Project Number: KLB – 0801

Design of Device to Stretch Tunable Stiffness Substrates for Cell Studies

A Major Qualifying Project

Submitted to the Faculty of

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In Mechanical Engineering

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Abstract

The aim of this project was to design and validate a device to cyclically stretch human cells on tunable stiffness substrates to enable the study of these two mechanical stimuli simultaneously. FEA indicated that squat stretching should yield near pure uniaxial behavior. Polyacrylamide (PA) hydrogel was utilized as the compliant substrate for tissue culture in a temperature controlled, sterile environment. Testing verified that PA gels have sufficient durability in squat configurations and can reach 20% strain at rates up to 10%/sec. This device represents the first method available for mechanobiological studies into the response to combined stretch and stiffness stimuli.

1.0 Introduction

Over 100,000 heart valve replacement procedures are performed in the United States every year as a result of a variety of different valvular diseases (Rosamond *et al*, 2008; Vesely, 2004). These conditions can often go untreated for years, but may result in structural degeneration causing congestive heart failure and thromboembolism, which could possibly lead to stroke in extreme cases (Bender, 2002). With these risks, it is important to more completely determine the mechanical behavior of heart valves and the effect this has on cellular expression in order to create new and safer replacement options.

Valvular interstitial cells (VICs) perform multiple functions within the heart and are able respond to changes in their mechanical environment. These reactions include changes in morphology (size, shape, orientation, etc.) and cell expression and can be affected by factors such as stretch direction, strain percentage, and the frequency of stretch (Butcher*et al*, 2006). To test these phenomena *in vitro*, cells are stretched on a pliable substrate. The stiffness of this medium can also cause morphologic and phenotypic changes in the cells (Yeung *et al*, 2005). It is clear to see that while stretching cells *in vitro* that there many variables and a wide range of combinations thereof. Currently, there are no devices available which can test these multiple permutations of these variables simultaneously, effectively and at a low cost.

The primary goal of this project was to design a device to cyclically stretch cell cultures on pliable polyacrylamide (PA) substrates. PA was chosen for its range of stiffness and reputation for favorable cell adhesion. This device will replicate *in vivo* stress that VICs would typically undergo and accommodate multiple samples. These samples can have different stretch parameters as well as varying substrate stiffnesses to gain a wider breadth of data from one test. In addition to stiffness, variable parameters will include: strain percentage, cycle frequency, strain pattern, and stretch modality. The device is able to function in an incubator for an extended (3-7 days) period of time to allow for complete, contaminant free data. With the wide range of permutations that can be tested on this device in just one experiment, the project team believes that this is a cost effective mechanically efficient method for testing cellular response to mechanical stimuli.

The second chapter in this report is a literature review that provides essential background on previous research and testing that has been done on relevant to this project including background research on mechanobiology and the different methods of stretching. The Project Strategy chapter introduces the reader to the initial client problem and the specific aims and tasks needed to solve it. This chapter also identifies the project's specific objectives, functions, constraints and assumptions. Preliminary validation was conducted and conceptual designs were generated and evaluated in Chapter 4. Chapter 5 discusses the validation of each designed subsystem, and Chapter 6 analyzes our results. The seventh chapter, presents the final design and its validation. Finally, in Chapter 8, conclusions are drawn about our design and recommendations are illustrated for future work.

2.0 Literature Review

2.1 Stretching Within the Body

Mechanobiology is the study of how mechanics affect molecular biology. This topic is growing increasingly popular as the technology and knowledge of the subject grows. With so many variables, like stiffness, strain, cycle rate, etc., to take into account, a multitude of experiments have been conducted that deal with these differences. Researchers have tested the effect of different types of stretching on different types of cells. Some studies have even the examined the effects of different strain rates on the same cells. However, by studying the research of other experiments, it can help show what sorts of effects to look for. Among these experiments, some of the most common results dealt with cell proliferation, Extra-Cellular Matrix gene expression, as well as protein expression. Looking at these experiments previous, it can clearly be seen which techniques worked best for each case.

In order for researchers to understand the types and magnitudes of mechanical forces to apply to cells, they determine what occurs naturally. This can be examined by conducting *in vivo* experimentation. For instance, in terms of strain, the magnitude of force on a muscle cell will be much greater than that of a brain cell. So by characterizing each type of cell and what sort of environment it is in, researchers can much more accurately imitate natural conditions, as well as expand on them.

One type of cell that research of this magnitude is lacking in is that of strain on valvular interstitial cells, or VIC's. These cells in vivo normally have a pressure gradient of about 80 mmHG. With such a gradient, this causes the valve leaflet to change in length in both the circumferential as well as radial directions. This intuitively means that the strain is increasing as well. However there is little data currently of the effects of altering the strain and what it does to the VIC. Researchers have found that the cells which were isolated from the pulmonary valve were less stiff than those that were isolated from the aortic valve, thereby showing that the stresses put on the cell affect its stiffness. This fact shows that VIC's are able to remodel their ECM in response to their surrounding mechanical environment (Butcher *et al* 2007). Currently these researchers are trying to apply this knowledge of VIC's to creating new in vitro samples. This is being done by taking cells from blood vessels and mesenchymal stem cells, and putting them into a PGA-PLLA copolymer. By using mechanical stimulation the team caused the cells to remodel the scaffolding and secrete an ECM. Though there are setbacks, and still some problems, this work still is promising in showing that by the use of mechanical stimulation, the properties of cells can be altered.

2.2 Replicating cellular response

Cells will respond to the changes in their mechanical environment in a variety of different ways. Examples of possible responses may include changes in the production of growth factors and vasoactive agents, an altered pattern of gene expression, and the remodeling of the extracellular matrix. (Ku *et al*., 2006). One of the major factors in the mechanical environment is that of strain. For instance, as a muscle is strained, it starts to tear. In the healing process, the body rebuilds these muscles, making them bigger, overcompensating in order to avoid further strains.

Another of the major factors is that of the stiffness that the cells are adhered to. This topic is one in which is currently being researched on its direct effects. One such study focused on stem cells and how the stiffness of the substrate affects them. Adam Engler, at the Pennsylvania Muscle Institute have found that these cells are extremely sensitive to the stiffness of the substrate. They found that, "Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic,"(Engler 2006). Making

these two factors adjustable in the same experiment is something that has never been done before. With the ability to study how these factors combined affect cells, and their alignment, new research can be done.

In vivo, VICs undergo mechanical stretching in various directions including uniaxial, biaxial, strip biaxial, and equibiaxial, as illustrated in Figure 1.

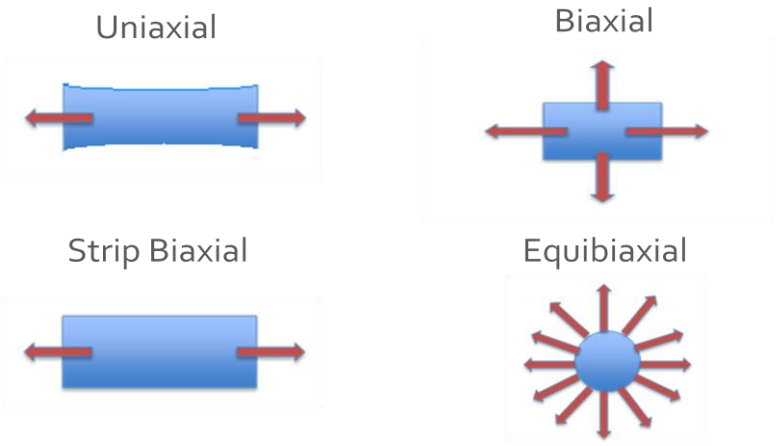


Figure 1: Examples of Stretching Directions

Cellular response will vary greatly on the type greatly on the type of stretch that is applied to it. Cell stretching devices available today in literature and commercially attempt to mimic the complicated mechanically stimulations that occur in vivo. Biaxial stretching devices stretch cells along two axes perpendicular to one another without shear stress. Uniaxial stretching devices stretch in only one direction, which creates a compression in the perpendicular direction of the cells. To prevent this, a strip biaxial stretch device applies a tension in the perpendicular direction that will prevent the Poisson effect from occurring. An equibiaxial stretch device applies uniform strain in all directions, eliminated the variable cell orientation. (Yost, 2000)

Another similar experiment conducted by Wang *et al* (1995) sought to compare the effects of stretching compared to static conditions. To do this, smooth muscle cells were subjected to different strains. The team examined the affect this would have on the orientation of the cells. In static conditions, the cells were randomly oriented with no pattern or uniformity. Once stretched uniaxially it was found that the cells all aligned to form an angle 65° from the stretch direction. As further research was done (Wang and Grood, 2000), the team found that the angle formed by the cells depended on the stretch magnitude. It was concluded that a large number of cells, when subjected to cyclic

stresses align in order to have minimum substrate deformation in order to subject themselves to the least amount of stress possible.

One of the more important variables being tested is the mode of stretching that the cells undergo. Researchers are trying to determine if one of these modes benefit cells in any way, or, perhaps, if one type of stretching is better for one particular end result, while another is better for some other desired end. One example of such a test was performed by Yang *et al* (2004) and involved human tendon fibroblasts. Researchers stretched these cells uniaxially over a range of 4-8% at a frequency of 0.5 Hz for 4 hours. It was found that under these conditions the cell proliferation increased, as did the type I collagen gene expression, and the protein production. The team discovered that the increase of these properties was directly related to the stretch-magnitude. They subjected the same material to biaxial stretch. Here, it was found that it was not the magnitude of the strain that determined the final properties, but rather the strain *rate*. This shows that different modes of stretch affect the cells in different ways and by different factors.

He *et al* (2004) conducted another experiment examining similar phenomenon while testing peridontal ligament fibroblasts. Here, the team was looking to determine if tensile strain testing would affect the cells differently than compressive testing. In the first case, the samples were stretched biaxially 10% for 24 hours. Upon examination, it was found that the fibronectin content increased five-fold, while no difference was found in the presence of type I collagen from that of the unstretched control sample. However, when the sample was compressed in the same manner, it was found that it had radically different effects. The Type I collagen mRNA decreased by two-thirds, and the fibronectin decreased by half. The data from this experiment showed that even one variable change can lead to drastically different effects.

Other researchers took a step back from this approach, and tested to see if the strain itself affected the results. Lee et al (1999) kept all variables constant while allowing the strain percentage to change. This would show if cells react differently when stretched to differing lengths. One of these tests was conducted by stretching adult cardiac fibroblasts to different strains. Initially the cells were cyclically stretched to 10% in the uniaxially. It was found that the levels of type I collagen and Fibronectin mRNA tripled under this strain. However, when the team increased the strain to 10%, it was

found that the amount of mRNA decreased compared to the levels from after the original test, while the type I collagen remained the same. This shows that even the amount of strain put on cells makes a difference in terms of how they will react to different stimuli.

By examining the different research, it was found that there are many mechanical variables that can affect cellular properties. By adjusting strain rate, strain percentage, and mode of stretch, desired cellular results can be achieved. With this being a relatively new topic, the effect of mechanical properties on cell response is not completely known. This uncertainty creates a particular gap in research that the result of this project is hoping to fill.

A common problem with uniaxial stretching is the compressive strains that occur perpendicular to the stretch direction that are created by the Poisson Effect. In this case, cells are subject to both of these strains and respond differently. To attain pure uniaxial stretch, also called strip biaxial, cells must on feel strains in one direction (Figure 2). While strip biaxial behavior is not physically possible for entire substrates, portions of

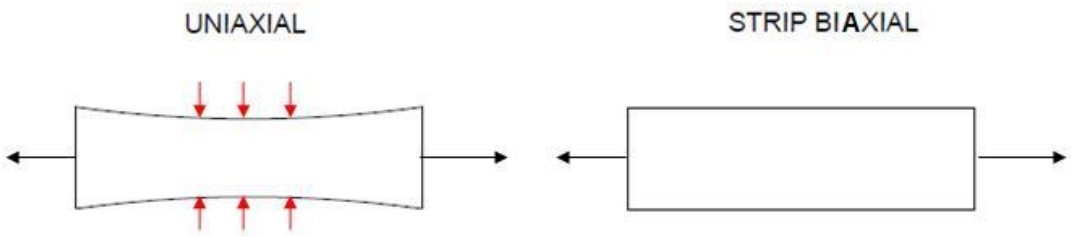


Figure 2: Free Body Diagram of Cells Under Uniaxial and Strip Biaxial Stretch

stretched membranes can approximately achieve strip biaxial properties. Wang *et al* (2000) achieved this by only seeded cells on a particular area of a large membrane. This area could be assumed to undergo homogeneous strain, thus accomplishing strip biaxial stretch. This project will take a similar approach as Wang *et al*, by trying to determine a more effective way to attain pure uniaxial stretch of substrates to gain pure data on the effect of strain on cell cultures.

3.0 Project Strategy

For this project, the design team followed the design process as presented by Dym and Little in "Engineering Design; A Project-Based Introduction." The process began with a basic project statement from the client, Professor Billiar, followed by a formal interview with him to help compile a list of design attributes. The list of design attributes would then be divided into project objectives, functions and constraints. Pairwise comparison analysis was then completely to prioritize these objectives. Finally, a function-means tree was created to graphically represent the primary and secondary functions of the design. Once completed, these guidelines were used to begin developing alternative designs. The section discusses the preliminary procedures to developing our design attributes to eventually create a revised client statement.

3.1 Initial Client Statement

The client presented the project team with the following preliminary project statement: "*Design and test a device to cyclically stretch a soft substrate in a tissue culture (temperature controlled and sterile) environment."* In order to clarify this statement, background research was conducted. This included, but wasn't limited to, scientific journals, previous Major Qualifying Projects, and existing patents. As a result of the question raised by this research, a secondary interview with the client was conducted to clarify the questions of the team (Transcript in Appendix A). This interview helped to create a list of design attributes, which could then be formed into objectives, functions, and constraints to help create the revised problem statement.

3.2 Objectives, Functions, and Constraints

The first step in the design process was to determine the requirements of the design and separate them into objectives (what the design should achieve), functions (what the design should do), and constraints (design limitations). The design attributes were expanded upon and detailed which resulted in the objectives, functions and constraints shown in Tables 1, 2, and 3, respectively.

Table 1: Project Objectives

Project Objectives:

1. Reliable
2. Durable
3. Inexpensive
    a. Minimize use of polyacrylamide, growth factors, & cultured cells
4. Easy to use
    a. Easy to Sterilize
    b. Quick to setup
    c. Portable
5. Effective
    a. Accurate and precise stretch

Table 2: Project Functions

Project Functions:

1. Stretching device will apply a 20-25% strip bi-axial (uni-axial without compression) stretch to the cells.
2. Strain should be adjustable in 5% increments. (5, 10, 15%, etc.)
3. Stretching device will be able to measure strain.
4. Stretching device will have an adjustable strain frequency between 0.1 and 2.0 Hz.
5. Strain rate will have different types of waveforms.
6. Stretching device will be able to hold polyacrylamide.
7. Stretching device will be able to be sterilized.

Table 3: Project Constraints

Project Constraints:

1. Must function in an incubator and fit on a shelf.
2. Cost to produce must be less than $500.
3. Stretching device much achieve a $1cm^2$ area of pure stretch.
4. Applied strain should be accurate to within 10%.
5. The device should not tear or plastically deform the substrate.
6. The device must remain sterile for at least 3 days and durable for a week.
7. All components that contact the substrate must be able to be sterilized.

In order to determine which designs best fit our objectives, the team assigned weighted these objective based on priority. In this way, it can be found which model fits best our overall concept and design parameters. The most important objective was

determined to be the effectiveness of the device. If the device itself does not work effectively, or with accurate results, then there is no reason to have it. The purpose of this device is to provide accurate and precise results. After this, the most important objective is that of reliability. The team felt it was very important that the device be able to give dependable, repeatable results. If the outcomes are not consistent from one test to another, it will be difficult to determine the accuracy of the results. The project team felt the remaining objective were all equally important, though lesser than the previous two. While low cost, durability, and ease of use are significant objectives, if the device is not effective and reliable the device would be considered a failure.

Table 4: Weighted Objective Tree

Objectives	**Weight**	**Sub-Objectives**
Effective	50%	• Accurate Results • Precise Stretching
Reliable	20%	
Durable	10%	
Inexpensive	10%	• Minimize use of polyacrylamide, growth factors, & cultured cells
Easy to use	10%	

A function means tree (See Appendix B) was created to organize the primary and secondary functions of the design. The top level of the tree begins with the functions that need to be met, with each succeeding level having primary and secondary functions to complete the top function. This graphical representation helps note which functions would be common to all possible design alternatives, or would be specific to just one design.

Lastly, a pairwise comparison chart was created using the project objectives to prioritize the importance of each objective. Professor Billiar, Angela Throm (one of the end users of the device), and the design team weighed each of the objectives. The results of this comparison can be found in Appendix C. This ranked the objectives (highest to lowest) as follows: Effective, Reliable, Durable, Easy to use, Inexpensive.

3.3 Revised Problem Statement

Once all objectives, functions and constraints of the design were established and organized, the project team was able to further clarify the initial problem statement. Using the analysis described above, the revised problem statement was expanded to:

"*Design and test a device to cyclically stretch soft substrate strip bi-axially in a tissue culture (temperature controlled and sterile) environment. This method should be able to apply a 20-25% strain and allow for adjustable strain increments of 5%. Additionally, the device should have an adjustable strain frequency between 0.1 and 2.0 Hz and be able to apply strain in different types of waveforms."*

This statement highlights the most important objectives, functions and constraints of the design and gives specific numbers that must be considered when creating design alternatives.

3.4 Project Approach

This section identifies the approach and development of device, which will stretch polyacrylamide samples of varying stiffnesses strip-biaxially. All assumptions made in the approach and methods for developing the final project statement and for reference at the conclusion of the project are identified. Specific aims and tasks are discussed to outline the guidelines and constraints used to determine what the project ultimately accomplished. This was creating a device, which was able to control levels of strain and stiffness of polyacrylamide samples.

3.4.1 Assumptions

In order to develop our method, the project statement was simplified using several assumptions. One of the preliminary assumptions was that the cells attached to the substrate are subjected to the same strain percentages and patterns as the substrate. This assumption would then later be verified during validation. The polyacrylamide was also assumed to be a flat, isotropic, and homogenous material in order to simplify analysis relative to changes in stretch stiffness.

