stainless steel cast, and Teflon top (Figure 13a). The mold creates circular bases, each with a pair of posts. The posts have a diameter of 800 µm and a height of 4 mm. On top of each post is a small cap, and between each pair of posts is a 3.5 mm gap. The posts, due to their small size, are capable of fitting within one well of a 96-well plate.

Importantly, the stainless steel cast has two sides, Side A and Side B (Figure 13b). Side B rests on top of the base. The three pieces (display in Figure 13a) were plasma etched with oxygen for 40 seconds. Then the pieces were silanized for a period of 24 hours. These steps were taken to
prevent the PDMS form sticking to the mold as the PDMS cures.

After salinization, two pieces are placed inside a vacuum casting chamber (Figure 13c). Two arms traverse one side of the chamber, running from the inside to the outside. On the inside, a zip tie is attached to the end of each arm to form a sling. The sling is used to secure a small beaker containing approximately 22 g of PDMS. The chamber is also integrated with a digital pressure gauge. With both the beaker of PDMS and mold inside the chamber (and the lid shut), a vacuum is applied until this pressure gauge reads 400-500 µm.

Applying the vacuum causes bubbles to form within the PDMS. The handle of the arm can be used to vigorously shake the beaker in order to break these bubbles. Once most of the air has escaped from the PDMS, it is poured into the mold. Upon entering the mold, the PDMS will again start to bubble. The release and intake valves, then, are used to increase and decrease the pressure within the chamber to eliminate these bubbles. After the majority of bubbles is removed, the vacuum is turned off and the release valve opened, which returns the pressure within the chamber back to room pressure.

At this point, the lid is opened, and the Teflon top of the mold is placed firmly on the two-piece base. The three pieces are then tightly clamped together using three C-clamps arranged in an alternating pattern. Finally, the mold (with the clamps) is placed in an oven. The temperature of the oven and the length of time the mold is left in the oven affects the ultimate stiffness of the PDMS posts produced. It was discovered that at a base to curing agent ratio of 20:1, the mold should remain in the oven for 40 minutes at 80°C in order to achieve a stiffness in the range of 0.1-0.2 N/m.

Upon taking the mold out of the oven, the clamps are removed and the Teflon top is carefully detached from the rest of the mold using a thick spatula. Small amounts of 70% isopropyl alcohol is applied to the top side of the stainless steel piece to remove excess PDMS. Next, the
Teflon base is carefully detached. Some posts stick to the base, fully intact, and can be used for experimentation. To free the ones stuck inside the stainless steel cast, forceps are used to circle the of each pair’s base. More isopropyl alcohol is applied to loosen the posts further. The forceps are used once again to then carefully remove the posts. Only posts with a base and cap intact are deemed suitable for experimentation.

One stainless steel (SS) ball (1.5 mm in diameter) is then glued using optical glue (Norland) to the top of each post so that the ball is centered on the cap. The combination of the posts and SS balls are then UV cured for 5 minutes. After curing, four of the posts are placed and again glued using optical glue in a standard 8-well strip, leaving one well empty between each post. The well plate is then UV cured for 5 minutes.

A lid was designed to hold the magnets over the posts in the 8-well strip. The lid was designed to fit securely over the 8-well strip while holding the magnet at desired distances from the stainless steel balls. Multiple lids will be used to achieve this purpose. The computer aided model and finished prototype can be seen in Figure 14. Aluminum was used to make the prototype because it is easy to manufacture, as it is a soft metal. The small dimensions in the lid design require a material that is both easy to manufacture and structurally stable. An issue may arise with using aluminum because it is cytotoxic.

The schematic below represents the fabrication and use of the PDMS and magnet method, beginning with PDMS post production and ending with cell culture (Figure 15).
Verification

Verification of this method will be discussed in Chapter 5 as this was chosen as the final design with which to move forward.
Chapter 5: Design Verification

This chapter focuses on verifying each component in the PDMS post and magnet system. Experiments were conducted to verify that the desired inherent stiffness of the posts was achieved as well as conducting experiments to assure that the magnets and stainless steel balls did not rust. Additional studies were conducted to find the appropriate magnet and stainless steel ball combination in order to modulate the posts to the desired stiffness conditions. Magnet interference was also investigated.

Stiffness Experiments

Several experiments were performed to verify the stiffness of the PDMS posts as well as the magnetic effects on the stiffness using different combinations of magnets and metal.

Verification of Measurements

Using a micrometer, electronic balance, and knife edge as pictured in Figure 16 below, the forces and corresponding displacements of the PDMS posts were obtained, and spring constant \( k \) was calculated using the slope of the line generated from this data.

![Figure 16 Knife edge and micrometer experimental setup](image-url)
Figure 17 displays how the posts are placed on the knife edge to calculate force as a function of the displacement measured by the micrometer.

To verify that the experimental setup was able to generate accurate $k$ values, the $k$ value obtained from a set of PDMS posts made with an 18:1 PDMS ratio and cured for 30 minutes at 122°C was compared to its calculated $k$ value obtained from Equation 2. The average $k$ value measured was $0.3451 \pm 0.0652$ N/m. The theoretical value is $0.3338$ N/m. This yields about a 3.4% error assuming the theoretical value is accurate. The theoretical value utilizes a length of 4.07 mm and a radius of 0.38 mm (both measured with calipers) as well as a Young’s modulus of 483 kPa measured with an Instron of a PDMS sample.

**Measurement of Low Stiffness**

Based on previous experiments by John et al. and Kural et al., the goal was to obtain a low stiffness of about 0.1 N/m. PDMS posts were fabricated at a 20:1 PDMS ratio and cured at 80°C for 40 minutes. Three posts were measured using the same method as described in the previous experiment. The $k$ value calculated experimentally was $0.1504 \pm 0.002$ N/m.

**Magnet and Stainless Steel Ball Combinations**

The next set of experiments was done to determine which combination of magnets or metals should be used to modulate the stiffness of the PDMS posts. Posts from the same batch with 18:1 PDMS ratio cured at 122°C for 30 minutes were used. For the first set of measurements, one 1.5 mm diameter spherical 440C stainless steel (SS) ball was glued to the top of each post using Loctite Gel Control Super Glue. The second set of measurements replaced the SS balls with 1/16” diameter by 1/32” long cylindrical N48 neodymium magnets. The stiffness of the posts was actuated using four types of cylindrical neodymium magnets all of 1/16” diameter but of varying heights and strengths as denoted by their $N$ value. Force and displacement values were obtained using the same experimental setup as the previous 2 experiments with the addition of a stand to
hold the pulling magnet concentric with the end of the post and vary its position relative to the end of the ball or magnet glued to the end of the post. The data is presented in Figure 18 below.

![Figure 18 Maximum stiffness values with stainless steel balls or magnets on end of posts; stiffness changes induced by forces of different magnets](image)

The maximum $k$ values were obtained when the magnets were held 1 mm from the end of the posts. Bringing the magnets any closer than 1 mm away from the stainless steel balls essentially achieves a rigid post which was not desirable. The distances where no magnetic effect was experienced varied and can be viewed in Table 5 below.

**Table 5 Distances of magnets with no effect on stiffness**

<table>
<thead>
<tr>
<th>Pulling Magnets</th>
<th>SS Ball on End</th>
<th>N48 1/16” x 1/32” Magnet on End</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4” long N52</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>1/16” long N52</td>
<td>9.5</td>
<td>24</td>
</tr>
<tr>
<td>1/16” long N42</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>1/32” long N48</td>
<td>4.5</td>
<td>13</td>
</tr>
</tbody>
</table>

It is also important to note that the deposition 150-200 mesh size nickel particles at the top of the posts before curing was utilized. However, there was no appreciable effect of the four magnets on the posts with nickel particles at the end, and therefore this method was discontinued.

**Stainless Steel Balls on Posts**

After analyzing the data and weighing the advantages of each combination of magnets and metals, we decided to move forward with the stainless steel balls on the end of the posts in conjunction with the ¼” long N52 cylindrical magnet to actuate stiffness. One 1.5 mm diameter
440C SS ball was glued with Bluestar Silicones Silibione® Medical Adhesive to the top of the posts made with 20:1 PDMS ratio and cured for 40 minutes at 60°C. The magnet was held at four different distances from the SS balls with the 10 mm distance effectively having no effect on the stiffness of the posts and 0 mm distance touching the SS ball and holding the post taut. The experiment was repeated three times and the average $k$ value of the posts with the magnet held at these four distances are reported in Table 6 below.

Table 6 Average stiffness values for posts with stainless steel balls at four magnet distance

<table>
<thead>
<tr>
<th>Average Stiffness Values (mN/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mm (no effect)</td>
</tr>
<tr>
<td>0.114 ± 0.050</td>
</tr>
</tbody>
</table>

Stainless Steel Ball Coatings

Research was conducted to determine a proper coating for 1.5 mm 440C SS balls that will be used in the system. Although SS 440C usually resists corrosion in mild environments, we wanted a coating that would be impermeable to prevent any corrosion or rusting that would leach out of the coating and kill cells [22]. Prior to experimentation, many coatings were investigated.

A very common silicone elastomer, PDMS, was considered for coating because it has good flexibility, temperature stability, is chemically inert, low cost and is permeable to gases, impermeable to water and nontoxic to cells [23]. It has been shown, however, that water escapes through PDMS and small molecules float through crevices in PDMS, which would not be ideal for this application [23]. Most researchers working with microfluidics want PDMS to have proper gas and nutrient exchange and add sugar particles to PDMS to make the pores larger [24]. Another problem with using PDMS in this application is how to coat the stainless steel balls in a uniform manner. Depending on how the PDMS is cured on the balls can change the coating thickness. Also, using a different ratio of base to curing agent could change the porosity. In this experiment, ratios of 5:1 and 1:1 will be attempted as potential coatings that would be less permeable [25].

Researchers have also used parylene coatings on top of PDMS to make it impermeable [26]. Parylene coatings are very expensive and require special equipment. Companies will typically coat a large quantity but parylene itself cannot be purchased for small laboratory use without proper equipment to use with it. As parylene is not produced or sold as a polymer, a vacuum application system is used that starts with a dimer, that is then placed in a vacuum system and converted to a reactive vapor of the monomer. This is then passed over room temperature objects and the vapor coats the object rapidly with a polymer. This results in an impervious, uniform finish that is impermeable and biocompatible [27]. This coating is much stiffer than PDMS at approximately 2700 MPa versus approximately 1 MPa for PDMS [28]. Although this coating holds much promise for future applications with the system designed, with the budget, this is not an option.

Another material considered for coating the stainless steel balls was Bluestar Silicones Silibione® MED ADH 4100 RTV, which is a medical grade high strength adhesive. It has a high
strength elastic bond of silicone to silicone and cures at room temperature for 20 minutes [29].

Another coating considered was Norland Optical Adhesive. This is a clear, colorless, liquid photopolymer that cures when exposed to UV light 350-380 nm for 1 minute to 60 minutes depending on the UV wavelength [30]. New Skin Liquid Bandage was also considered because it is antiseptic, flexible, waterproof, low cost and easy to obtain [31].