3.4.2 Specific Aims and Tasks

Completion of specific tasks was required in order to develop a device to control strain on various stiffnesses of polyacrylamide. These tasks included:

- Selecting a stretch type
- Creating a way to interface with polyacrylamide to have strong substrate attachment
- A method for curing and attaching the substrate to the stretch device.

The combination of each of these aims and tasks allowed the cellular environment to be subjected to controlled levels of stretch and stiffness.

4.0 Alternative Designs

4.1 Feasibility Studies

4.1.1 Failure Testing of Polyacrylamide

In order to determine if polyacrylamide gels could withstand the strains that the device would impose on them, a failure test was conducted. The objective of this testing was to see how polyacrylamide could withstand cyclic stretching of different strains and strain rates. A #11 PA gel (E=153600 Pa) was attached to the Instron MTS grips by porous polyethylene sheets (PE) cured into each side (as represented in Figure 3). The sample was cycled at 1% per second and easily reached a 5% strain, but broke when the parameters were increased to 2% per second with a 10% strain, this however was a result

Figure 3: Diagram of a PA sample with PE grips

of a pre-existing tear in the failure area. The test cycled #6 PA gels (E=4800 Pa), except this time the sample were mounted to the Instron hooks using small pieces of drywall sandpaper and small clips. Although more difficult to attach, the PA gels held up better because there was no place to easily rip on the grip edge. Starting at just a 5% stretch at 1% per second, the project team was able to eventually achieve a 40% stretch at 8% per second for 10 cycles without breaking. Finally, the PA was stretch at 57% without failure. The limits of the stretch environment of the test were the only limit to how much the gels could be strained. While, the actual failure is not known, the polyacrylamide was

cycling well outside the ranges that this device will put on it. This test served to validate that, as a material polyacrylamide could be stretched to or beyond the required strains and frequencies that the client desired. These results, however, we gained with a gripping method that was timely and difficult, so an easier method must be determined.

4.1.2 PE Mesh as a Gripping Alternative

To remedy the difficult gripping situation the project team looked into alternatives to the sandpaper and clip configuration. Porous polyethylene sheets have been shown to cure well in polyacrylamide, so the project team investigated other polyethylene geometries to determine if they provide any advantage over porous sheets. The desirable quality of the PE sheets was there porosity, so the project team decided to validate PE meshes as analogs of porosity (albeit large). The tests conducted were largely qualitative and were carried out by attaching wire hooks through the PA samples and gently pulling them by hand to roughly 20%, as it was already shown the material should with stand this. The first samples used a natural HDPE cut into small rectangles and cured on each side of the PA. Two meshes were cured in #9 PA gels. Both were high density PE (HDPE) with different mesh densities. These we cured with the grids parallel and biased (rotated 45°). The finer, denser, mesh appeared, visually, to set well into the gels, while the looser mesh (seen in Figure 4) had varied results.

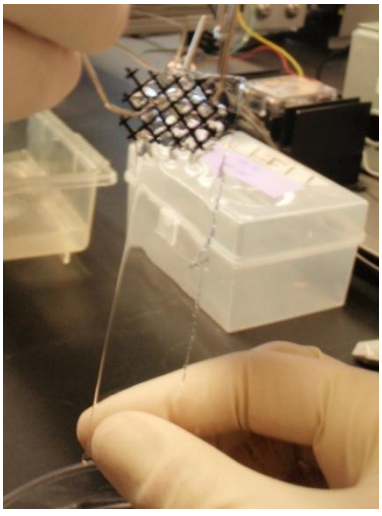


Figure 4: Polyacrylamide cured with HDPE Biased Loose Mesh

Stretching also provided mixed results. The denser mesh consistently failed at the edge of the grips and barely reached 20% strain. As this was a fault of the grip it must be discredited. The looser mesh did show some promising results. However, because of the varied curing quality, it was hard to verify is this was repeatable. Some tests had the gel fail at its center after 20% strain and others had the mesh slip out entirely and no difference was seen between perpendicular and biased configurations. It is difficult to determine how well it could work, but the loose HDPE mesh definitely deserves a closer look.

4.1.3 Preliminary Validation of Squat Stretching Method

FEA analysis conducted by the team proved that squat stretching would garner results that closely resemble strip bi-axial behavior. Tests were conducted to verify if PA gels with this type of dimensions could be strained to 20% in such a small dimension. The tests showed that the gels have sufficient durability in this configuration and easily reached 20% strain, and more importantly could withstand strain rates as high as 10% per second, which is well beyond the necessary parameter.

4.2 Sample Size Validation

4.2.1 Determination of Sample Size

In order to determine an effective scale for the device is was necessary to first determine the size of the samples that will be stretched. The finite element analysis program ANSYS was used to determine an accurate model for polyacrylamide, evaluate load patterns to replicate stretch modes, and to arrive upon a sample size with sufficient usable area and near-strip biaxial conditions.

4.2.2 Finite Element Model of Polyacrylamide

To correctly determine the nature by which polyacrylamide sees strain, an accurate model of the material must be made in finite element software. These parameters, outlined in the below table, determine such factors as the dimensions of the sample, its stretch nature, as well as how the software interprets its results. For the purposes of this project, it was necessary to model the samples as membranes with minimal thickness to closely mimic the shape of the samples generated in the lab. A

solid element type was chosen and given a thickness of 1.5mm as this is the easiest

Table 5: Model Characteristics

Parameter	Description	Note
Element Type	Solid, Quad 4node 42	Behavior: Plane Strs w/ thk
Real Constants	Thicnkess	1.5mm
Material Model	Structural > Linear > Elastic > Isotropic	E = 4800 Pa; ν = 0.35

thickness to make in the current lab conditions. To approximate the mechanical properties, the project team decided to use the data for polyacrylamide gel 6, which has mid-range stiffness. The physical characteristics of PA gels vary with measurement techniques, but typical values will be used for this experiment. A Young's Modulus of 4800 Pa (Engler *et al*, 2004), and a Poisson's Ratio of 0.35 (Li *et at*, 1993) will be used, as these values are the close to the average of values available in literature..

4.2.3 Modeling Uniaxial Stretch

Once an accurate material model was determine, preliminary tests were run to verify if this model returns results that are typical of isotropic materials under uniaxial stretch. The most satisfactory attempt to model uniaxial stretch is pictures in Figure 5. Here, the sample is elongated a total of 20% along its width by displacing each vertical edge by 10% of the original width. It was determined that this method yields identical results to holding one edge fixed and displacing the opposite edge 20% of the original

Figure 5: Stretching Parameters

width. This was a key finding to simplify the design process. To ensure pure uniaxial stretch the corners of the samples were constrained to only move in the lateral direction. This assure that the entire edge is displaced the full 20%.

The aforementioned parameters were applied to a 2 x 3 cm ANSYS sample with a 0.25 mm mesh size to gain a high resolution. The total mechanical strain in the lateral direction was plotted (Figure 6) and examined. This plot shows a substantial region (Yellow-Green: 0.2025- 0.205) that is homogeneous, predictable, and within the

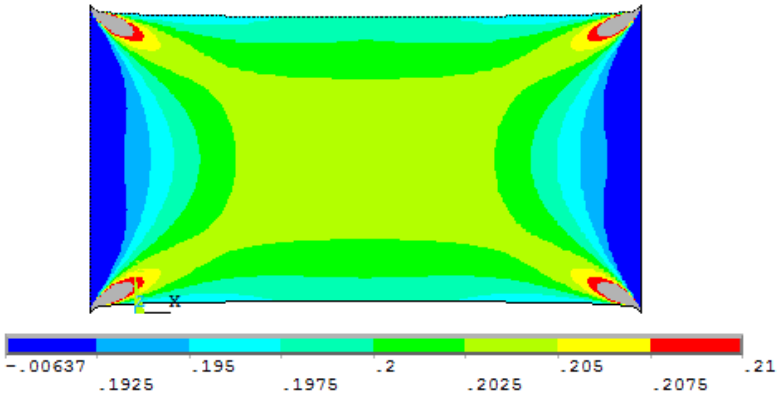


Figure 6: Total Lateral Mechanical Strain

tolerances given in the design objectives. Also, the strain pattern is symmetrical about both the lateral and transverse midlines, which shows that this is indeed a proper model of an isotropic material. Having a valid material and stretch model identified a method of gaining strip biaxial stretch needed to be determined.

4.2.4 Attaining Strip Biaxial Stretch

There were two methods of possibly attaining strip biaxial stretch that needed to be tested. The first was the application of hooks along the bowing edges of a sample, and the second is squat stretching. Each of these was evaluated to determine if the method produced results that could mimic strip biaxial stretch. An accurate method should be able to create a transverse strain of less 2% and ideally zero as set forth by the design criteria.

4.2.4.1 Hook Method

The hook method uses evenly spaced hooks to counter the Poisson Effect by keeping the edge being stretched in tension. While this solves the issue of altering the strain in transverse direction is creates another problem longitudinally. Because polyacrylamide is an isotropic material the Poisson Ratio is the same in any direction of stretch. By pulling on the stretched edge, the same effect occurs in the longitudinal

direction. This causes distortion in the lateral strain pattern (Figure 7) and limits the amount of homogenous area. To more closely model strip biaxial elongation, more

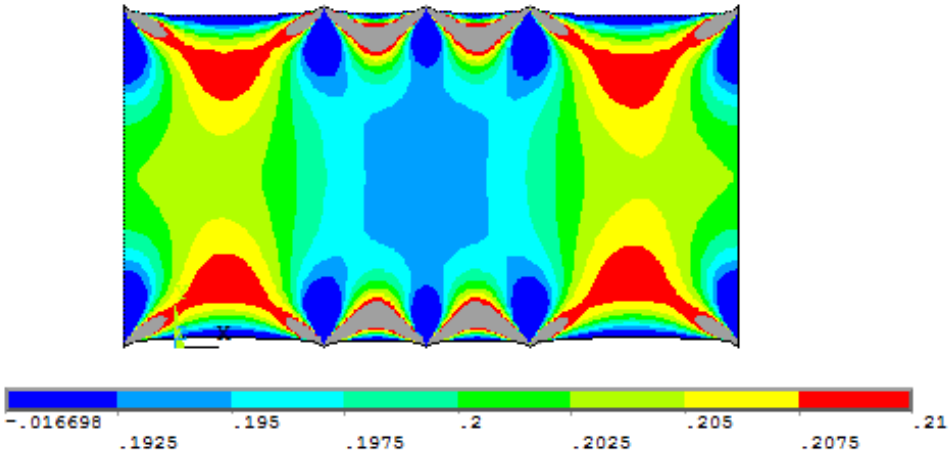


Figure 7: Lateral Strain with Hooks

hooks would have to be added to the sample. It can be seen that as the number of hooks increases, the amount of usable area reduces significantly. It is because of this and, perhaps more importantly, design feasibility that hooks were discredited as method of creating strip biaxial stretch.

4.2.4.2 Squat Stretching Method

The method of squat stretching employs the Poisson Effect to attain near-strip biaxial conditions. In this situation the smaller dimension of a long, thin sample is stretched (Figure 8). Because of the length is so large as compared to the width, the of lateral edge, if properly constrained, causes homogeneous and predictable local strain

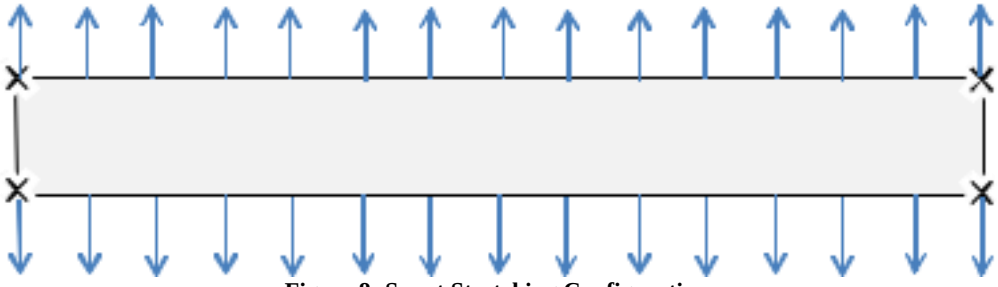


Figure 8: Squat Stretching Configuration

that is very close to the overall strain. The longer the sample gets, the closer the transverse strain gets to zero as it gets further and further away from any curved edges that could cause fluctuation. The edge effects (Figure 9) of this configuration make up so

little of the overall area that the vast majority of the sample is usable. This proves to be a suitable method to precisely control the transverse strain values to ensure near-strip biaxial conditions.

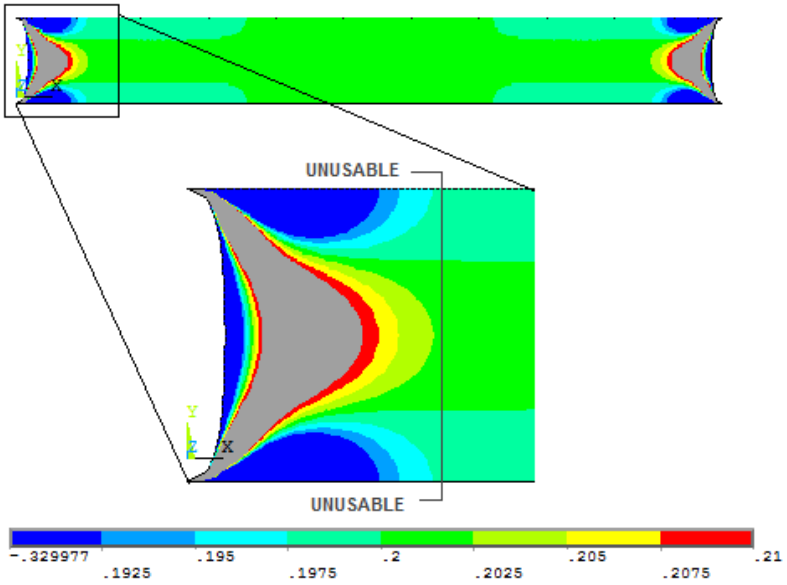
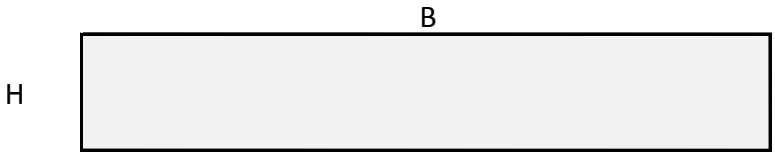


Figure 9: Definition of Unusable Area

4.2.5 Analysis

With the stretch configuration established and an accurate model of polyacrylamide functioning, it was necessary to determine the final dimension of the samples that the device will ultimately stretch. For a sample to be considered usable, there must be a region of at least 1 cm^2 of homogenous strain that is as close to the desired strain as possible and have a transverse strain the is within 10% of the desired axial strain. The smallest geometry that meets both these criteria should be considered the optimal sample size for this device.

A sample is considered to exhibit strip biaxial capabilities when the transverse strain is reduced to a point such that it is 10% of the desired axial strain, in this case that means attaining a strain value of less than 2%. To determine this, a study (Data in Appendix E) was done to determine the effect of normalized width (as defined in Figure 10) of a sample has on this strain at the center of the sample. In this study, multiple combinations of unit length and width were squat stretched and the results were analyzed. It was shown that unit length had no impact on the strain pattern and the central



Normalized Width = B/H

Figure 10: Normalized Width Definition

transverse strain depended on the width of the samples. After width normalization and data regression it was found that normalized width (W) and central strain (ε_{Tc}) were related exponentially according to Equation 1. It is important to note that

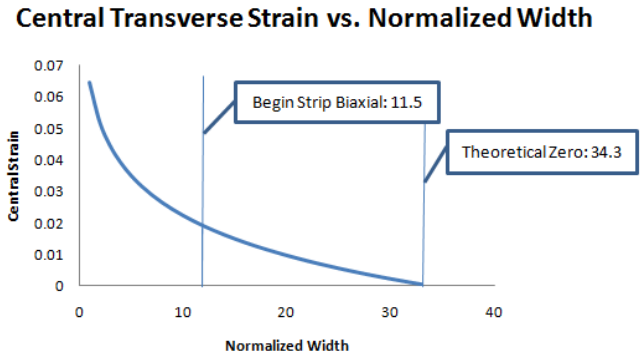


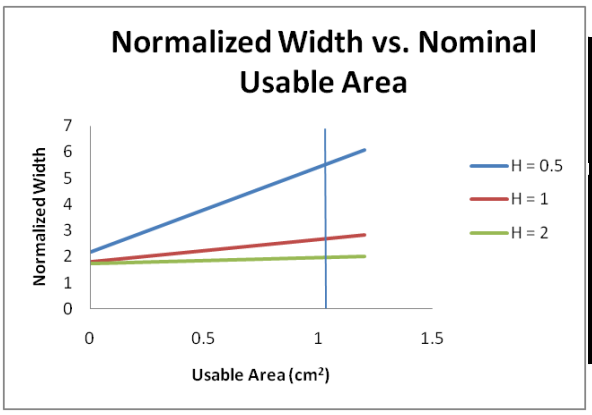
Figure 11: Central Strain Data Analysis

$$\varepsilon_{Tc} = 0.086 \ln W - 0.0648 \quad (1)$$

the actual transverse strains are negative, and should be input thusly, however in the above graph they are displayed as positive. By plotting this formula (Figure 12) it can be shown that a sample begins to show strip-biaxial behavior at a normalized width of 11.5 and should theoretically reach pure strip biaxial stretching at a value of 34.3. With these values in hand, it was necessary to determine the most efficient use of area.

4.2.6 Size Considerations

Having the above definitions, a final decision on the size of the samples needed to be made. An ANSYS study (Data in Appendix C) of samples with various H-Values and normalized widths was conducted to determine when a squat sample attains 1cm^2 of usable area, as defined previously (Figure 9). In Figure 12 it is shown that a sample's H-Value increases the sample reaches the desirable area more quickly, which was expected. It should also be noted that even the smallest H-Value reaches 1cm^2 of usable area before



A=1 cm^2	
H	W
0.5	6.08
1	2.82
2	2.00

Figure 12: Usable Area Assessment

it even begins to exhibit strip biaxial behavior. With that said, the samples should have an aspect ratio close to 1:11.5 as to not waste media. However, some other constraints make it necessary to make the samples a little larger than the minimum to increase ease of use and ensure reliable data from the end device. This includes a 1mm offset from all edges to account for the presence of edges effects. Also, as samples needed to be seeded with cells, extra usable surface would be beneficial. Because of this, as well as the rest of the data in this section the project team has chosen to deal with samples that are 0.5 x 6 cm with a thickness of 1.5 mm. With this parameter determined the overall size of the device can be minimized to ensure the most efficient use of space on an incubator shelf.