Coating Durability Experiments

After researching various coatings for the stainless steel balls, an experiment was designed and conducted to determine the rate of rusting for each coating based on images and which coating would be best for this cell culture application. First, stainless steel balls were glued to the top of PDMS posts using 1:1 PDMS and cured. Two stainless steel balls were used for each of the following conditions:

- No coating (control)
- 1:1 PDMS
- Liquid Bandage
- Norland Optical Adhesive
- Bluestar Silicones Silibione ® MED ADH 4100 RTV

PDMS with a curing ratio of 1:1 had a low viscosity and tended to drip down the posts. To address this problem, 1:1 PDMS was cured for 24 minutes at 60ºC, and was then used to coat the stainless steel balls as it was almost cured. These posts with coated balls were placed back in the 60ºC oven to cure for 10 more minutes to ensure the PDMS coating stayed on the balls. Liquid Bandage was more liquid than the PDMS and was even less viscous. It dripped down the posts slightly, but took less time to cure/dry. Norland Optical Adhesive is very viscous, thick and adhered well to the stainless steel balls. It cured in less than 5 minutes with 365 nm UV light. Bluestar Silicones medical glue was sticky, thick and difficult to work with, especially within the tissue culture hood.

Each of the PDMS posts with a coated ball was placed in a well of a 24-well plate. During experimentation, the wells were filled with PBS as salt accelerates rusting. The stainless steel balls were imaged prior to incubation. The 24-well plate containing PBS and stainless steel balls with coatings was incubated and reimaged once per day over nine days to observe any macroscopic rusting. After one week of incubation, there was no rust visible on the SS balls with any coating using a Canon EOS7D camera. A neodymium magnet was added to the experiment in a separate well with PBS to compare to the SS balls. Rust was visible on the magnet within twenty-four hours. This proved that any rust would be visible to the naked eye and no rust was visible on the SS balls, coated or uncoated. Therefore, the stainless steel balls can be used in the system with no coating and cause no adverse effects due to rusting. Representative images of the SS balls with and without coatings, on Day 9 of incubation, are shown below in Figure 19.
Magnetic Interference Experiment

A set of stiffness experiments was conducted to prove that the magnet affecting one post would not interfere with the stiffness of the other post. A pair of posts with an inherent stiffness of 0.258 mN/mm was tested with only one post on the knife edge (Figure 20).

A ¼” long N52 cylindrical magnet was placed 3 mm away from the post off the knife edge, and the resulting stiffness of the post on the knife edge was found. Comparing this value with the inherent stiffness, the magnet demonstrated a negligible effect on the opposite post (Table 7 below).

The magnet was then placed 3 mm away from the post on the knife edge. As expected, the magnet increased the stiffness of the post. The stiffness calculated was 0.326 mN/mm. Finally, a test was done in which two magnets were used. A magnet was placed 3 mm away from each post, and the resulting stiffness of the post on the knife edge was 0.275 mN/mm. This decrease in stiffness of the post on the knife edge suggests that there is magnetic interference when using two magnets. Thus, subsequent calibration stiffness experiments utilized two magnets to account for this effect since the system was designed to use two magnets for cell culture experiments. Table 7 depicts the experiment visually.
Table 7 Representative image of PDMS posts on knife edge with two magnets to determine interference

<table>
<thead>
<tr>
<th>Left Post</th>
<th>Right Post</th>
<th>Both Posts</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
</tbody>
</table>
| • $k=0.258$ mN/mm  
  • Inherent stiffness: $k=0.203$ mN/mm | • $k=0.326$ mN/mm | • $k=0.275$ mN/mm |
Chapter 6: Discussion

The overall goal of this design project was to design a system that would uniaxially induce varying levels of tension exhibited by valvular interstitial cells in a 3D fibrin gel matrix. While there were many challenges associated with this aim, a system was developed that fulfills this goal as well as the objectives and constraints. The system has been designed to utilize PDMS posts with stainless steel balls glued to the post heads. A set of two PDMS posts is placed in each well of a 96-well plate. A lid was designed to hold magnets above the PDMS posts, in a sterile manner, to affect the stiffness of the posts. As the distance between the magnets decreases, the stiffness of the post increases and vice versa. This was proved through post stiffness experiments (results shown in Chapter 5). The stiffness range of the post obtained by using the magnets was from 0.2 N/m to 0.8 N/m or completely rigid. These values were consistent with the desired stiffness ranges of soft to stiff boundary conditions shown in previous research. These stiffness values were achieved by varying the ratio of PDMS and the curing time. Cell-populated fibrin gels were then cultured on the posts in each well and the tension generated by these cells was modulated by holding the magnet device at different heights above the stainless steel balls. By measuring the displacement of the posts, cell-generated forces were quantified. The objectives to be fulfilled by this design were: safety, reliability, versatile function, high-throughput, user friendly and cost effective. The system designed thus far has fulfilled the most important objectives, as it is being designed to be compatible with aseptic technique and to be reliable. The constraints included that the device must fit in an incubator, be standardized to a well plate, have a 90% success rate and last for at least 100 experiments. The system designed will fit in an incubator and is standardized to a 96-well plate (or an 8-well strip of a 96-well plate). In cell culture experiments, the goal was to achieve a 90% success rate and to conduct several experiments utilizing the system.

The system designed is unique because it mechanically modulates the tension generated by cells in a 3D gel. Researchers have designed several devices to study the effects of cells’ mechanical environment on cell tension and to understand the mechanisms that govern cell-generated tension. 2D and 3D models have been utilized to study these mechanisms; however, 2D models do not accurately mimic in vivo conditions. 3D models utilize gels to overcome the limitation of 2D models. However, current research models do not focus on modulating cell-generated tension by mechanically varying the stiffness of the substrate (PDMS posts in this system designed). This project sought to enhance understanding of this research area.

Project Impacts

When conducting a design project, it is important to take into account economics, environmental impact, societal influence, political ramifications, ethical, health, and safety concerns, manufacturability and sustainability of the project. The system designed in this project will only be used for research purposes and thus, the main concerns include safety of the cells cultured and manufacturability of the design.
Economic

The results of this project would not likely influence the economy of everyday living. The system designed in this project is for research purposes and use in the laboratory as a tool to understand mechanisms behind mechanoregulation of disease. However, the PDMS post system is economically advantageous as compared to the clamped beam method as the PDMS post system costs $9.81 to produce whereas the clamped beam system cost $89.81. The design chosen for the ultimate completion of the project was the PDMS post system which is significantly less expensive than the other alternative and other current research devices.