4.3 Conceptual Designs

The project team had several ideas of how to attach the stretching device to the samples of polyacrylamide. One of the first ideas was the use of clips. Previous experiments done using polyacrylamide used a combination of drywall sandpaper as well as small toothed hair clips. The team found these were hard to manipulate onto the samples. Even once they were put on, it was even more challenging to get both sides to be symmetrical, to yield accurate results. For this reason the team designed several clips which they felt might make this process easier.

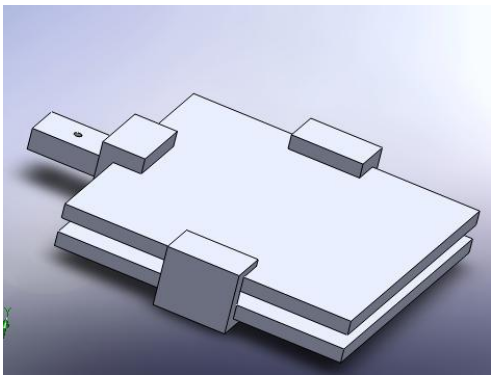


Figure 13: Toothless Clamp

The design in Figure 13 uses pressure and friction in order to hold the sample in place. This clamp works by placing the sample along the bottom, then folding the top down. The two side clips then clamp down tightening the clamp, making it hold the sample. This was a simple concept, however the team was nervous that it would not be secure enough in order to hold the sample.

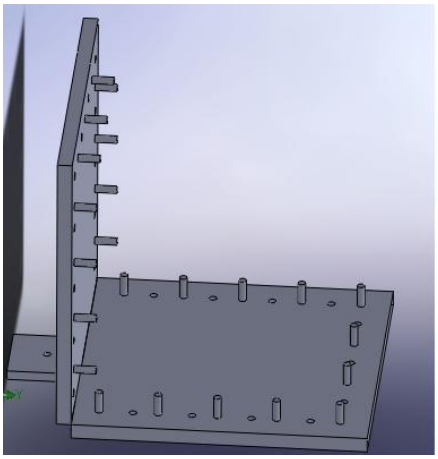


Figure 14: Toothed Clamp

The first clip design was a modified technique of what was being used with the hair clips. By flattening out the clip and tailoring this idea specifically for our samples, the gripping process would prove much easier (Figure 14). When closed, this grips teeth would puncture the sample and then proceed to the corresponding whole. This clip can then easily be attached for stretching through the bracket on the back. One downside of this design is in ensuring that both ends are clipped in the same manner. If one side is clamped straight on, while the other has a slight angle, it would cause unequal stretching, thereby throwing off the results.

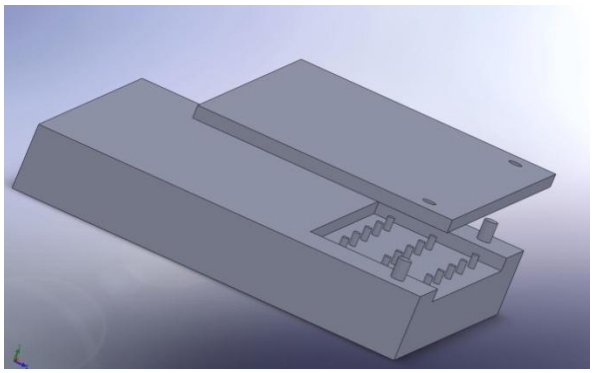


Figure 15: Shaped Tooth Clamp

By making a slight alteration, the design in Figure 15 uses the same idea of a toothed grip, however this one would provide more consistent clamping. The toothed section has been cut into the clamp. This means by placing the sample against the back of the cut section, you guarantee that it is straight on, and the same as other samples. The top plated would then come down to ensure that the sample stayed in place. With this as well as the previous model, the problem of tearing was discussed. By having the teeth dig into the samples, the chance of tearing increases, which would mean a ruined sample

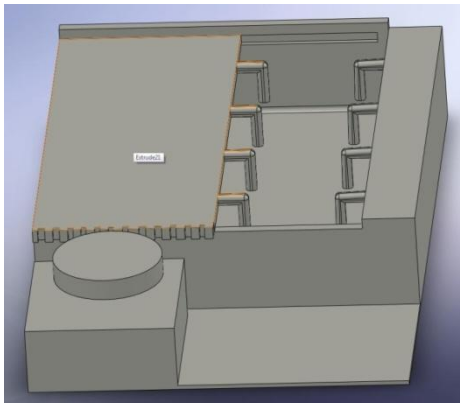


Figure 16: Gear Rack System Alternative Design

One of the earliest models created, utilized a gear rack system in order to convert the rotational movement of the motor into linear motion (Figure 16). This would be able to be created simply by attaching a gear to the shaft of the motor and placing a gear rack on the edge of the slide. This slide would have hooks attached on the end which would drop into the bath. On the opposite side also dropping into the bath are fixed hooks attached to the wall. This means as the slide would move, the hooks attached to them would pull one side of the sample, while the other end would remain fixed. This means that it is the hooks that are moving, and not the samples. This type of device would work

primarily for samples which are being stretched parallel to the samples longest side. One of the major advantages of such a design is its simplicity. Very little machining would have to be done in order to create the parts. Also in terms of moving components there are so few, it would be easy to troubleshoot if there was a problem. However there were also several downsides to such a design. One of which dealt with contamination. By having one large bath with multiple samples, the risk of one sample becoming contaminated and spreading to the others is quite high. However, if each sample was separated, then if one did become contaminated it would be an isolated incident meaning that the others would still be usable. Another downside of such a design dealt with having a motor on only one side. With such a case, if there was any sort of friction the sides would not move equally meaning some samples might stretch differently than others.

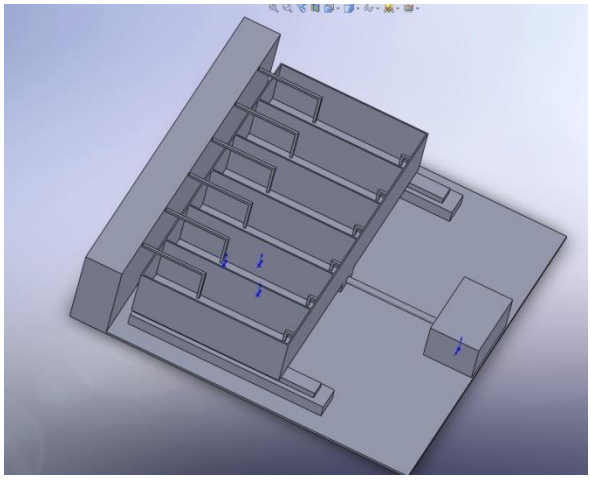


Figure 17: Moving well Alternative Design

The model shown in Figure 17 was created by trying to compensate for the downsides of the previous model. In terms of the interface between the samples and the device, hooks are still being utilized. One difference to this design is the system of wells. In order to prevent cross contamination of samples, individual wells are being used. This can all be connected as one unit, or can simply be made up of individual wells, which are placed next to one another. Several steps were also taken in order to deal with the issue of friction. One step taken was to adjust the way the rotational movement of the motor was translated to linear motion. With this model, it is the wells that are moving away from the hooks. This is done by the use of a threaded rod or ball screw placed under the

center of the wells. As these would turn with the motor, it would cause the wells to move pulling them away from the hooks. In order to do this, the wells would be placed on ball slides in order to assure friction free movement. This device while having its advantages over previous models also had flaws in it. One such flaw dealt with the contamination of samples. Though the team did have individual wells for each sample, while being in the incubator the system needed a way to cover it. This would prove challenging because the hooks would be moving making a static cover hard to be successful. Another issue dealt with having the wells move instead of the hooks. The team was concerned that with the movement of the wells, the medium within them would shift, which could affect the experiment negatively.

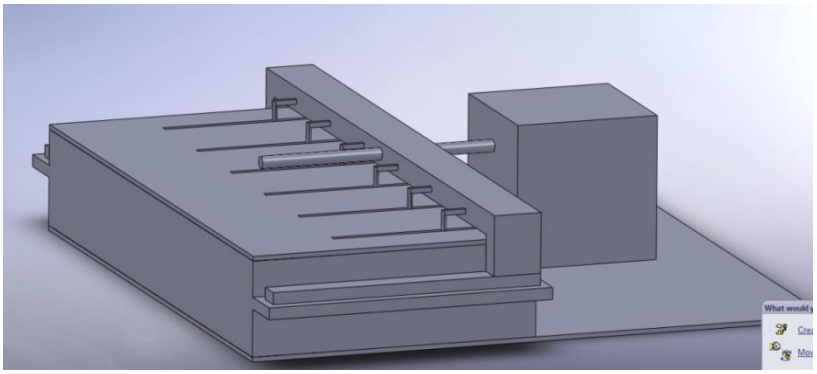


Figure 18: Threaded Rod Alternative Design with Cover

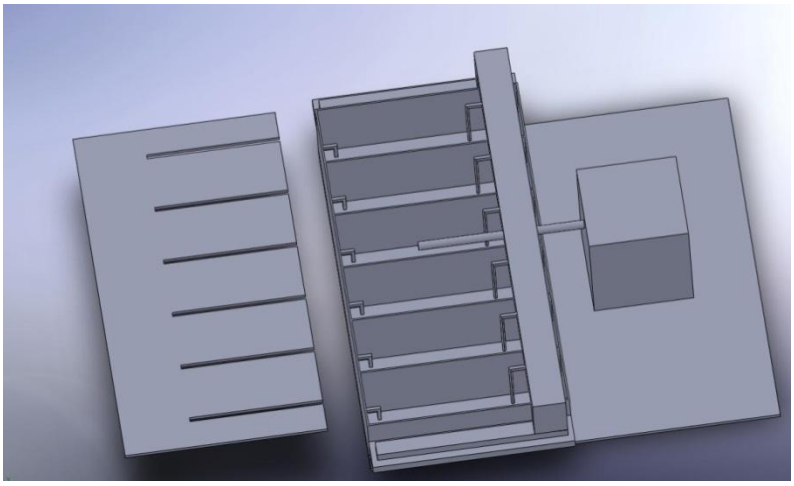


Figure 19: Threaded Rod Alternative Design without cover

The next generation prototype attempted to alleviate the flaws of the previous models (Figure 18 & Figure 19). In order to stop the issue of the medium sloshing in the wells, the means of stretching was altered. The team returned to the idea of moving the hooks relative to the wells, instead of moving the wells themselves. In this case however, the ball screw is attached through the center of the slide which has the hooks protruding from it. Again as this screw rotated, it would move the slide on top of the ball slides, thereby stretching the samples. By using this technique, friction would not be a problem while the samples are stretched. The other issue which was addressed was that of a cover. In this case, the team felt that perhaps a slotted cover would work. Though simple, while placed on top of the wells, it would prevent contamination, while still allowing the hooks to move.

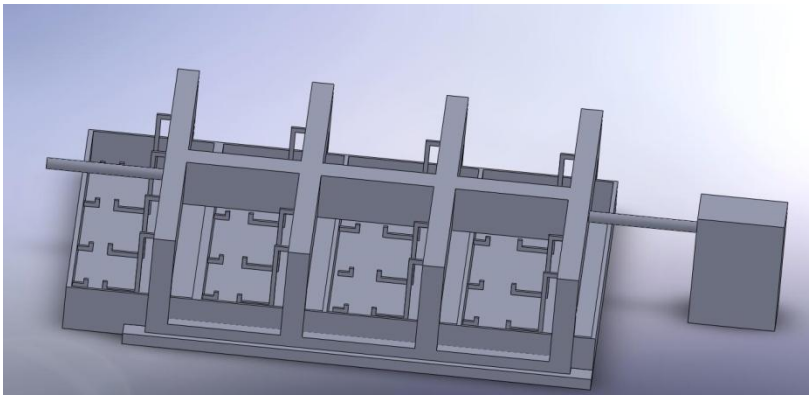


Figure 20: Threaded Rod Alternative Design

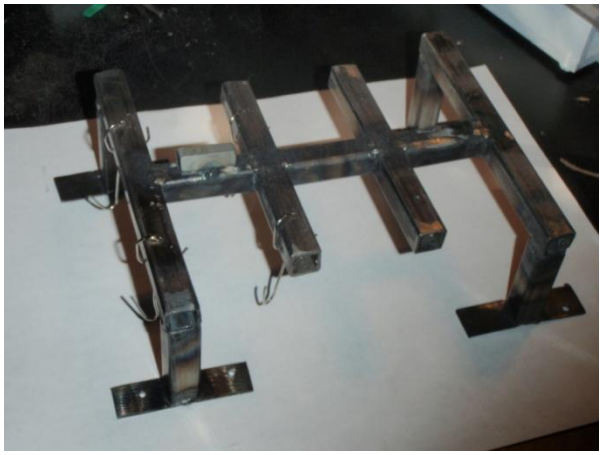


Figure 21: Threaded Rod Prototype

One concept different than the others was to stretch the samples parallel to their shorter sides. None of the previous model configurations addressed this idea, for they all stretched length-wise. The model in Figure 20, though fundamentally similar to the others, had some different features. This concept has the stretching device simply be placed over the system of wells. The backbone of the device branched off into side arms, which had hooks. These hooks would attach to the sample and coincide with fixed hooks which are located in the wells. These side arms then attach to ball slides along the wells, which keep the device moving smoothly. As the ball screw moves, it would move the backbone, and in turn stretch the sample. A steel prototype of this model was welded together and attached to four ball slides. Using this model as a visual reference, the project team concluded that this concept's major problem dealt again with the issue of covering. By having the stretching occur directly over the wells, it proves difficult to cover the samples to prevent contamination.

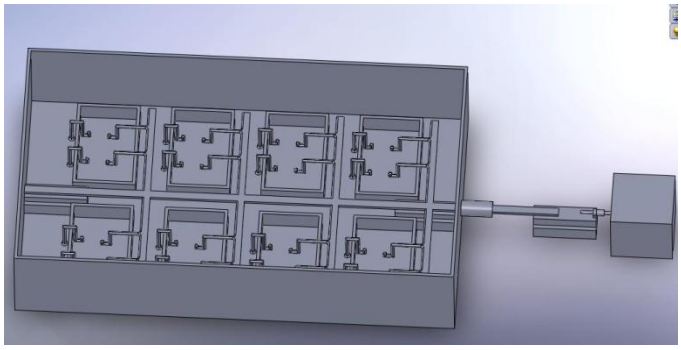


Figure 22: Covered Threaded Rod Alternative Design

One of the biggest challenges with all the models is the issue of covering. By having moving components, the way in which you arrange the system determines the covering system. By enclosing the wells all within one unit (as seen in Figure 22), a cover can be placed over the whole thing, while all the moving components are self-contained. The motor is located outside which is connected to a ball screw. This connects to a ball slide, which in turn is connected to the slide. This enters the unit through a small hole in the outside walls, which also has a small bellow to deal with the slide moving. As the ball slide moves horizontally, it moves the ball slide in turn moving the slide. This slide branches into the hooks, which attach to the samples. Inside the unit

at the front and back there are also ball slides, which help alleviate the friction of the slide moving within the unit.

A second-generation mock-up prototype (Figure 23) of the threaded rod design was created using two ball slides and a welded piece of steel. A drill was attached to the threaded rod to simulate a motor to see how the movement of the threaded would move the entire device. This prototype helped the project team visualize how the stretching arm would move cyclically. The team also decided that a design without ball slides on each side of the arm would be more efficient. It also helped bring attention to the possibility of a balance issue along the arms. Using this model, the team was able to cyclically stretch a rubber band using the drill as a motor.

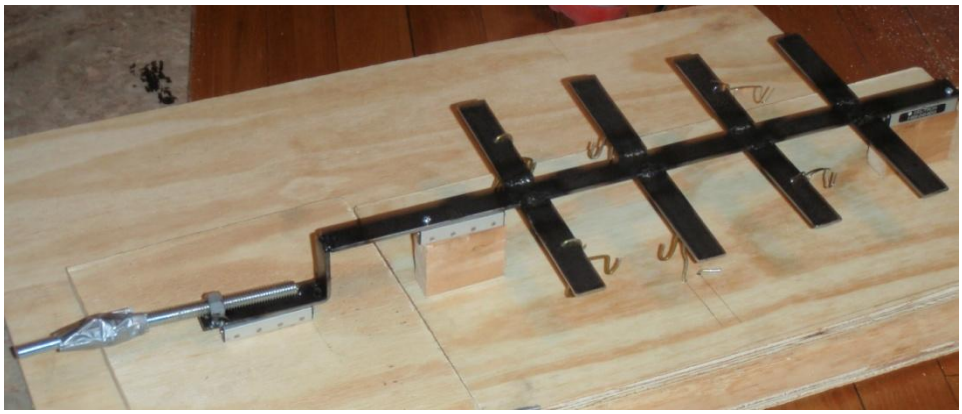


Figure 23: Second Generation Threaded Rod Prototype

4.4 Design Calculations

One of the first material issues dealt with was that of the polyethylene being cured in to the polyacrylamide. Past work showed that it cures in effectively, however with the strain being put onto the polyethylene, the interface between that and the polyacrylamide must be strong. There were two options of what type of polyethylene to use. The team initially thought that the polyethylene mesh, as seen in Figure 4, would work better than the previously used porous polyethylene sheet in Figure 3. After mechanical testing of both scenarios, it was found that the polyacrylamide cured into the mesh was not strong enough to handle the strain put onto it. The polyethylene sheet however was able to hold up to 57% stretching. This is when it was decided to use the sheets over the mesh.

Due to the specific environments, which the device would be subjected to, the materials used would be crucial. One example of this is the fact that cells will be contained within the device. This means that the internal structure of the device must be sterilized in order to not contaminate the samples. The most common way to do this is to use an autoclave machine. What this does, is use high pressures and temperatures to kill any contaminants present. This was one of the major factors in finding an appropriate material. One part this directly affected was that of the slide arm. It also had to withstand the stresses of cycling and be strong enough that it would not deform, for this would cause the cells to stretch non-uniformly. For these reasons, it was decided that steel would be used to create the arm. However, there are several different types of steel as can be seen below in Table 6. One of the main reasons 316 Stainless Steel was used was because it is considered Surgical Stainless Steel meaning it is easily sterilizable. It also has the best corrosion resistance of all the steels.

Table 6: Stretching Arm Material Selection

	303 Stainless Steel	**304 Stainless Steel**	**316 Stainless Steel**
Corrosion Resistance	Low	Excellent	Best of all Steel
Weldability	Poor	Good	Good
Additional Comments	Free machining version Not produced in flat rolled product	Most common grade Good for being drawn	Best for flat rolled product Surgical Stainless Steel

Once the device is ready to run, it will then be placed in an incubator, meaning that it will then be subjected to controlled temperatures, humidities, and other factors. If the casing would deform when brought to high temperatures, then it would be useless. This material also had to have the basic capabilities of being relatively inexpensive as well as strong enough to be structurally sound. Included with these factors is again the ability to be put into an autoclave machine. There were several plastics, which were looked into as seen in Table 7, each one having its own properties. It was determined that polypropylene would suit the needs the best, mainly due to its chemical un-reactivity as well as high temperature resistance.

Table 7: Case Material Selection

	Polyester	Polystyrene	Polyetherimide	Polypropylene	Polysulfone
Translucent	Clear w/ no tint	Translucent w/ white tint	Translucent w/ yellow tint	Translucent w/ white tint	Translucent Amber
Lowest Temperature	*-99-(-1)F	0-60 F	*-99-(-1) F	0-60 F	*-199-(-100) F
Highest Temperature	100-200 F	100-200 F	301-400 F	201-300 F	301-400 F
Operating Temperature	*(-30)-150 F	40-148 F		32-210 F	*(-150)-320 F
Autoclavability	Yes	Yes	Yes	Yes	Yes, but for only a few cycles
Expensive	No	No	No	No	Yes
Additional Comments	High Energy Absorption	Limited flexibility Can compress in autoclave		Chemically un-reactive, High temperature resistance	Stability at high temperatures Resistant to oxidizing agents Not resistant to organic solvents

4.5 Design Decisions

4.5.1 Motor Selection

With the selection of the final design and controller coming together, the project team needed to select a motor that would connect to the threaded rod. When selected a motor, the project team knew they would be choosing between a servo motor or a stepper motor. A brushed D.C motor would not work for the application because it only rotates in one direction unless the current from its power supply is alternated. Servo and stepper motors provide a much more accurate and precise position based on the location given by the controller.

A DC stepper motor works very similarly to a brushed DC motor. As opposed to the brushed motor, a stepper motor has several negative and positive poles. This means as the armature rotates when current is applied, it does not necessarily have to rotate a full 180°. This feature has both positive and negative aspects. With such a system, this type of motor has more precise control over where it stops. As the number of poles within the motor increases, so does its accuracy. For example, if there are 90 poles surrounding the internal shaft, then there will be able to rotate the motor in 4° increments. It also, with multiple poles has a high holding torque. Stepper motors tend to be more inexpensive in comparison to a servo motor. One of the disadvantages to using a stepper motor is that it does not have a smooth cycle when in continual rotation.

A servo motor uses a potentiometer that gives the position feedback signal. These two signals both enter a summing operational amplifier, which then creates the error signal, or the difference between the actual and desired output position. This signal is then put through a power amplifier, since most traditional operational amplifiers cannot provide enough power to move the motor. This signal then proceeds to the motor, which then can move to the precise output position defined by the user. This system leads to a highly sensitive and accurate motor. The more accurate of a servo motor however, the more expensive it tends to be.

All Motion, the company that manufactures the controller selected by the project group, recommended that motors produced by Portescap worked very well with their controllers. Using this information, the project team called an engineer at Portescap and, after explaining the application to them, were told that an inexpensive stepper motors would work for the device. After studying the products distributed by Portescap, and following the advice of their engineer, the project group decided to select a stepper can stack motor with a 26mm shaft. The reason behind this decision was because it was very inexpensive and could be used to experiment with until the controller was fully programmed and properly working.

Figure 24: Stepper Can Stack Motor

After several weeks testing the motor, the project team decided they were unsure how reliable to stepper motor would be attached to the threaded rod. Looking into other options for motors, the project team discovered a linear actuator motor in Professor Billiar's lab at Gateway. The motor was produced by the same company, Portescap, as the stepper motor previously used. Therefore, it was also compatible with the controller and driver already purchased from All Motion. Using the linear actuator eliminated the need of the threaded rod and allowed the project team to bring the actuator shaft in and out exactly the distance needed. Being low cost and spatially efficient, the linear actuator motor proved to be a perfect choice for the application.

Figure 25: Linear Actuator Motor

4.5.2 Controller and Driver Selection

While deciding upon a motor, the project team was also researching what controllers and drivers were available to govern it. Engineering catalog websites were consulted for different controller companies. The project team researched the product line, prices and help support with each individual company until the company All Motion was decided upon. All Motion had a very user-friendly website with a Help Desk to submit questions when having difficulty with the controller. After explaining the proposed device to the company's Help Desk, the project team received a timely and helpful answer on what model to choose and why. This exceptional customer service helped finalize the decision. The model chosen was EZHR17EN Stepper Motor Controller. This controller was one of the higher end models All Motion offered. It could be programmed to all the motions the project team desired included a sinusoidal wave motion. Most importantly, it was also capable of stand-alone operation with no connection to a PC needed. Once delivered, the project team was able to set up the controller and having it working in just a few hours.

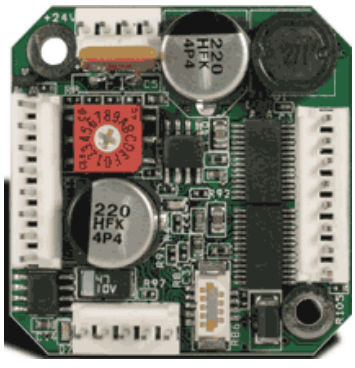


Figure 26: EZHR17EN Stepper Motor Controller

4.6 Optimization

The optimization process of this design focused primarily on the environment that the device had to operate in. This presented issues of both material selection and of size. This section details the iteration process for designing around these parameters.

4.6.1 Dimensional

The driving parameter for the size of the device was the shelf size of the incubator that it will ultimately operate in. The shelf is roughly 18" x 18" with adjustable heights. As this device will be in the incubator for extended periods of time, it should take up as little space as possible while still stretching a considerable amount of samples. Because of this, the project team sought to maximize the number of sample along the width of the shelf and minimize the height. The depth of the device was kept and roughly half the width of the shelf as well. This, along with the minimized height could allow two devices to be operated simultaneously, either stacked on top of one another or run side-by-side.

4.6.2 Material Selection

Most materials used in the device had to be corrosion resistant and any parts that enter the culture environment needed to also be autoclaved. This limited the list of materials that the project team had to work with. All metal components were to be made of 316 Stainless Steel or other corrosion resistant metals. Plastics were a bit more difficult to select, however. The high pressures and temperatures in the autoclave can cause some plastics to warp or fracture, not optimal for a device that would need to be autoclaved before each use. Plastics such as polypropylene, polyester, and delrin could withstand such use.

5.0 Design Verification

5.1 Well Verification

In order to move forward with the design, the team needed to confirm that the newly created well system would work. In past experiments, the gels were cured vertically within glass plates. This new system has them being cured horizontally, which had never been done before. In order to test this, the team created a test well, made of glass slides as seen below. Then in creating the gels, are the same steps were taken as if

we were curing them horizontally. The test was successful in that the gels were created, and displayed the same properties as gels made in other techniques. This meant that the team could continue with their design based around horizontal based wells.

5.2 Motor System Verification

One of the main aspects of the motor system is that it must be durable. The device is designed to run for 3-5 days in the incubator. So one of the first tasks was to ensure the motor and controller could run for that long without any problems. In order to test this, the team tested the motor and controller cyclically for two days The team applied a small load to the motor and had it run for two days. It was found that both the controller and the motor were able to handle this load.

One aspect of the motor that needed to be validated specifically was its precision. Since the device is only stretching a small amount, if the motor is not precise it would greatly affect the results of the project. So in order to measure the displacement of the linear actuator, a digital dial indicator was used. The Mitutoyo Absolute dial indicator used was accurate to one thousandth of a millimeter. The motor was able to move its desired distances to within 0.5%. This was well within the tolerances of the device.

Another validation test conducted was that of the heat given off from the controller. The entire device needs to be in an incubator, which is a very controlled environment. If the controller was giving off a lot of heat, then this could alter the environment and in turn alter the results of the experiment. By using a thermocouple the team analyzed the heat given off by the controller. It was found that the temperature ranged between 20-25°, or just around room temperature after a day of running continuously.

6.0 Discussion

The overall objective of this design project was to design and test a device to cyclically stretch a soft substrate in a tissue culture (temperature controlled and sterile) environment. While there was many design difficulties along the way, the project team believes they were able to successfully complete the client statement given to them. The device built is able to stretch eight samples of polyacrylamide simultaneously with a

theoretical strain of 20%. FEA analysis confirmed that the polyacrylamide would stretch homogeneously if the stretching region were the correct dimensions as discussed in Chapter 4. However, the project team was not able to confirm the actual strains of the polyacrylamide due to lack of time and a corruption on the HDM computer. The actual validation will be conducted by a biomedical engineering undergraduate during the summer of 2009.

One of the major difficulties in designing this device was there need for extremely precise machining. Most of the parts built had to be accurate to 0.01 inches. The machining equipment at Worcester Polytechnic Institute was not intended for such small, accurate machining. To overcome this, the project group special ordered new wells and casting wells from a plastic machining company that could accurately machine parts to a tolerance of +/- 0.01 inches. The quotes have been submitted and the parts will arrive in time for validation in the June 2009.

Overall the project was quite successful, and even with the difficult machining the project team was able to produce a professional looking, effective device to meet the client's needs. The validation of each subsystem in the device is discussed in the following chapter.

7.0 Final Design and Validation

After multiple design iterations and compromises a final design was decided upon that would not only meet the needs of the client, but also be easily made and used for a relatively low cost. The following section details the last design decisions by subsystem, but does discuss specific dimension. To view these and other part characteristics see Appendix D.

7.1 Curing System

The polyacrylamide samples will be cured and stretched in the same well, which allows for the samples to make it to testing with the least amount of damage possible. The wells are efficiently designed with little wasted space to not only save material costs, but also to allow for the largest number of samples to be stretched on one incubator shelf.

The wells themselves (as seen in Figure 27) have four walls made of polypropylene with a glass bottom. The glass bottom serves two purposes. Firstly, it lets

the polyacrylamide be cured directly into the well, as polyacrylamide cures well when surrounded by glass. Secondly,

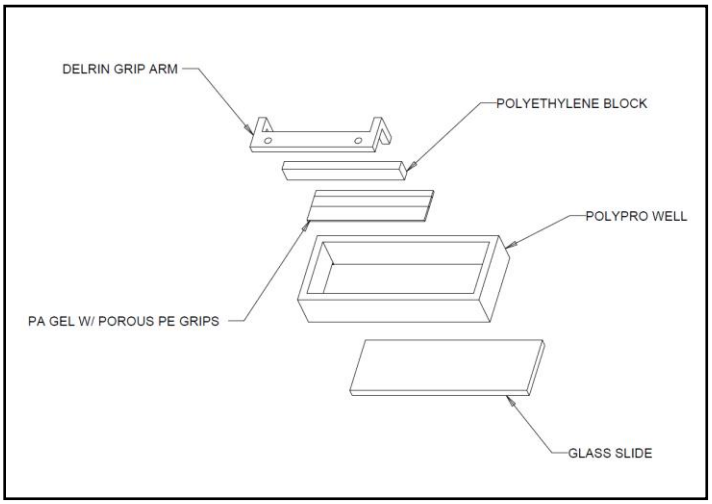


Figure 27: Well Configuration

glass allows the wells to be placed under a microscope which is important to the future applications of this device. A clear polyester lid was also added to each well to aid in monitoring of the cell cultures.

The porous polyethylene grips have a block of polyethylene glued to them that, in turn, are fixed to the delrin grip arms via steel hooks. During well transportation, these arms are fixed so that the gels only see strain once the device begins to function. Once placed into the device, these delrin arms will attach to the driving mechanism.

7.2 Driving Mechanism

The physical means of stretching the gels is outlined in Figure 28 below. This right side view shows just the moving parts (with the exception of the motor, which is stationary) of the

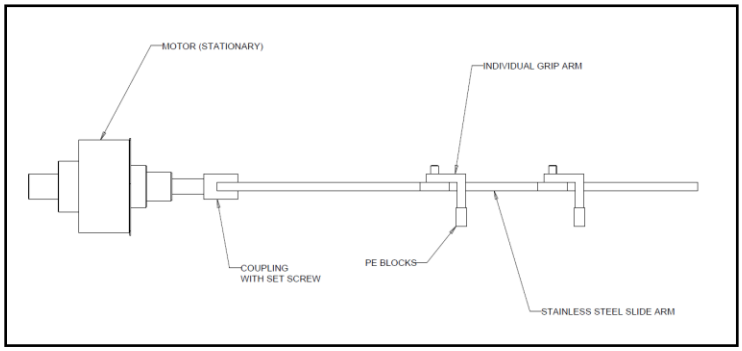


Figure 28: Driving Mechanism

device minus the polyacrylamide samples. The linear motor drives the coupling assembly, which in turn moves the slide arm back and forth. The motor is attached to the coupling by existing threads on its shaft and the arm is fixed to the coupling by a set screw. Individual grips arms that enter the cell culture environment are attached to the slide arm by threaded stand-offs and nuts. The ball slide was included to reduce the friction of the device and limit the power that the motor must output. All surfaces that mate with the device case will have acrylic-adhesive Teflon tape applied to them (as well as on the corresponding surface on the case) to further limit the friction in the system.

7.3 External Casing

Two key characteristics of the casing of the device are that it must be autoclavable and that its interior must be air tight to avoid contamination. To adhere to the first characteristic, the parts are made of polypropylene, which performs well after repeated autoclave sessions. The case (Figure 29) must also not allow the passage of particles from the outside environment into the cell culture environment. Silicon sealant or plastic welding should be used to ensure that the case itself is sealed properly. Though difficult,

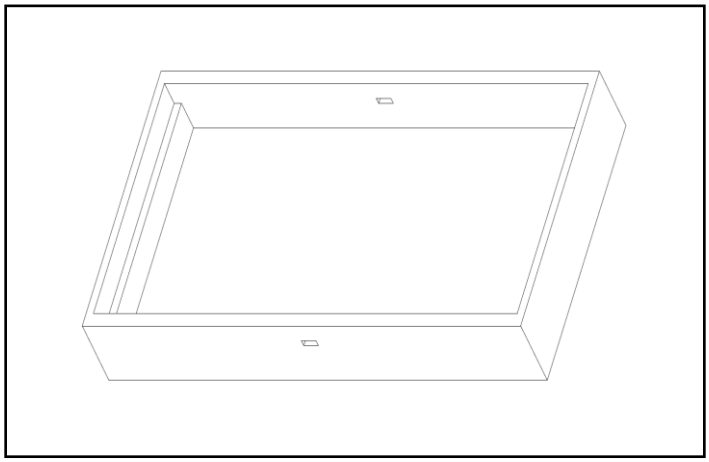


Figure 29: Device Case

the project team recommends machining the case out of one solid block as it produces more predictable results and a cleaner look. The case also has a removable, clear polyester lid which is gasketed to guarantee that minimal amounts of particles, if any, get into the culture environment.

An area of contamination concern in the case is where the slide arm enters the cell culture environment. The project team originally sought to use bellows to fulfill this task, but after an exhaustive search, no bellows were found that met our parameters as well as

the size constraints of the device. Because of this, it was necessary to design a "bellows like" system to achieve this end. The result is depicted in Figure 30. A short section of collared PVC tube is fixed around the opening in the case. A thin membrane is fixed to the inside of this tube, left with slack, and a hole slit is cut into it. The slide arm is fed through this and the hole is adhered to it in such a way to allow for sufficient motion of the device. When the device is extended (left side of figure) the membrane is slackened and when the arm is at home position (right side of figure) then membrane is slightly tensioned.

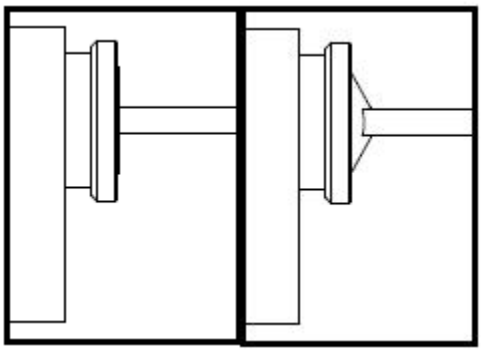


Figure 30: Bellow Replacement

7.4 Electronic Components

The electronic components list of this device consists of the motor, motor controller, USB adaptor, a computer for programming, and the power supply. These are configured as shown in Figure 31. The power supply is a 3 A/ 13.8 VDC supply which power the controller.

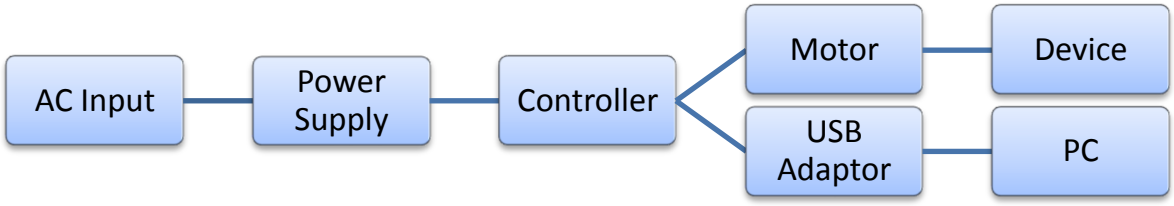


Figure 31: Electronics Configuration

The controller in turn is linked to the USB adaptor and powers the linear motor. The adaptor allows for an easy interface between the controller and the user, while allowed

the motor to run while unattached from the source PC. To aid in the mobility of the device, the wire from the motor was shortened and had a connector added. This will connect to an extension cable, which will then attach to the control case, which includes the controller and adapter. A shorter extension cable connects this controller box to the power supply, which would be situated nearby. These connections allow for the device to be easily transported in segments to avoid large amounts of excess wire.