Environmental

The results of this project could have an eventual positive impact on the natural environment. The system designed uses a few simple, common materials whose production has little negative impact on the environment. The system is proposed to further researchers’ understanding of how cells react to a dynamic change in the stiffness of their environment. This knowledge gain could lead to an eventual cure for diseases including cardiac fibrosis of the heart valves or cancer. A cure for these diseases would reduce the number of surgeries occurring as well as resources utilized. Reducing the number of surgeries could lower carbon emissions from material production and transportation.

Societal

The system designed could have an influence on ordinary people in society. Once the system has been modified and optimized, the knowledge gained from experiments conducted on the PDMS post system could lead to a cure for diseases such as cancer or cardiac fibrosis. This implies the system would be very well accepted into society as it could indirectly save many lives in the future. It can be marketed to researchers in the field of mechanobiology.

Political

The PDMS post system could influence the global market indirectly. It could eventually be utilized to discover the mechanical mechanisms behind disease. Like any medical discovery, this could lead improved treatments or cures. This would have a global effect, but the actual system designed in this project will not have a global use. Whichever researchers purchase the system and choose to use it to understand disease will have an impact globally, but the actual system will not affect the global market as a whole or the culture of other countries.

Ethical

This PDMS post system can only have a positive effect on society. It is a research tool that can ultimately be used to understand what dysregulation in cellular processes that lead to disease. The ultimate end goal of the system designed can only lead to an outcome of a prolonged and satisfying life for those affected. The greatest ethical concern is if this research is not conducted. If scientists and engineers do not use this PDMS post system to understand disease pathologies, then cures are less likely to be discovered. It would be more unethical if this research
was not conducted and researchers did not attempt to understand the mechanisms behind various diseases. Additionally, the valvular interstitial cells utilized for experiments on this device thus far were from heart valves which were going to be discarded. By using cells that were going to waste, the cell culture experiments in this system utilized waste for something positive.

A potential ethical concern for future applications of this system would be the study of stem cells’ reaction to changes in mechanical environment and conversion between phenotypes. Stem cell research that utilizes human embryonic stem cells has both ethical and political implications that should be taken into consideration.

**Health & Safety**

In terms of safety of the cells cultured in the system, it was designed to maximize sterility of the cell-populated gels. The system does not cause harm to any person as it is solely a laboratory research device with which *in vitro* cell culture experiments can be performed. However, if sufficient knowledge is gained from experiments performed in the system, a cure could be discovered or a mechanomedicine designed that would positively impact the medical community and ultimately save lives.

**Manufacturability**

The manufacturability of the device will be more challenging as the PDMS posts and stainless steel balls are difficult to work with due to their small size. However, a system can be designed to automate the fabrication of PDMS posts with SS balls attached. Additionally, the lids would need to be machined with a plastic, such as polycarbonate or acrylic, or biocompatible metal instead of the cytotoxic aluminum currently used. The design created in this project can easily be reproduced. The PDMS posts used in the system are manufactured on a large scale as the equipment to make the posts was donated by Myomics. The manufacturing process should be modified slightly to make it easier to extract the PDMS posts from the molds as well as attach stainless steel balls with glue. This entire fabrication process should be automated. In terms of the amount of materials used for manufacturing, each iteration of the system requires very few materials (PDMS, stainless steel balls, lid material, magnets, and 8-well strips). Using fewer materials allows for less overall waste produced.

**Sustainability**

Sustainability is an important aspect of a design project. The production of the PDMS post system should not negatively affect biology/ecology in terms of renewable energy. Producing the PDMS post system to be used by researchers could eventually lead to greater sustainability because if cures for diseases are discovered, surgeries and other medical necessities can be eliminated. There will be less material wasted on finding cures for diseases because this system could help understand the mechanism behind various pathologies. This design is more sustainable than the clamped beam method which was not chosen as the final design. The system designed is also sustainable because the PDMS posts and 8-well strips can be reused after each experiment.
Overall, this design has several limitations to overcome but appears promising to accomplish the goal, objectives and constraints originally determined. The validation of each component of the design is discussed in the following chapter.
Chapter 7: Final Design and Validation

The curing conditions, stiffness of the posts, lid design, cell-gel production, and imaging techniques were validated to ensure that the PDMS post and magnet system produces reliable and reproducible data.

PDMS Curing Conditions

The ratio of polymer base to catalyst has a significant effect on the stiffness of PDMS. In addition, the temperature at which PDMS is cured, as well length of time it is given to cure, directly affects stiffness. In general, a higher base to catalyst ratio will yield a lower stiffness value. Higher temperatures and longer curing times will yield greater stiffness values. There is a balance to be found among these parameters when attempting to achieve a certain stiffness.

Numerous curing conditions were tested in order to achieve an inherent post stiffness of approximately 0.1 N/m (Appendix G). It was validated that PDMS at a 20:1 base to catalyst ratio, cured at 80°C for 40 minutes yielded posts with a sufficient stiffness value of 0.152 ± 0.020 mN/mm. Therefore, the conditions under which these posts were made were deemed optimal.

PDMS Post Stiffness Experiments

The stiffness of the PDMS posts were validated using a unique experimental setup (refer to Figure 16 in Chapter 5). The setup was designed in such a way that the deflection of the PDMS posts could be controlled, and the force associated with that deflection could be calculated. Testing was done on posts after SS balls had been attached, using Norland Optical Adhesive®. Experimentation was carried out as follows:

The bases of the PDMS posts were adhered to the very end of a cantilever platform, so that the posts, themselves, were oriented horizontally and extended from the platform. The platform, and therefore the posts, could be raised or lowered in small increments by turning an adjustment knob. To start experiments, the height of the platform was adjusted so that the SS balls attached to the posts rested on a knife edge, located on an electronic balance. Additionally, adjustments were made to position the posts perpendicular with the knife edge. From here, the platform was lowered a certain distance. This displacement was measured using a micrometer, which was attached to the platform/post assembly. The change in distance would cause the posts to deflect against the knife edge. The force generated was calculated by multiplying the acceleration due to gravity (~ 9.81 m/s²) by the scale reading at that point of deflection. Displacement was increased approximately 0.2 mm, a minimum of five times, for each experiment. Displacements and corresponding forces were plotted against one another. The slope of the best fit line generated by the data equates to the spring constant, $k$, for the post tested. By such methods, the inherent stiffness values of the PDMS posts were validated.

The same experimental setup was used to test magnetic effects on stiffness. A magnet holder was used to position magnets at various distances from the posts. A camera placed directly above the experimental setup was used to take high resolution images of the posts, knife edge, and magnet. Using these images, the distance between the top of the SS ball and magnet was validated with ImageJ software. The images also ensured the alignment of the magnets with the SS balls, and
that only the SS balls rested on the knife edge.

Such stiffness experiments were conducted to validate modulation between a minimum of three stiffness conditions. The stiffness of posts cured under optimal conditions was tested with a ¼” long N52 cylindrical magnet held at 1 mm and 2 mm. The last condition required no magnets, relying on the inherent stiffness of the posts. Force was calculated at five points of displacement, for each case (Figure 21). The slopes of the force vs. displacement curves were calculated, and therefore the stiffness values determined. With the magnets at distances of 1 mm and 2 mm away, the posts exhibited stiffness values of 0.801 mN/mm and 0.413 mN/mm, respectively. The inherent stiffness of the post was 0.204 mN/mm (no magnet condition). Therefore, the experiment was able to demonstrate successful modulation between three stiffness conditions, achieving a 3.9-fold increase between the lowest and highest stiffness condition.

![Figure 21 Varying stiffness conditions depending on distance between stainless steel ball and magnet](image)

**Figure 21 Varying stiffness conditions depending on distance between stainless steel ball and magnet**

**Lid Design**

The original lid designed (refer to Chapter 5) had the potential to hinder gas exchange which is required to culture viable cells. Upon further modifications, the lid was designed to maintain sterility while allowing for gas exchange. The top of the 8-well strip is not flush with the underside of the lid as the two extruding knobs allow for a small space between the top of the strip and the lid. Magnets are placed in the desired holds and fit tightly within these holes preventing them from falling into the 8-well strip (Figure 22).

The lid was also designed to sit above the wells at a distance that prevents the media required for cell culture from touching the lid. This contributes to the sterility of the system.
Imaging Techniques

Consistent imaging of the PDMS posts was validated by running a mock experiment, in which no cells or media were used. To conserve resources, the wells were filled with deionized water instead. The test well-strip was placed on a lab bench top, and imaged using a Canon EOS7D camera on a tripod setup. Where the well-strip was placed was marked, and the distance from the camera lens to the well-strip was measured at 4”. After an image was taken, the well-strip and camera were moved well out of position. This was to simulate the events of an experiment involving cells, during which the well-strip would need to be taken in and out of the incubator for imaging.

The well-strip and camera were then repositioned in the manner they were originally, and another image was taken. This process was repeated so as to attain three images of the well-strip at three different times. For four separate wells, the distance between the centers of the SS balls on each post was measured using ImageJ software (Figure 23). These measurements were made using each image. The average distances for each well are presented in Figure 23. The coefficient of variance in all cases was 5% or lower, ensuring the accuracy and repeatability of the imaging technique.

<table>
<thead>
<tr>
<th>Average Distance (mm)</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Well 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.51</td>
<td>3.54</td>
<td>3.41</td>
<td>3.55</td>
</tr>
<tr>
<td>Standard Deviation (mm)</td>
<td>0.17</td>
<td>0.19</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>5%</td>
<td>5%</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Cell Gel Production

In order to conduct experiments involving cell culture, a procedure was designed for producing fibrin cell-gels. The detailed procedure can be found in Appendix H. Pluronic is applied to wells to discourage gel attachment to the walls. Gels were populated with approximately 250,000 cells/mL, and 100 µL of cell-gel solution was dispensed into each well, just enough to submerge the stainless steel balls. Approximately 100 µL of media is then added to each well to sustain the cell-gel culture.

Feasibility of System

A preliminary cell culture experiment was conducted using the PDMS post system to
ensure that the system could be successfully used in cell culture experiments. Before cell culture, the system including the 8-well strip with posts and the lid must be properly sterilized. This was completed using 70 percent ethanol and UV light inside the cell culture hood. VIC-populated fibrin gels were cultured in four wells of an 8-well strip. The remaining four wells contained control gel with no posts or stainless steel balls. In two of the four wells containing posts, magnets were held 1 mm above the posts using the custom lids. During culture, one of the two posts in each well attached to the magnet in the lid; thus, one of the posts was held rigid for each set. The other two wells utilized the lid with no magnets for the most compliant condition. Cell-generated forces were compared for wells without magnets and wells with magnets held 1 mm above the SS balls after 19 hours in culture. With no magnets, both posts were assumed to be the same stiffness of 0.204 mN/mm. For one post held rigid, the stiffness was assumed to be 7 mN/mm as previously determined experimentally while the stiffness of the other post 1 mm from the magnet was assumed to be 0.801 mN/mm. The equivalent stiffness $k_{eq}$ [N/m] of two posts attached to one gel is calculated like two springs in series utilizing Equation 6 below with $k_1$ [mN/mm] being the spring stiffness of one post and $k_2$ [mN/mm] being the spring stiffness of the other post.