7.5 Design Evaluation

By combing these subsystems, the total device is shown in Figure 32. The small, four-well based design for PA gels to be cured, seeded with cells and transported to the incubator without subjecting them with strains until the device begins its cycles. With this design also comes the ability to run multiple test groups in different cases to allow for greater variability in different experiments. Based on the size of the incubator shelf, up to four devices could be run off of on motor and with the motor controller's ability to control multiple motors at once, it is possible to conduct experiments with upwards of thirty samples.

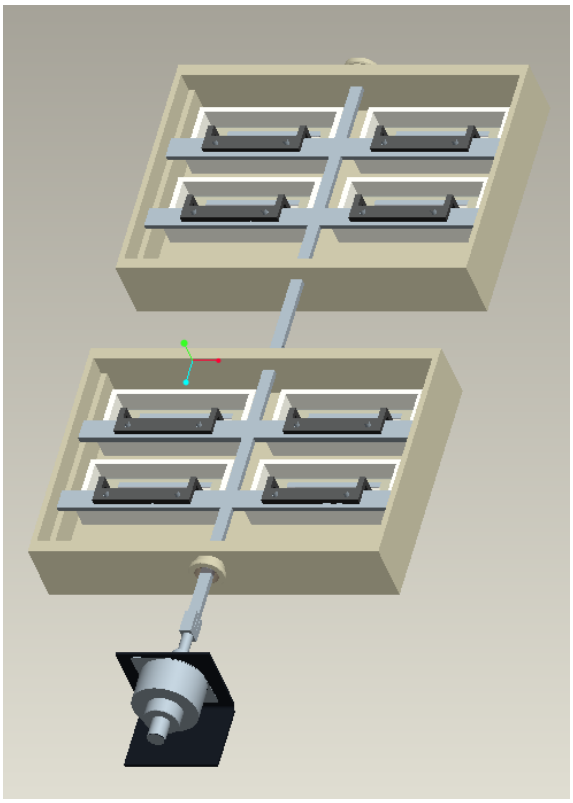


Figure 32: Final Cyclic Stretch Device

The final device met or exceeded the preliminary specifications put forth in the client statement, and in that regard it is a success. With a completed design in hand, prototyping must occur to validate the various subsystems and the device as a whole.

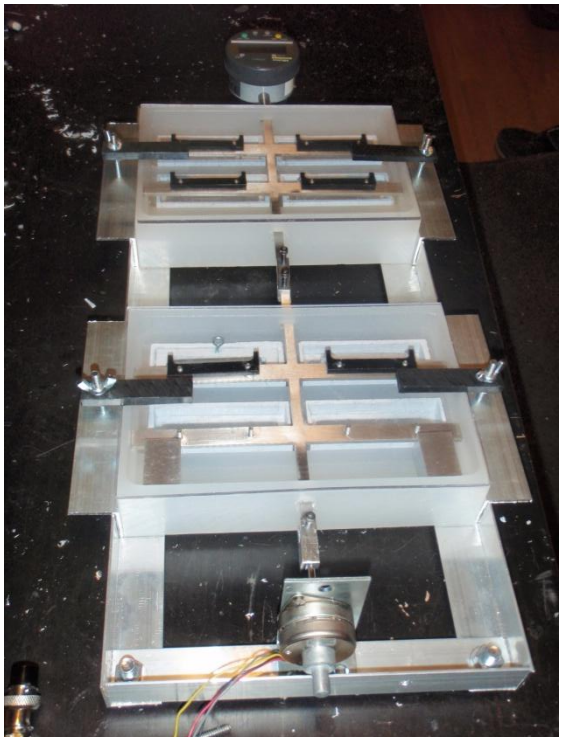


Figure 33: Final Prototype

8.0 Conclusions and Recommendations

8.1 Conclusions

A device to cyclically stretch polyacrylamide gels for extended periods of time was successfully designed. While the device itself still needs some validation the project team is confident that, upon testing, all subsystems will perform satisfactorily. The device met the key functions as set forth by the client statement and all constraints were accounted for. Cyclic strains can be applied to gels in such a way that will not tear and will allow for favorable cell growth. This, along with the high number of samples that could possibly be run at one time makes this device ideal for the types of experiments that it will be used for.

Before experiments can be conducted, however, the device needs further validation of the actual strains the device applies to the cells. The project team is

confident that completing a more complete analysis will show that the membranes will see strain similar to those in the Finite Element Analysis. Once this is completed and the reproducibility of the individual samples is in order then this device will be able to run experiments that will test combined stretch and stiffness of membranes on cell cultures.

8.2 Recommendations

This section illustrates the suggestions that the project team believes will improve the current designed device. These recommendations include ideas for validation of the device and for cell adhesion as well as other ideas for possible future manufacturing. While the project team is very proud of their final design, they provide these suggestions to possibly improve the effectiveness of the device and better control the stiffness and stretch of the polyacrylamide.

Performing strain and cell adhesion tests would be the obvious first step in continuing this design project. While in theory our device should stretch and keep cells alive based on our FEA analysis and material selection, human error can always occur making it possible that the strains and cell behavior might vary from the expected. Testing these would ensure that our device runs as it is supposed to.

Finding a more reliable adhesive would be very beneficial if wells ever need to be constructed. During the manufacturing process, the project team had a very difficult time with the adhesive provided to us from Gateway that would not harm the cells. However, it seemed this adhesive would wear overtime and made our wells frequently leak. Having a stronger, more reliable adhesive that would not harm the cultured cells would prevent any similar problems from occurring again.

More working space between wells would make curing polyacrylamide much easier. When the stainless steel arm was originally designed and built, the project team wanted to be able to fit as many samples as possible into the case while still keeping it a reasonable size. After necessary redesigns of the model, the user is no longer able to remove the wells once the stretching arm is put into the device and the PA is cured. This makes removing the casting wells more difficult because the spacing between each stretching arm is exactly the same size as the width of the wells.

Improving the well lid covers will improve the sterility of the device and would allow the cell experiments to run for a longer period of time. Currently, the lids only cover approximately one half of the wells, leaving room for the stretching arm to move cyclically. Creating a lid that could cover the entire well while moving with the stretching arm would be most effective.

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Appendix A: Interview with Professor Billiar

What are the specific functions this project must obtain?
My current today is strip biaxial stretching it is going to be I think relatively straight forward and be able to do experiments more short term equibiaxial is a lot harder how to attach things, if it breaks, get uniaxial. Equibiaxial systems exist, but hard to work with.

Heart Valves go at very high strain rates and strains; I would like to be able to go to 20-25 percent stretch, if only fifteen and works all other ways but if you can go 25 percent that would be ideal for strip biaxial.

Do you want it to be adjustable in terms of the strain?
I would like it to be adjustable 5,10,25,20.25 and each one being plus or minus ten percent of their strain. So if you are at 25 percent it is plus or minus 2.5 percent. Then what if you are doing 25 percent strain and the other axis goes to .95 it's not zero, its .5 percent. That's 10 percent of zero so in that axis you want to be within 10 percent of your stretch axis so if you stretch to 25 then in the latter axis if you are within 2.5 percent

As long as within the area it is within those specs, you made it. I'm going to say a 1 cm squared area is what I want then to get those cells you are going to need five of theses to be enough so if you can go to five cm squared on 1 that would be ideal or for another assay that's fine so there we have to talk to her as a client because then you have a big five cm one it's a big thing now you can only have five but if their smaller you can have 25 of them, if it's a square thing then she could pool five together from one assay for all the other it satisfies. It has to go with the design i'm envisioning different type of designs, if you start making them bigger and bigger, you are going to use a lot of material, you have to talk to the client about what they need. My feeling right now is 1 cm squared is the minimum but not at the expense of using a lot of material or not being able to run a lot of experiments.

Things that cost money cells culture mediums and growth factors. So we will have to deal with that as an issue. The medium and the cells, and if you need ten million cells growing them up and using them sometimes it matters sometimes it doesn't if you only feed it in a small area, if you define well where that homogeneous area is so you only functionalize that part and only allow cells to attach to that part you can make up a restraint around it so only the media stays in that area, if you need to cover this large thing with media it will be too expensive.

The function is to stretch it the device has to stretch at a certain rate, high as strain rate as possible

Constraints
.1-2 hertz, if not possible just 1 hertz well have to deal, wouldn't be as useful, if you could put it whatever strain rate you wanted hold it and bring it back down and do any

waveform you wanted that would be ideal, but I want low cost also and easy to use and reliable.
It has to do all that but we know it has to be able to hold onto without tearing the soft gels, that's not external objective, its within there, a sub objective, of course limiting the amount of media used would be very good lets say you have a design where you could pull on it and it stretch beautifully if it had walls that stretched with it and the media was just within that area then you could use 1 ml and do the test and do the whole test and do each one independent, if you had 5 in a bath 1 L, each one is taking 200 ml of everything else is in the bath and has a chance of getting infected.

Each sampled use less than 10 ml of media

What is the consistency?
More like soft jello to stiff jello, if you put too much water in the jello or you left it out in the fridge, and the top layer is stiff, were up to that its that same goopy when you make it soft so its kind of hard to hold on its more like soft jello. So if you do reology in that, you take a simplest one is a parallel plate and it oscillates and it measures shear stress and strain and you calculate stiffness, but when ins hear its shear stiffness, g, there's g prime and g double prime g prime is the elastic component g double prime is the viscous component so if something is liquid it is more g double will be high, if more elastic, then g prime will be higher. It usually changes as frequency changes so something that is pretty liquidy when you cycle it fast it acts more like an elastic it doesn't have time to flow, with fluid you'll see the stretch is proportional to the strain rate not to the actual strain so not elastic at all.

You should go make some with Angie

You need to go in there and play with the stuff with Angie

I didn't even think about the volume before, that would be a very beneficial design to have a limited volume for a lot of reasons

The polyacrylamide is very goopy and when it gets soft it probably gets more viscous however it is considered by people that use it as elastic solid. They're just real soft; it's a fully elastic matrix just very soft. And new would be made each time, casting it, it will not cure against plastic so if you make this neat little mold for it oxygen radicals will stop it from solidifying , so between two pieces of glass it works very will in a vacuum or in nitrogen without any oxygen it will be much better we know much more about that now because we were trying to get it to attach to the surface and we get a nice gel and it slides around what you are trying to do is attach a liquid to something, liquids don't attach they're not getting a good bond with he liquid, under nitrogen we could get them to attach we could actually not put a glass cover slip above it and just do it under nitrogen and get a flat surface, you don't have to worry about taking the cover slip off and ruining the surface.

You might just make one in-between two pieces of glass pull off the glass slap it in there and grab it with grips and go and there's no problem at all or you might need to solidify it into something now that material is plastic and doesn't work then you do it and start stretching it and it comes off the grip, she actually knows porous polyethylene material are very porous especially with the vacuum will suck in the pores and cure.

She has a lot of knowledge you could use right away,

Again this stuff is porous, so if you made a will out of it then put a well in there so underneath has to be media too if it is really soft, it might just bow.

Optimized design based on finite element to find out what would be the shape of the pulling thing to get the largest homogenous stretch for size and make the bath as small as possible around that is going to minimize your volumes for everything you won't have to have much media above ore beneath it so these are all details

Incubator you need to talk to Angie in term of what performs best, metals that rust are not good, but you need to sterilize things you are going to use more than once so you need to deal with what can be sterilized and Angie knows that and some things can be sterilized and if you do it too many times they degrade. Polypropylene can be sterilized and doesn't react with any chemicals real soft.

Talk to Angie about it has to fit on the shelf in the incubator
Minimizing size is critical, stacking certain ways, and being creative with the design

No power restrictions

Polyacrylamide, it comes back to original size, who does it cyclically no one it might be until 100 times then plastically deform, have a silicone membrane, stretching these things and they start waffling

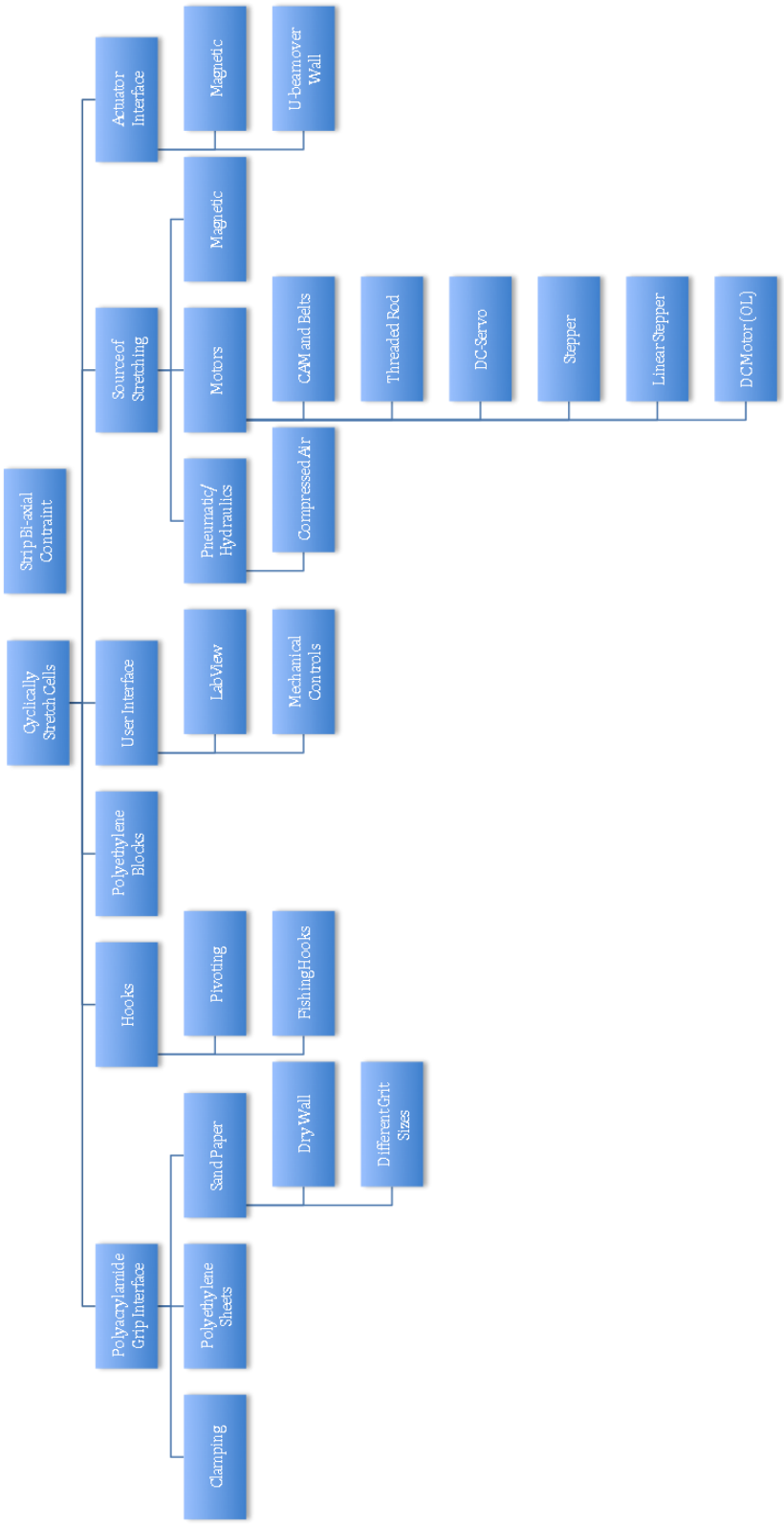
1 a sec, ten hours 36000 cycles, 24 hours, 80000 cycles a day, a high strain, this stuff might totally stretch out after one hundred, would like to stay sterile for three days, objective as long as possible, be durable enough to handle a week, but if it can only handle a day, well have to do experiments in a day but if that's the limit of the stuff. Is it the attachment or the material gets stretch out can we do something about it or put a backing on it, then we have to deal with its limitations, then we could look at PEG, I have a college at Georgia tech we have all the materials, and he does it all the time, so attaching cells will not be a problem, if you could get it stretching, he can give us the surface chemistry to get it going, the polyacrylamide has had all sorts of problems with it, maybe peg can be stretched, when you learn how to make polyacrylamide you cane make some peg to and just play with them and get them to be the same stiffness and pick one. If you have to make a bunch, if there's an easier way to do, within the constraints of the problem.

Might have to precycle the material to avoid plastic deformation while cells on it

Fluid stresses will affect growth of cells, could use baffolds to break up fluid movement Anything contacting tissues needs to be sterile, the polyacrylamide can't be sterilized, not a sterile system,

What if you could use magnets, have magnets outside, metal inside sterilized, and little posts of how far it could move, you turn it on and the thing inside is totally sealed and its just the magnet going through the wall moving the way you want it, no motors, that starts being a very big thing, it must be sterilized must have this functions of doing things, but must be done in a aseptic way.

Appendix B: Functions Means Tree



Appendix C: Pairwise Comparison Charts

Objectives: Project Group

	Reliable	**Durable**	**Inexpensive**	**Easy to use**	**Effective**	SCORE
Reliable	X	1	1	1	0	**3**
Durable	0	X	1	1	0	**2**
Inexpensive	0	0	X	0	0	**0**
Easy to use	0	0	1	X	0	**1**
Effective	1	1	1	1	X	**4**

Objectives: Professor Billiar (Client)

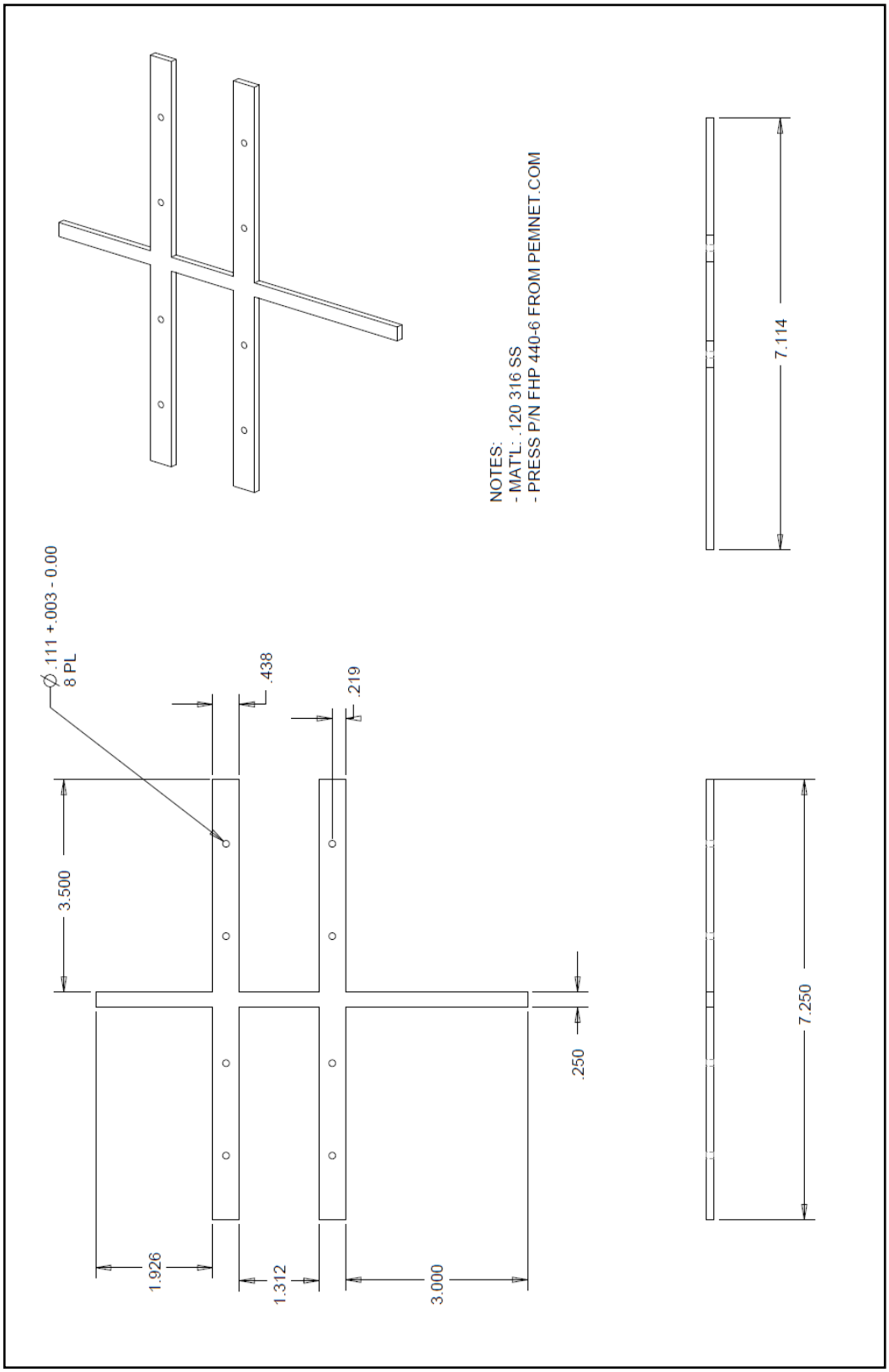
	Reliable	**Durable**	**Inexpensive**	**Easy to use**	**Effective**	SCORE
Reliable	X	1	1	1	0	**3**
Durable	0	X	1	1	0	**2**
Inexpensive	0	0	X	0	0	**0**
Easy to use	0	0	1	X	0	**1**
Effective	1	1	1	1	X	**4**

Objectives: Angie Throm (Client)

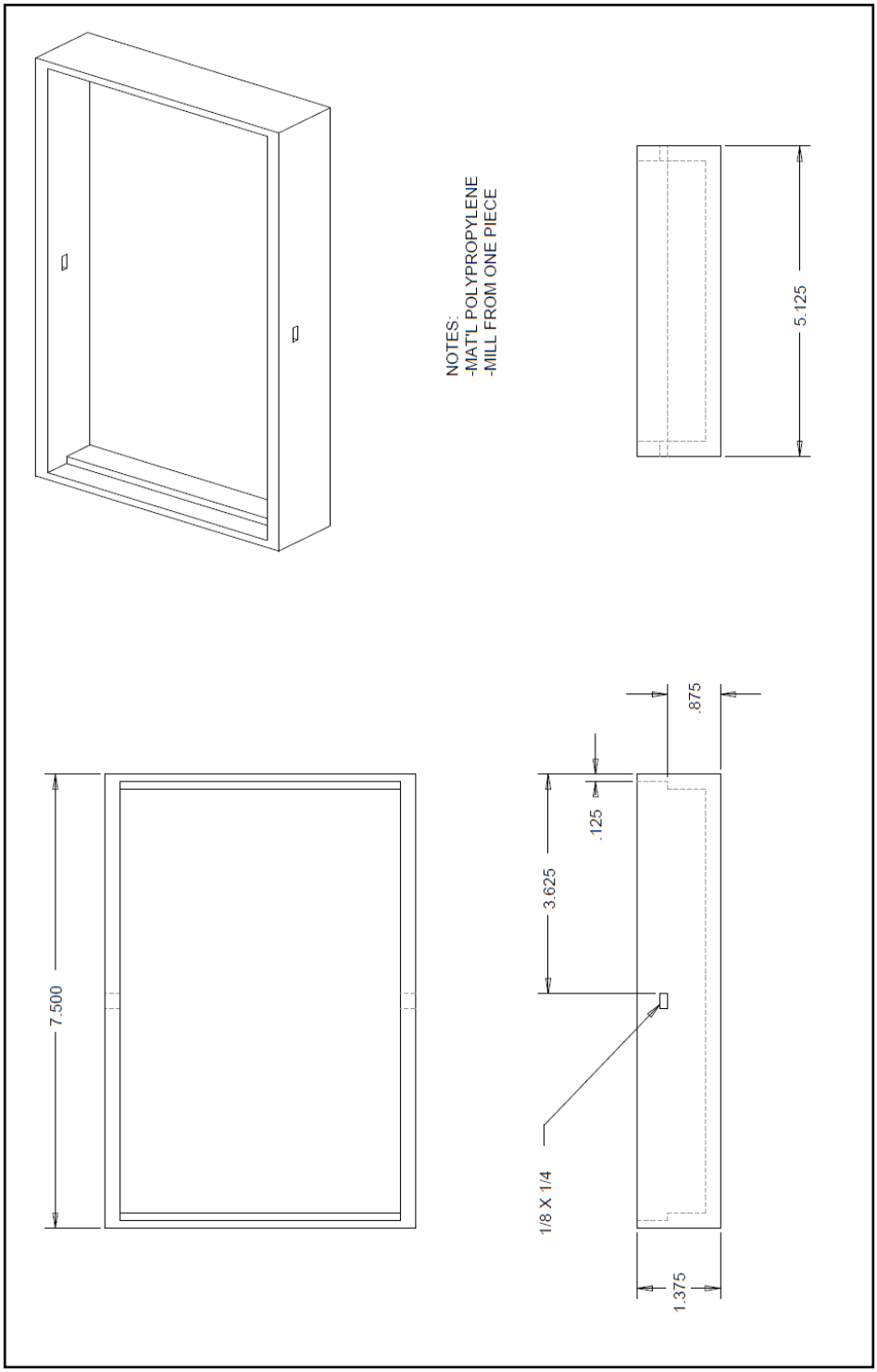
	Reliable	**Durable**	**Inexpensive**	**Easy to use**	**Effective**	SCORE
Reliable	X	1	1	1	0	**3**
Durable	0	X	1	1	0	**2**
Inexpensive	0	0	X	1	0	**1**
Easy to use	0	0	0	X	0	**0**
Effective	1	1	1	1	X	**4**

Appendix D: Dimensioned Drawings of All Custom Parts

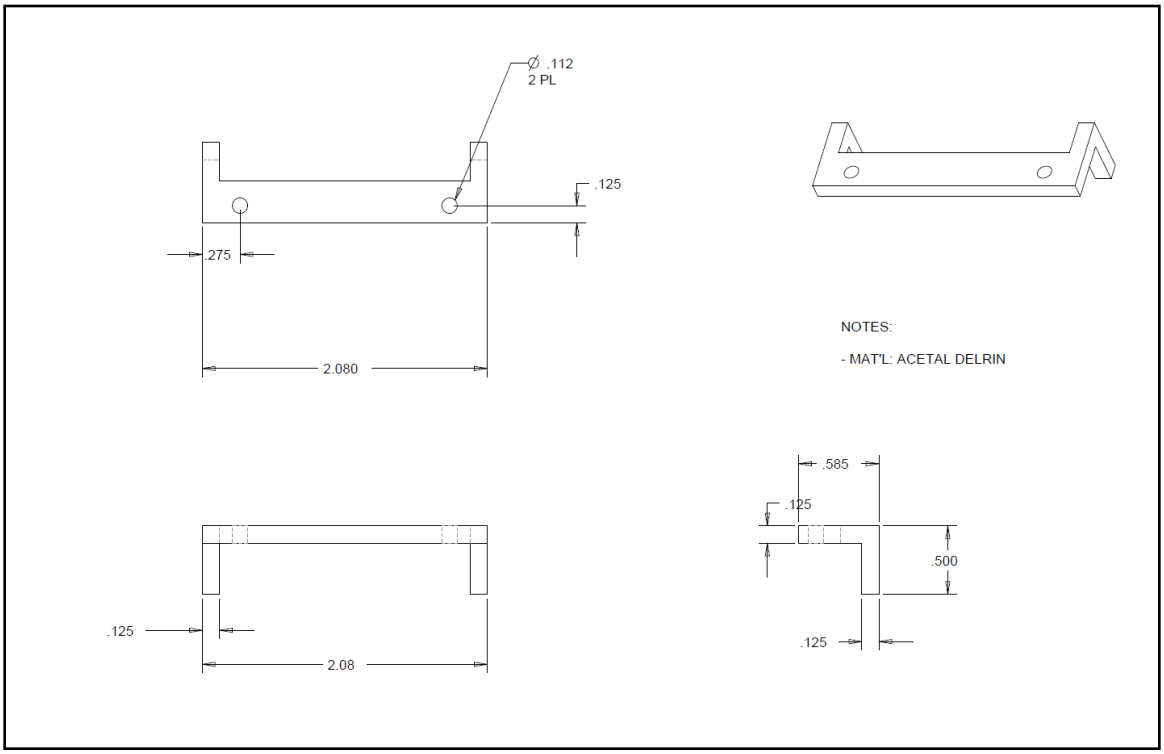
1. Slide Arm
2. Case
3. Well Walls
4. Grip
5. Coupling



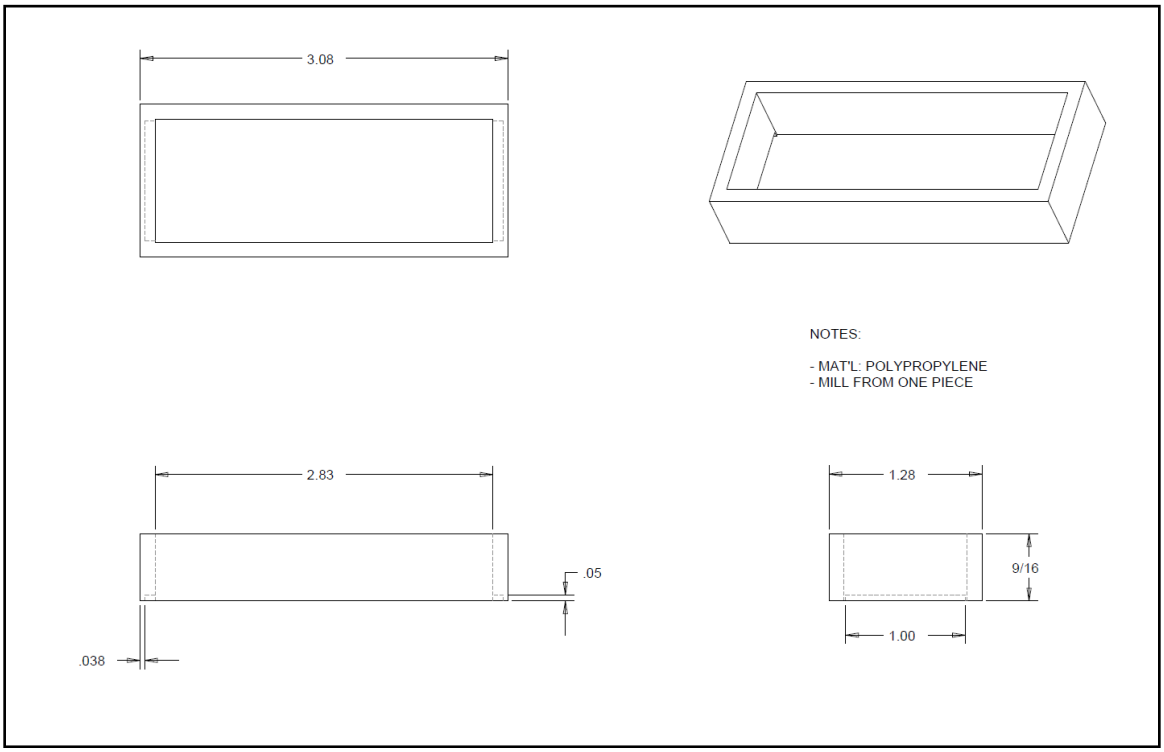
Slide Arm Drawing



Case Drawing



Grip Arm Drawing



Well Drawing

Appendix E: Finite Element Analysis

Goal: To determine the effect of the dimensional ratio on the strain maps of polyacrylamide (PA) gel 6 using ANSYS software. Samples are stretched 10% in either direction creating an overall stretch of 20%. Though pure strip-biaxial stretch cannot be achieved with given volume parameters, we can get a rough approximation to within 10% of the length-wise stretch percent.

Standard Model Parameters:

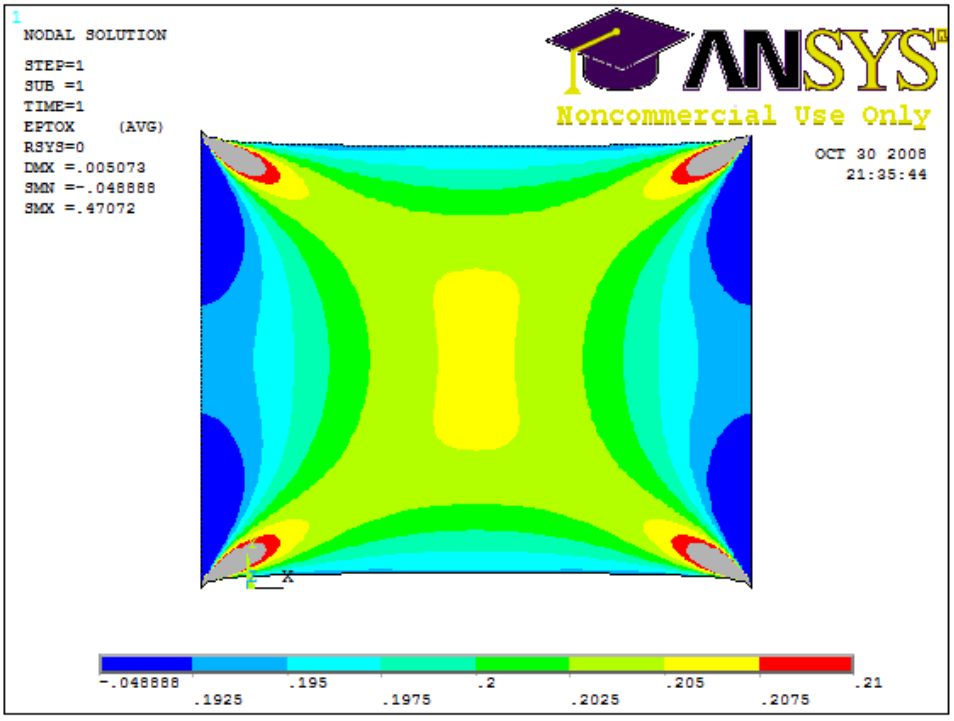
- Thickness: 1.5mm
- E = 4800 Pa
- $\nu = 0.35$
- Element Type: Solid, Quad 4node 42
    - Behavior: Plane Strs w/ thk
- Real Constants: 0.0015m thk
- Material Model: Structural > Linear > Elastic > Isotropic
- Mesh Size: 0.0005m
- Loading:
    - Each vertical edge displaced 0.10*L in UX
    - Corners are constrained in UY

Sample Ratios Shown:

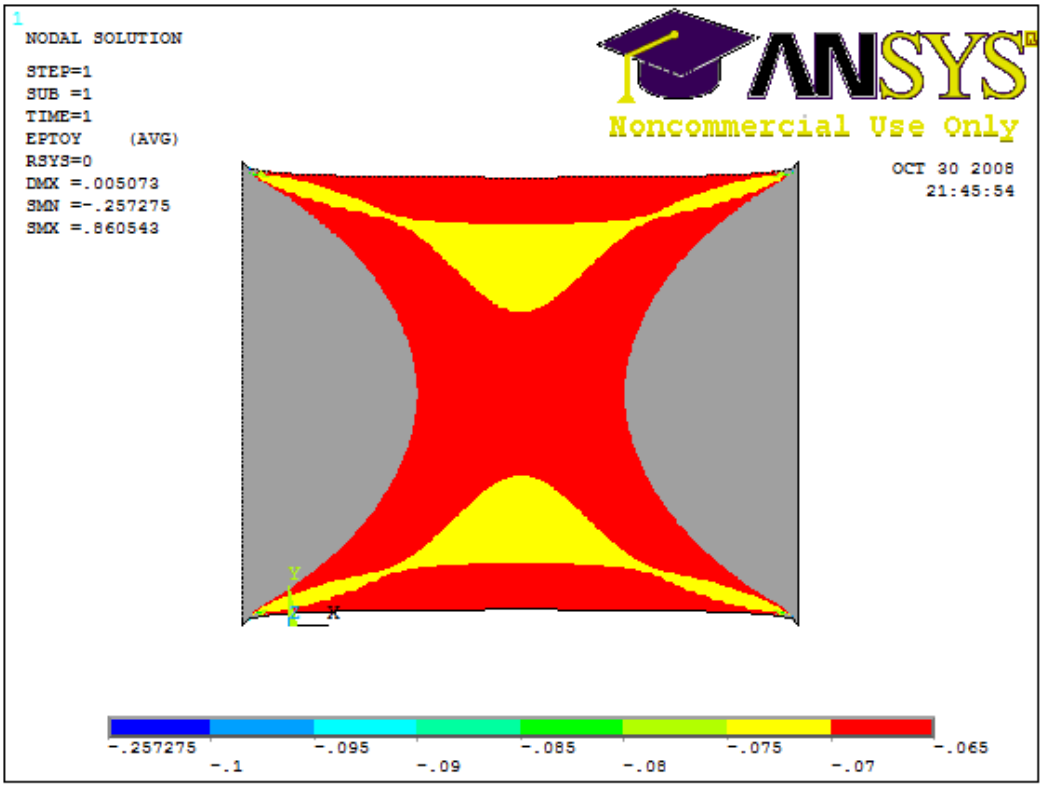
- 1:1
- 1:2
- 2:1
- 1:3
- 3:1
- 1:4
- 4:1
- 13:1
- 16:1