$$\frac{1}{k_{eq}} = \frac{1}{k_1} + \frac{1}{k_2} \quad (6)$$

After calculating the equivalent stiffness, the following equation was used to determine the force per cell where $F$ [mN] is the force per cell and $\Delta x$ [mm] is the change in distance between the SS balls from $t=0$ to $t=19$ hours. The number of cells per well was approximately 25,000 based on the cell count and gel volume per well.

$$F = \frac{k_{eq} \Delta x}{\text{# of cells per well}} \quad (7)$$

The average force per cell at the lowest stiffness was $3.4 \pm 0.5$ nN/cell while the force per cell at the highest stiffness condition was $15.5 \pm 6.5$ nN/cell. The results show that the cells that contracted around the stiffer posts generated higher forces per cell because they produce more tension in response to a higher stiffness (Figure 24).

<table>
<thead>
<tr>
<th>Average Force per Cell after 19 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Least Stiff</strong> (no magnets)</td>
</tr>
<tr>
<td>3.4 ± 0.5 nN/cell</td>
</tr>
</tbody>
</table>

The p-value for these results was 0.059. This value indicates a definite trend but cannot be used for statistical analysis due to the small sample size and high variability. Although this experiment had a small sample size of $n=2$ for each condition, it holds promise for future cell
culture experiments because it proved that gels can be successfully cultured with the system. The cells were viable when observed under an Olympus CK-2 Microscope with 4X and 10X magnification and contracted around the PDMS posts as seen in the representative image above (Figure 24).
Chapter 8: Conclusion

The final system was able to successfully modulate the mechanical environment of a 3D biopolymer gel in real time. Currently, no other system has been able achieve more than a single change between quantifiable stiffness values. Yet, such a system could be used to study cell differentiation, as well as cells' ability to convert phenotypes. The PDMS post and magnet system achieved a 3.9-fold change between the highest and lowest stiffness conditions while maintaining a sterile culture environment. The stiffness ranged from 0.204 mN/mm to 0.801 mN/mm, which is consistent with the levels of stiffness used in current research methods. All components of the PDMS post system were validated to ensure the system would function as designed. Additionally, imaging repeatability was validated to ensure the deflection image results would be reliable.

In a preliminary experiment (n=2), the VIC populated fibrin gels showed varying levels of force production as the mechanical environment was modulated from a low to high stiffness. Future modifications for the PDMS post and magnet system were identified, and following future optimization, the system could provide further knowledge into understanding the mechanobiology of cell behavior.

Future Recommendations

In the future, there are several aspects of the assembly and operation of the system that can be optimized. The fabrication of the posts proved difficult because the system from Myomics was design for stiffer PDMS. With the more compliant PDMS curing conditions used in this system, the posts were difficult to remove from the molds and resulted in a low yield of intact posts with each batch produced. Additional protocols for post fabrication using silanization of the molds should be thoroughly investigated to optimize post yield.

In terms of system assembly, some tools, guides or molds could be created to more easily and accurately glue the stainless steel balls to the top of the posts. Position of the balls, amount of glue, and position of the posts within the wells should all be controlled perhaps even by an automated process in the distant future.

In future iterations of the lid design, it should more adequately allow for gas exchange with the environment and be machined out of stainless steel or another bioinert, noncytotoxic material. Coatings could be utilized to achieve this biocompatibility. Additional cytotoxicity experiments should be performed on the lids as well as the stainless steel balls and PDMS posts after employing the same sterilization methods performed prior to cell culture experiments. A different mechanism for changing magnet height above the posts could also be designed using screws or notches similar to the clamped beam method.

Further understanding of the orientation and strength of the magnetic fields would be ideal for the future optimization of this system. The effects of adjacent magnets should also be explored further in experimentation as well as theoretically. With these stiffness experiments, a more reliable setup should be used to achieve more repeatable results with the knife edge, magnet position, electronic balance, and micrometer.

In order to fully validate the system, further cell culture experiments must be performed
using the three different lids containing the various magnet heights. It should be ensured that the magnets at the 1 mm distance do not touch or pull the stainless steel balls off of the posts so the various desired stiffness conditions can be achieved using some sort of spacer or different method for placing the lids. Investigation into cell viability and compatibility with the system should be explored due to the inconsistency and challenges encountered with cell culture in this project. A modification to the experimental protocol could also include imaging from the top of the strip to avoid distortion from the concavity of the wells. This could be achieved by using a clear lid or placing the magnets on the side of the posts if the appropriate range of stiffness values can still be achieved in this orientation. Other more accurate imaging techniques using inverted microscopy or other techniques should also be explored.

**Future Opportunities**

An understanding of the mechanoregulation of cell behavior can aide in furthering research fields such as cancer cell research, stem cell research and the development of mechanomedicines. Our system can be used as a tool to further contribute to these fields of study.

Normal cells are capable of producing tension dependent on their phenotype and the tissue in which they live. This prevents them from crossing tissue types, as they cannot deform the collagen fibers of the other tissue type to migrate efficiently. Comparably, cancer cells are able to change their morphology, which allows them to produce varying levels of tension. Cancer cells can then cross tissue types, which leads to metastasis. It is believed that cancer cells are soft compared to healthy cells, however it has not yet been proven. Hence, Wirtz et al. (2012) discuss the need to know the stiffness of cancer cells and understand their ability to pass on physical attributes from generation to generation. The system designed in this paper could contribute to this topic of study, as the behavior of cancer cells could be modulated and quantified in real time as the stiffness of their environment changes [32].

As mentioned previously, the stiffness of the environment surrounding cells directly affects their behavior, including differentiation. It is not well understood how the mechanical environment effects the differentiation of naïve stem cells [33]. Understanding this mechanism could further contribute to controlling the differentiation of stem cells to grow desired tissues types. The mechanical modulation of stem cells using the designed system could provide data collection in real time to understand how the stiffness of the environment affects the resulting phenotype of naïve stem cells. Additionally, this data could further lead to developing scaffolds with tailored mechanical properties to grow natural tissues or heal damaged tissue with a desired phenotype *in vivo* [33].

Stimuli-sensitive drug delivery vehicles have become a popular area of research, changing environmental conditions including temperature, pH, and the chemical environment to elicit the drug release. There is limited research focused on developing drug delivery vehicles sensitive to mechanical stimulation.
References


[16] Lecker, Stewart H., et al. "Multiple types of skeletal muscle atrophy involve a common
Appendix A: Current Devices

There are a variety of ways researchers have been able to measure cell-generated forces and also manipulate the stiffness of 3D gel matrices through changing boundary conditions. For example, experiments done by John et al. utilized cantilevered beams (stiffness of 0.048-0.64 N/m) to alter the boundary stiffness of 3D collagen gels seeded with 3 million human foreskin fibroblasts. A series of stainless steel (SS) cantilevered beams of different diameters were attached to the boundaries of the gels to alter their stiffness over a large range and in a graded manner (see Figure 1).

The beams were compliant, and able to bend as cell-generated forces acted upon them. The resulting deflections in the beams were determined through digital imaging, and the cell-generated forces along each axis were calculated using the equation:

\[ F = k \times \frac{\Delta x}{2} \]  

where \( k \) is the spring stiffness and \( \Delta x \) is the change in distance between the two beams along one axis. Increases in force correlated with increases in boundary stiffness, and the force per cell was about 17 to 100 nN. This experiment was novel in that it measured cell contractility within 3D gels between the standard fixed and free boundary conditions.

Researchers are able to quantify cell-generated forces in a number of ways through boundary condition manipulation. Culture force monitors (CFM) were first developed to measure forces generated by cells in cell-populated gels. A CFM has a cell-populated gel suspended in media between an isometric force transducer and a fixed anchor so the tension can be measured. Powell et al. developed a mechanical cell stimulator that applies forces similar to the body to engineered tissues. A force transducer is attached and measures internally-generated and externally-generated forces (see Figure 2 below).
A multi-station CFM system has also been developed by Campbell et al. which has four vertical cantilever beams with semiconductor strain gauges and computerized data acquisition units to measure contractile forces within a collagen gel. Another CFM system was developed by Peperzak et al. which studies mechanobiological responses of cells in collagen gels with a cantilever beam and semiconductor strain gauges [22].

An experiment conducted by Boudou et al. utilized microelectromechanical systems (MEMS) technology and generated arrays of cardiac microtissues that were embedded in 3D micropatterned matrices. This system utilized microcantilevers that constrained cardiac microtissue contraction and also reported forces generated by the tissue in real time. Boudou et al. also independently varied the mechanical stiffness of the cantilevers and collagen matrix which showed that the forces of contraction and static tension in the tissue increased with boundary and matrix rigidity (see Figure 3 of the device below).

MEMS technology was also utilized in a study by Legant et al. in which arrays of microtissues were created. These microtissues were made of cells within 3D micropatterned matrices. Microcantilevers were used to constrain collagen gel remodeling and measure the forces generated by the cells (see Figure 4 below). Legant et al. also independently varied the
stiffness of the cantilevers which showed that cellular forces increase with boundary or matrix rigidity.

A bio-magnetomechanical microtissue system was developed by Zhao et al. for use in the magnetic actuation of 3D microtissue arrays with microcantilevers (See Figure 5 below). This system allows the user to measure the contractility and stiffness of engineered tissue and dynamically stimulate the microtissues.

Another study conducted in the Chen lab developed an array of silicone elastomer-based microwells (<1 mm in length) each containing PDMS cantilevers (micro tissue gauges, or µTUGs). This system was used to culture cell-populated collagen gels between compliant anchors and in a high throughput way. The system is advantageous because of the small size of samples which leads to low material costs, lowered diffusion limitations, and the ability to use
powerful microscopes for imaging. This system was used to measure force per cell of different cell types and measure changes in tension in response to stimuli. Also using this system, the intrinsic stiffness of microtissues could be measured using magnetic beads attached to one of the cantilevers in each well pulling the tissues with magnetic force (the previous system described).

Thus, the effect of stiffness on cellular functions continues to be thoroughly investigated in 3D cell culture matrices. However, researchers have yet to investigate the effects of modulating stiffness dynamically in real time on cell characteristics and behaviors.

References:


## Appendix B: Pairwise Comparison Chart

<table>
<thead>
<tr>
<th></th>
<th>User-friendly</th>
<th>Safe</th>
<th>Reliable</th>
<th>Versatile</th>
<th>Cost-effective</th>
<th>Group</th>
<th>Advisor</th>
<th>Grad Student</th>
</tr>
</thead>
<tbody>
<tr>
<td>User-friendly</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Safe</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Reliable</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Versatile</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cost-effective</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix C: Objectives and Functions/Means

Ranked Objectives

1. Safe
2. Reliable
3. Versatile
4. User-friendly
5. Cost-effective

Functions-Means Tree
Appendix D: Organizational Tools

Gantt Chart

Work Breakdown Structure

Design a Device to Mechanically Modulate Cell-Generated Tension in a 3D Tissue Model
## Appendix E: Cost Analysis