Strain Plots

Ratio 1:1

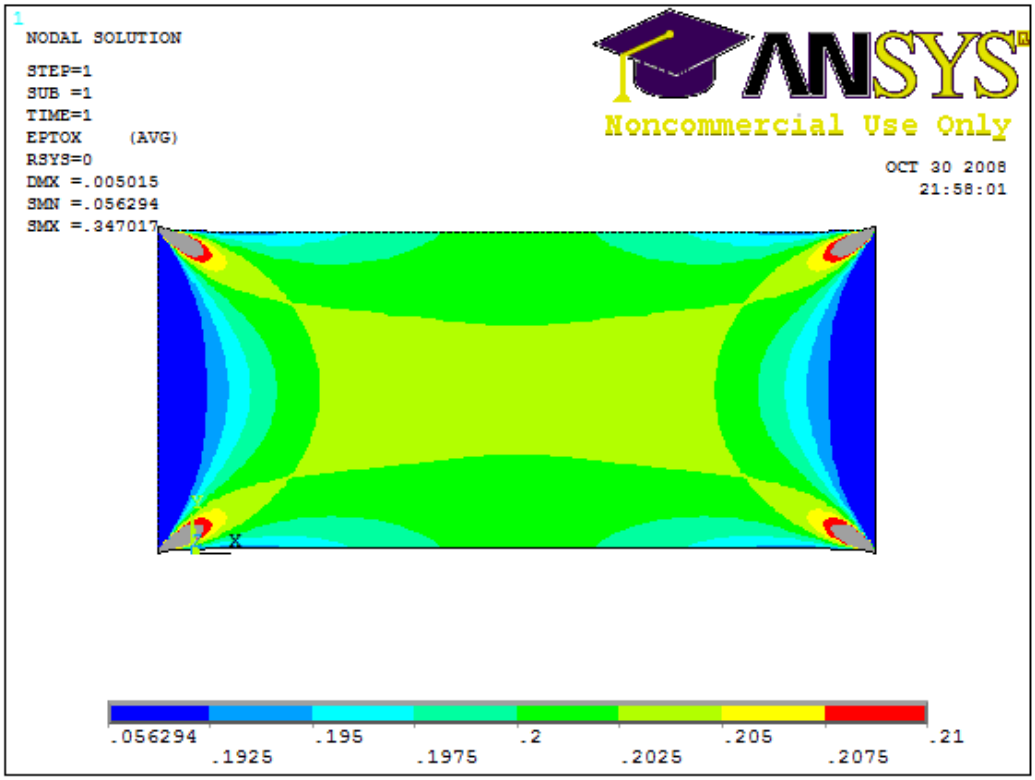


1:1 UX-Strain

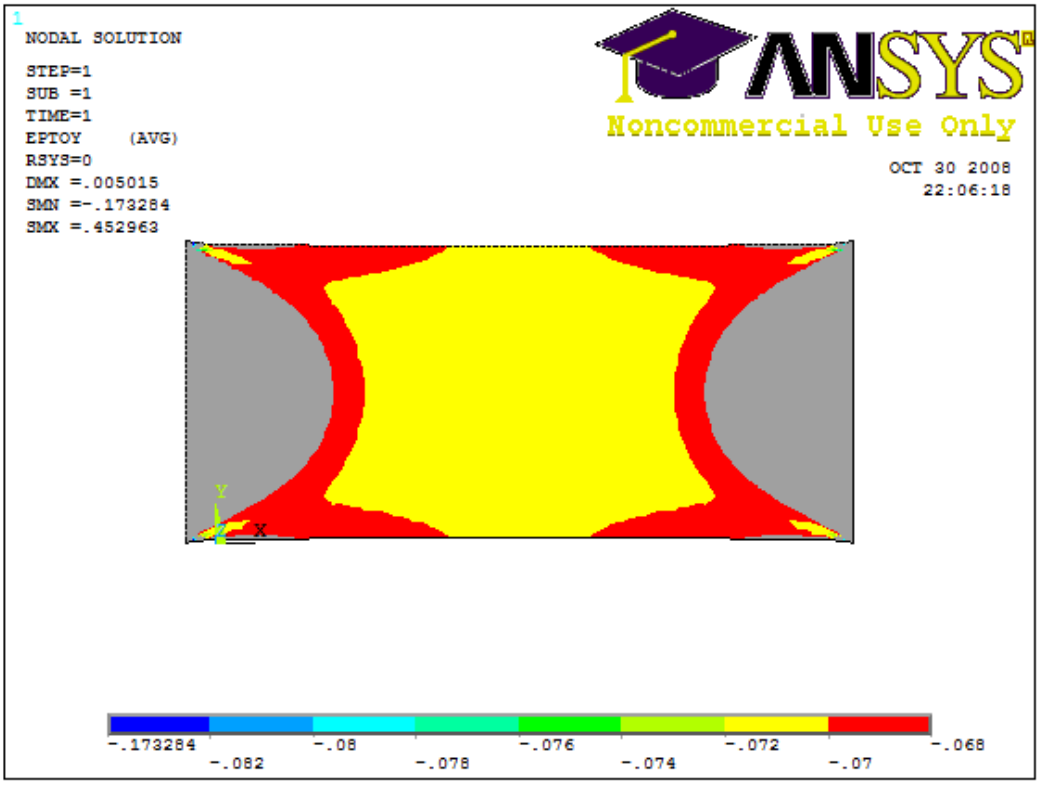


1:1 UY-Strain

Ratio 1:2

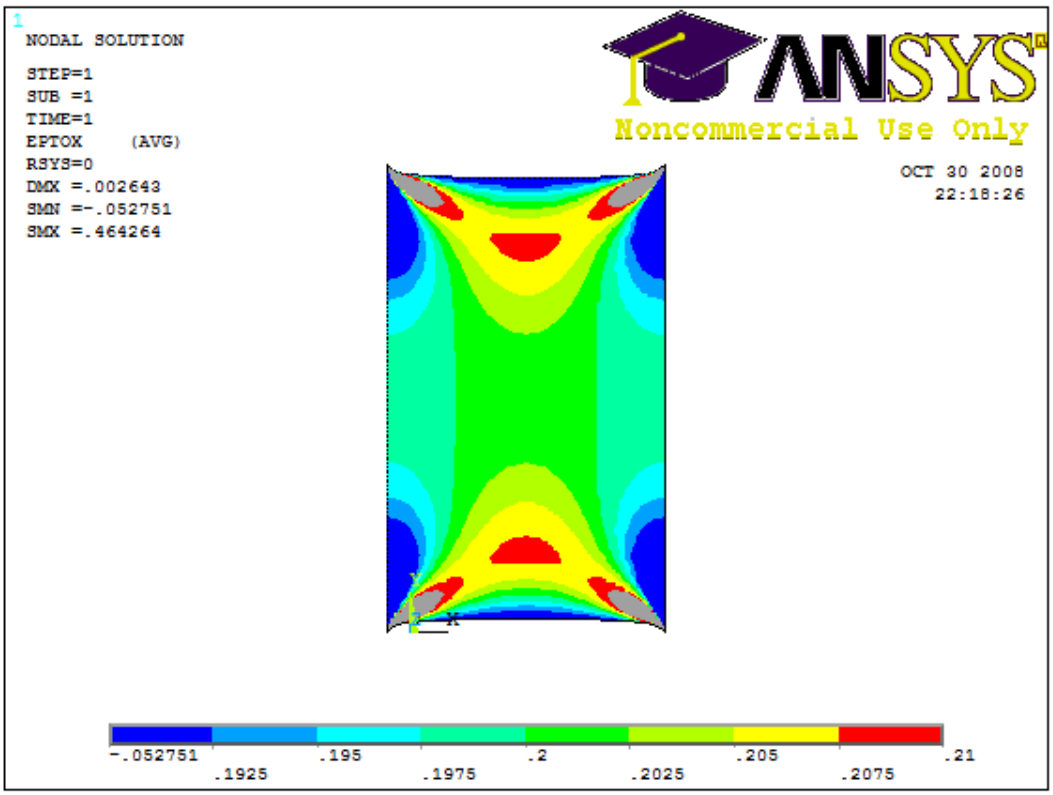


1:2 UX-Strain

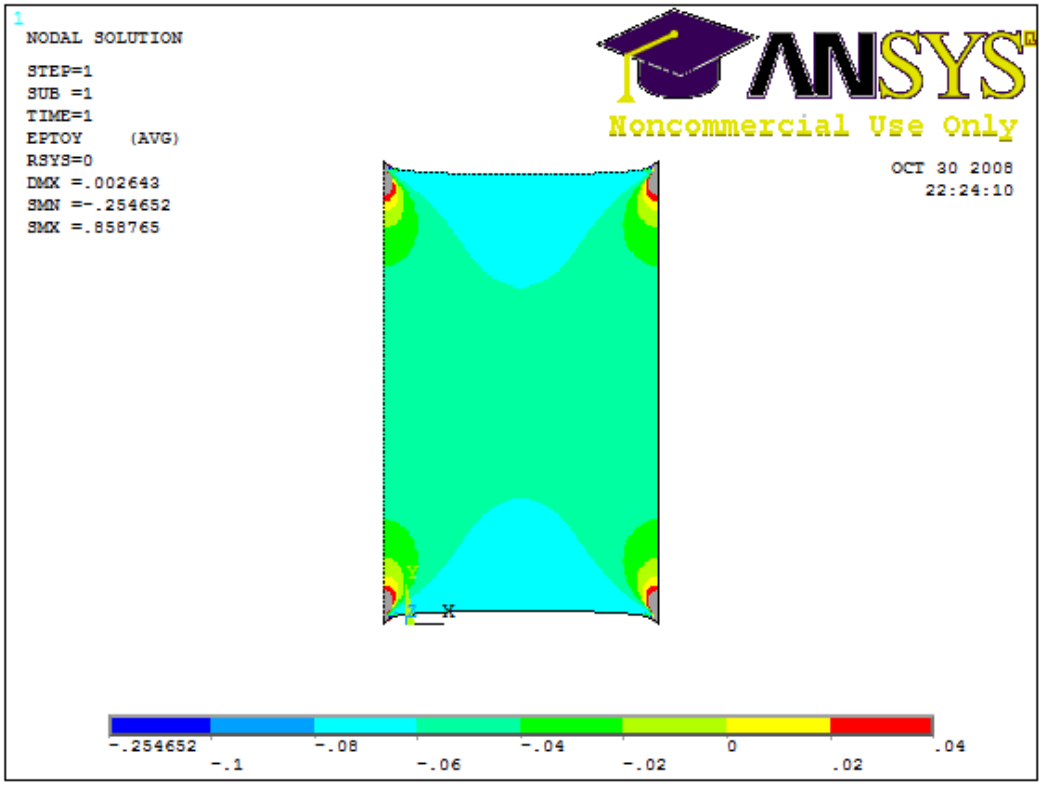


1:2 UY-Strain

Ratio 2:1

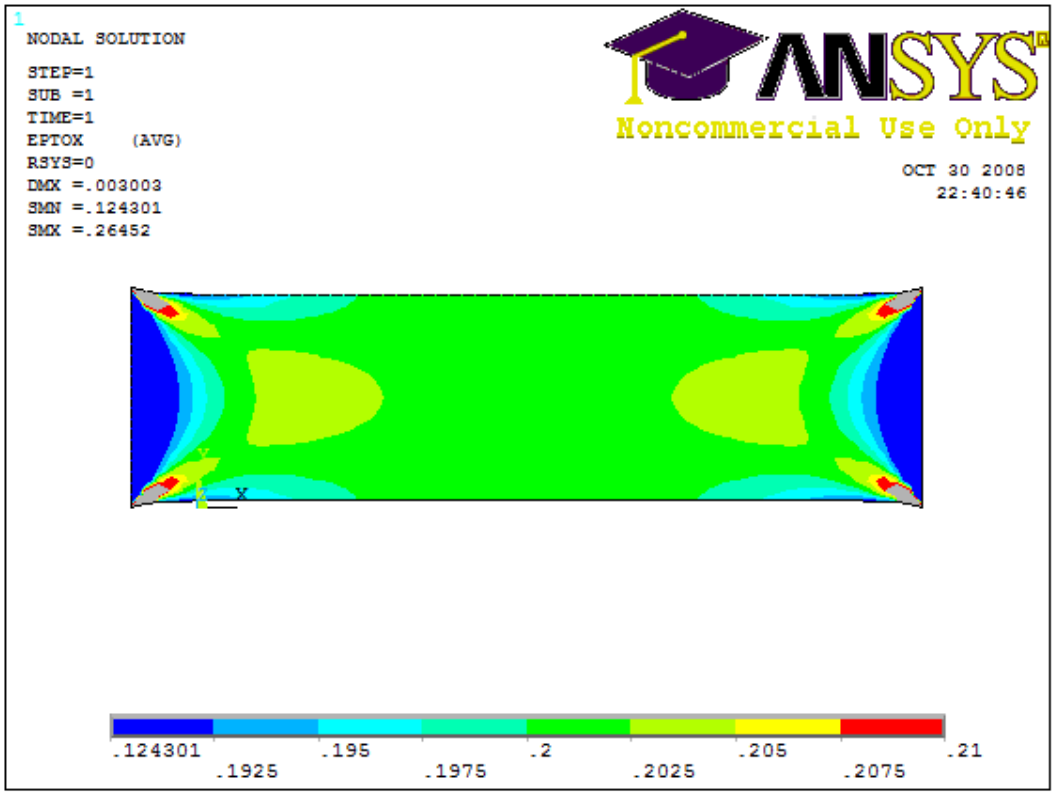


34. 2:1 UX-Strain



2:1 UY-Strain

Ratio 1:3



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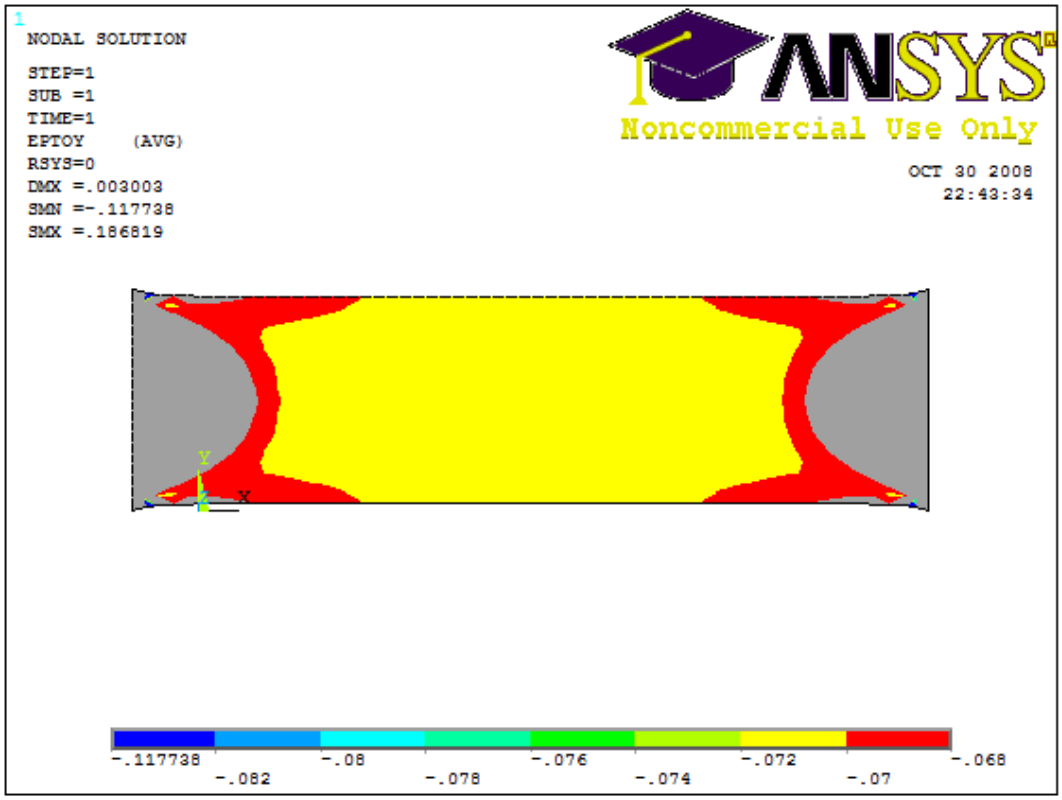