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Cost</th>
<th>Material</th>
<th>Quantity</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beams</td>
<td>120&quot;</td>
<td>$14.67</td>
<td>PDMS</td>
<td>0.1</td>
<td>$5.05</td>
</tr>
<tr>
<td>Acrylic</td>
<td>3 sheets</td>
<td>$62.50</td>
<td>Magnets</td>
<td>8</td>
<td>$1.60</td>
</tr>
<tr>
<td>PDMS</td>
<td>0.25</td>
<td>$12.63</td>
<td>SS Balls</td>
<td>8</td>
<td>$0.39</td>
</tr>
<tr>
<td>Screws</td>
<td>2</td>
<td>$0.01</td>
<td>8-well strip</td>
<td>1</td>
<td>$0.61</td>
</tr>
</tbody>
</table>

**Total Cost**

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norland Optical Glue</td>
<td>minimal</td>
<td>$1.00</td>
</tr>
<tr>
<td>Lid</td>
<td>1</td>
<td>$0.37</td>
</tr>
</tbody>
</table>

**Total Cost**

$89.81

$9.02
## Appendix F: Design Evaluation Matrix

<table>
<thead>
<tr>
<th></th>
<th>Beam Clamp</th>
<th>Altering Structural Material</th>
<th>Pneumatic/Hydraulic Method</th>
<th>Piezoelectric Actuators</th>
<th>PDMS beams with or without Magnets</th>
</tr>
</thead>
<tbody>
<tr>
<td>C: Incubator Size</td>
<td>X</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>C: Autoclave Size</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>C: Durability</td>
<td>X</td>
<td>O</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>C: Success Rate</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>O: Safe</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>O: Reliable</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>O: Versatile</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>O: User friendly</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>O: Cost effective</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>39</td>
<td>36</td>
<td>29</td>
<td>30</td>
<td>39</td>
</tr>
</tbody>
</table>
## Appendix G: Curing Conditions

<table>
<thead>
<tr>
<th>Date</th>
<th>Conditions</th>
<th>E (kPa)</th>
<th>K value calc</th>
<th>K value exp</th>
<th>K value max</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/22/2014</td>
<td>20:1 @60°C 20 hrs (1mm diam)</td>
<td>180 kPa</td>
<td>0.041</td>
<td>0.142</td>
<td>0.389</td>
</tr>
<tr>
<td></td>
<td>18:1 @122°C 30 mins</td>
<td>483 kPa</td>
<td>0.480</td>
<td>0.848</td>
<td>(2.5x increase)</td>
</tr>
<tr>
<td>2/19/2014</td>
<td>20:1 @122°C 30 mins</td>
<td>490 kPa</td>
<td>0.357</td>
<td>0.330</td>
<td>0.674</td>
</tr>
<tr>
<td></td>
<td>20:1 @60°C 40 mins</td>
<td>95.5 kPa</td>
<td>0.06</td>
<td>.1280 +/- .0324</td>
<td>0.3</td>
</tr>
<tr>
<td>2/25/2014</td>
<td>5% @100°C 32.5 mins</td>
<td>375 kPa</td>
<td>0.155</td>
<td>0.323</td>
<td>0.333</td>
</tr>
<tr>
<td>2/26/2014</td>
<td>20:1 @90°C 35 mins</td>
<td></td>
<td></td>
<td></td>
<td>(2x increase)</td>
</tr>
<tr>
<td>3/17/2014</td>
<td>20:1 @75°C 40 mins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/18/2014</td>
<td>20:1 @110°C 23 mins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/18/2014</td>
<td>20:1 @75°C 30 mins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix H: Gel Procedure

List of Materials
- PDMS Post and Magnet System
- 70% Ethanol
- F-127 Pluronic (Life Technologies, 1546589)
- Fibrinogen from bovine plasma (Sigma, F8630)
- HBSS [HEPES Buffered Saline Solution (with 20mM HEPES), made from HEPES (OmniPur, 5320)]
- M199 10X with Earle’s salts and L-glutamine and sodium bicarbonate (Gibco, 11825)
- Thrombin from bovine plasma (Sigma T7513)
- N Ca$^{2+}$ [made from CaCl (Sigma, C-4901)]
- TGF-β1 Human (Sigma, T7039)
- Porcine valvular interstitial cells
- Media*
- Ice
- 15 mL conical tubes
- Trypsin-EDTA 1X (Gibco, 1459814)
- Trypan blue
- Eppendorf tube
- Hemocytometer

*Media = DMEM 1X (Gibco, 1535882) +10% FBS + 1% Anti-Anti + 1 ng/mL TGF-β1 Human (Sigma, T7039) + 33.3 µg/mL Aprotinin + 50 µg/mL L-Ascorbic Acid (Sigma, 71K1379)

Fibrin Cell-Gel Production
1. Apply 1 mL of 5% Pluronic to each well
2. Wait 5 minutes, then aspirate Pluronic
3. Get container of ice and prepare 2, 15 mL conical tubes
   a. Tube A
      i. 1576.3 µL HBSS
      ii. 200 µL fibrinogen
   b. Tube B
      i. 200 µL M199 10X
      ii. 20 µL thrombin
      iii. 1.7 µL 2 N Ca$^{2+}$
4. Prepare porcine VICs: 250,000 cells/mL
   a. Aspirate media
   b. Add 2-3 µL trypsin and incubate approx. 5 minutes
c. Add 7-8 µL media with serum

d. Mix equal amount of trypan blue and cell suspension in Eppendorf

e. Apply to hemocytometer and count cells

f. Centrifuge cell suspension for 5 minutes at 1.1 X 1000 rpm

g. Aspirate supernatant

5. Mix contents of both conical tubes in a new conical tube to create fibrin gel solution

6. Add gel solution to tube with cells and pipet up and down to resuspend cells. The amount of gel solution added should be

7. Add enough cell-gel solution to each well such that the balls are completely submerged (~100 µL/well)

8. Add ~100 µL/well cell-gel solution to a separate 8-well strip (or 96-well plate), to serve as a control group.

9. Let gels polymerize 30 minutes – 1 hour in the incubator

10. Check tube of gel

11. Add ~100 µL of media to each gel.