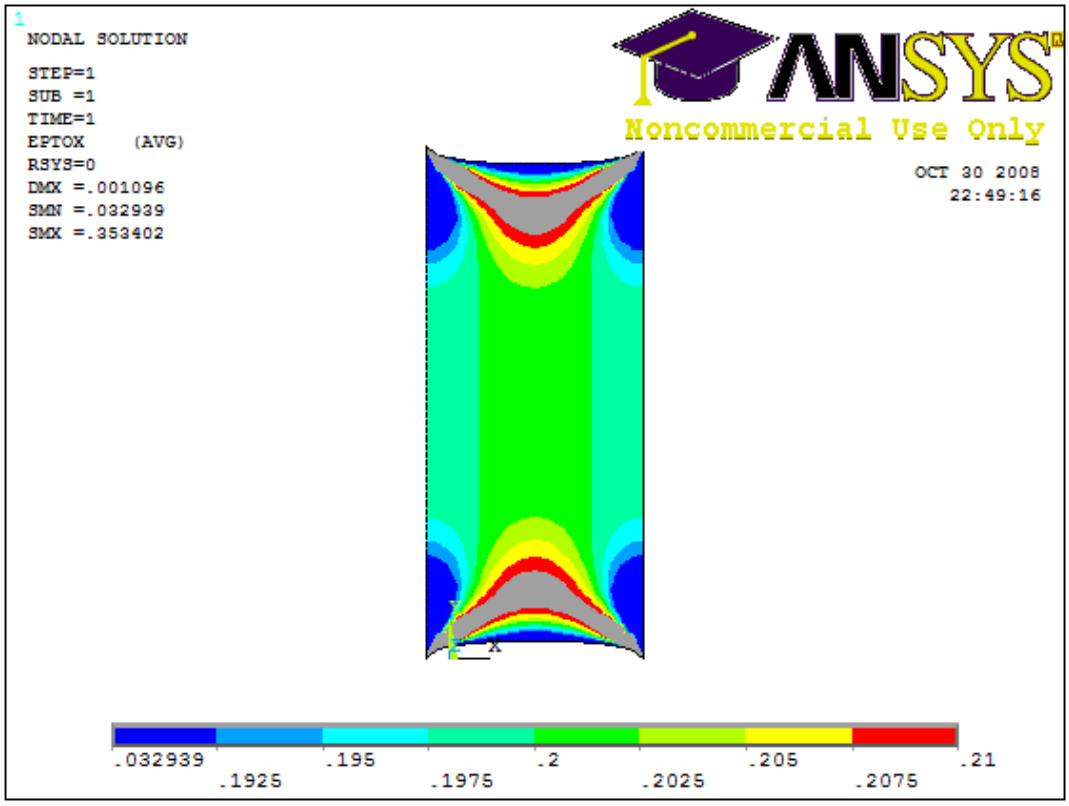
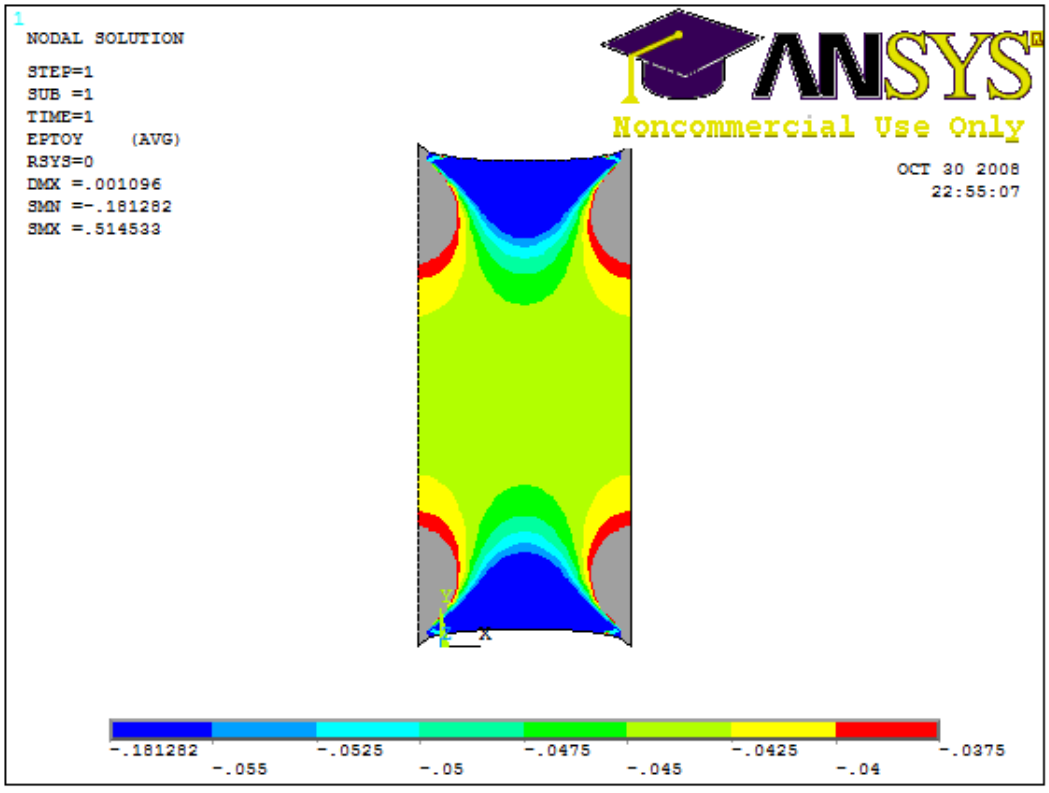


1:3 UY-Strain

Ratio 3:1

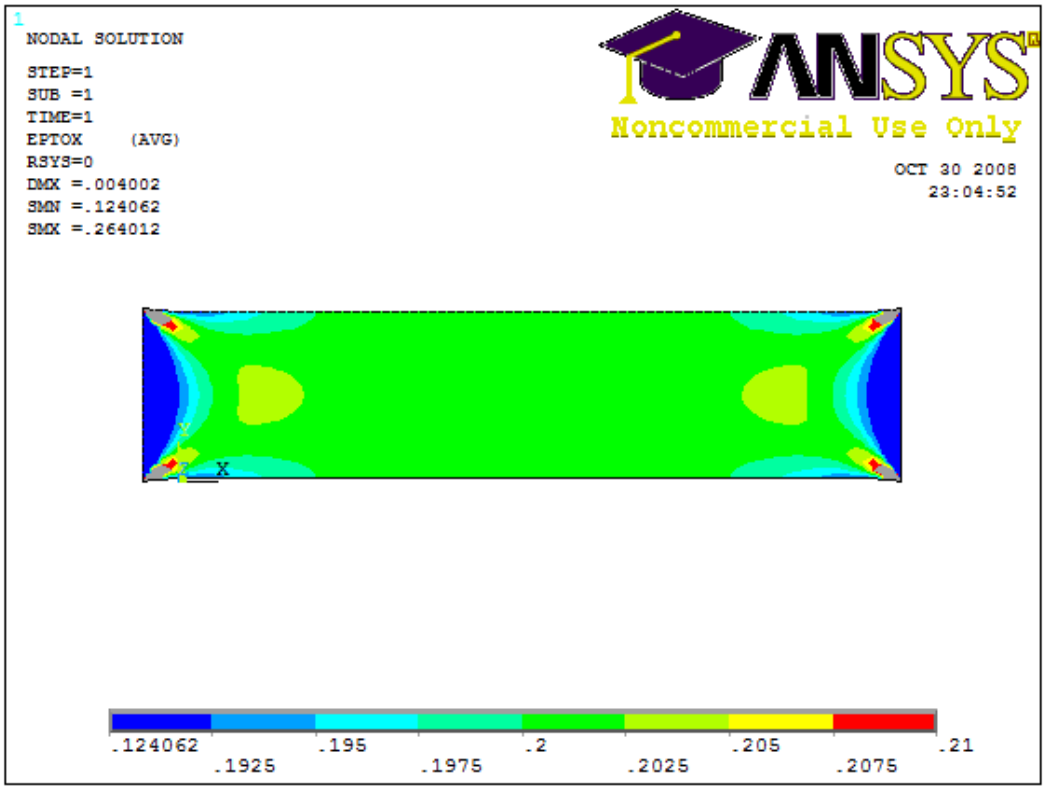


3:1 UX-Strain

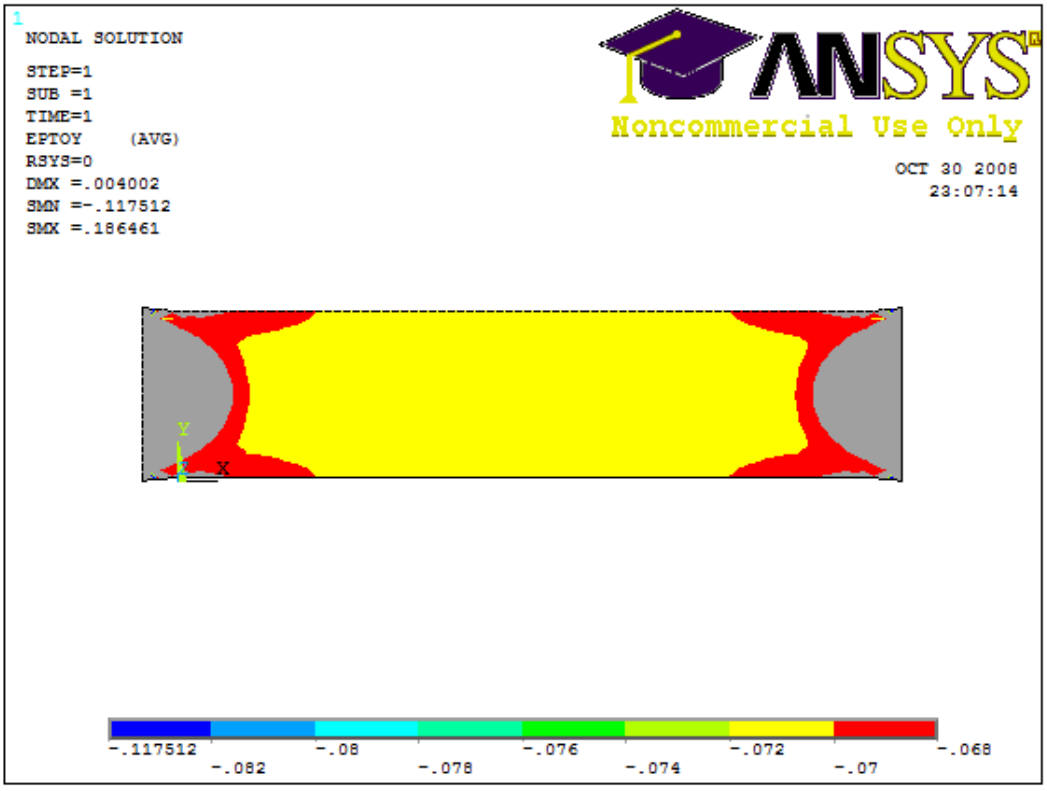


3:1 UY-Strain

Ratio 1:4

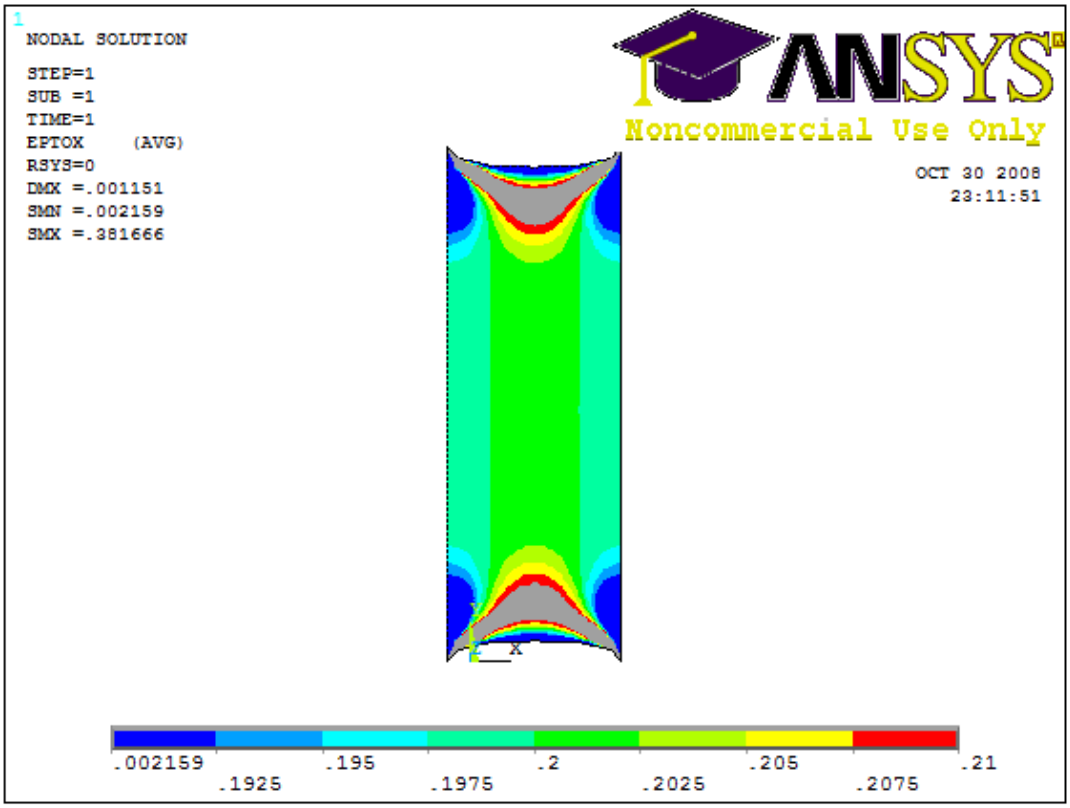


1:4 UX-Strain



1:4 UY-Strain

Ratio 4:1



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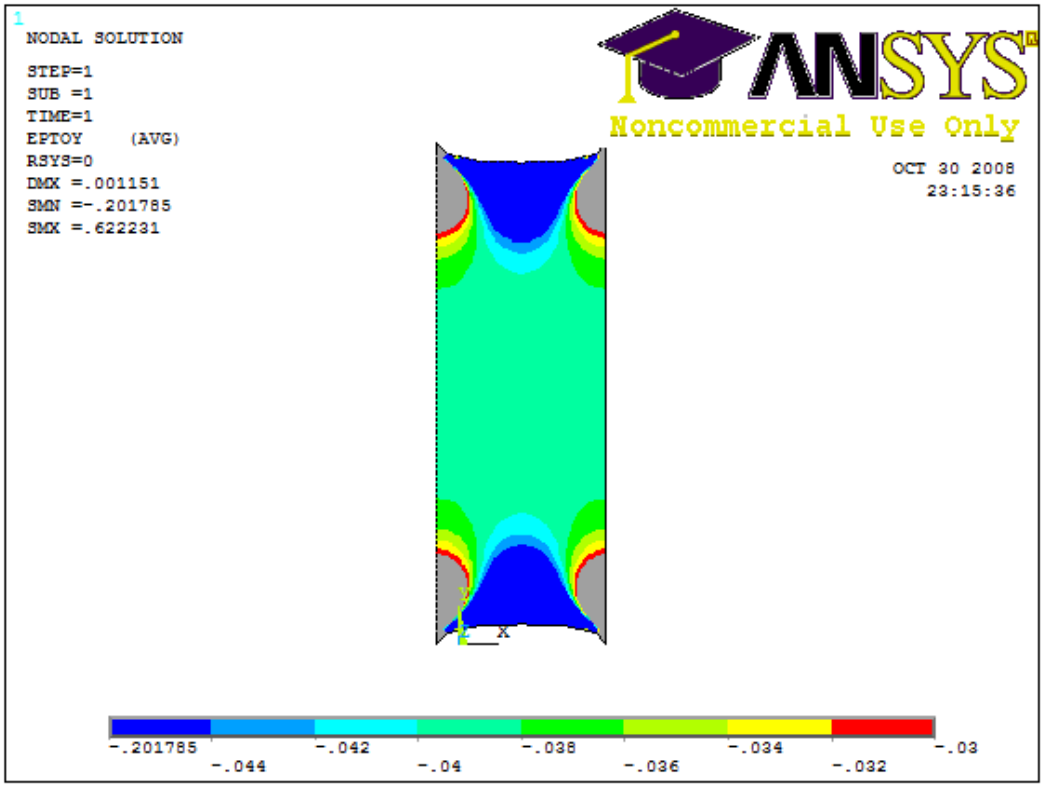
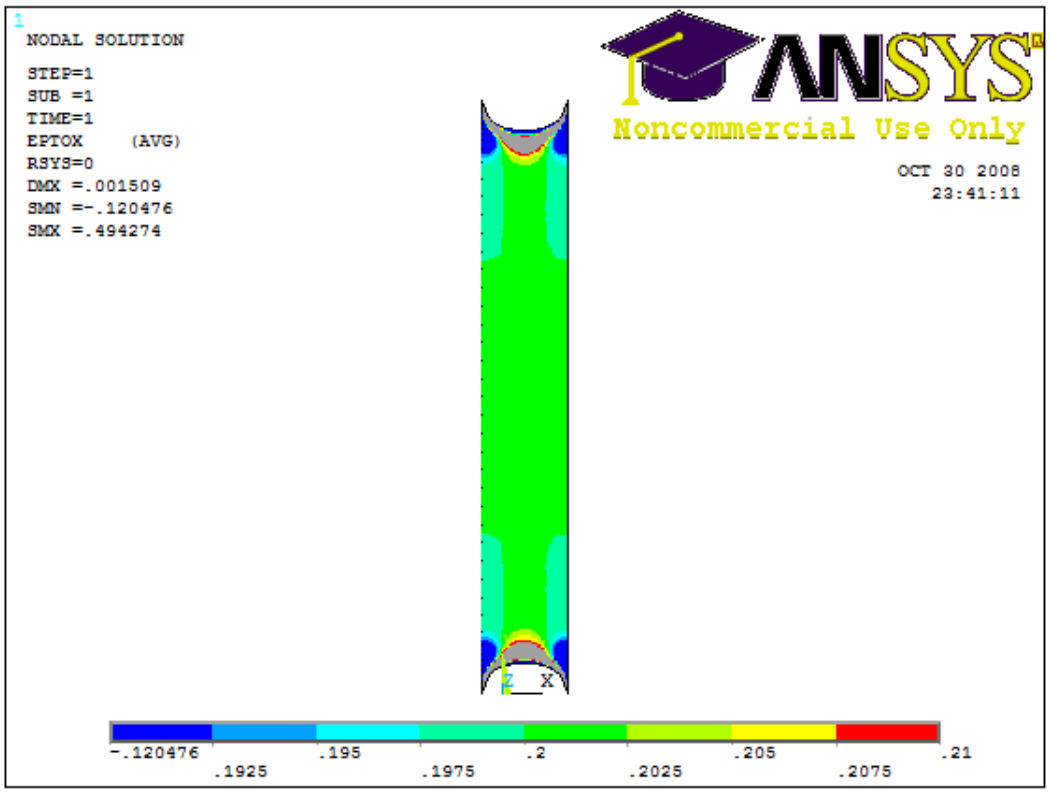
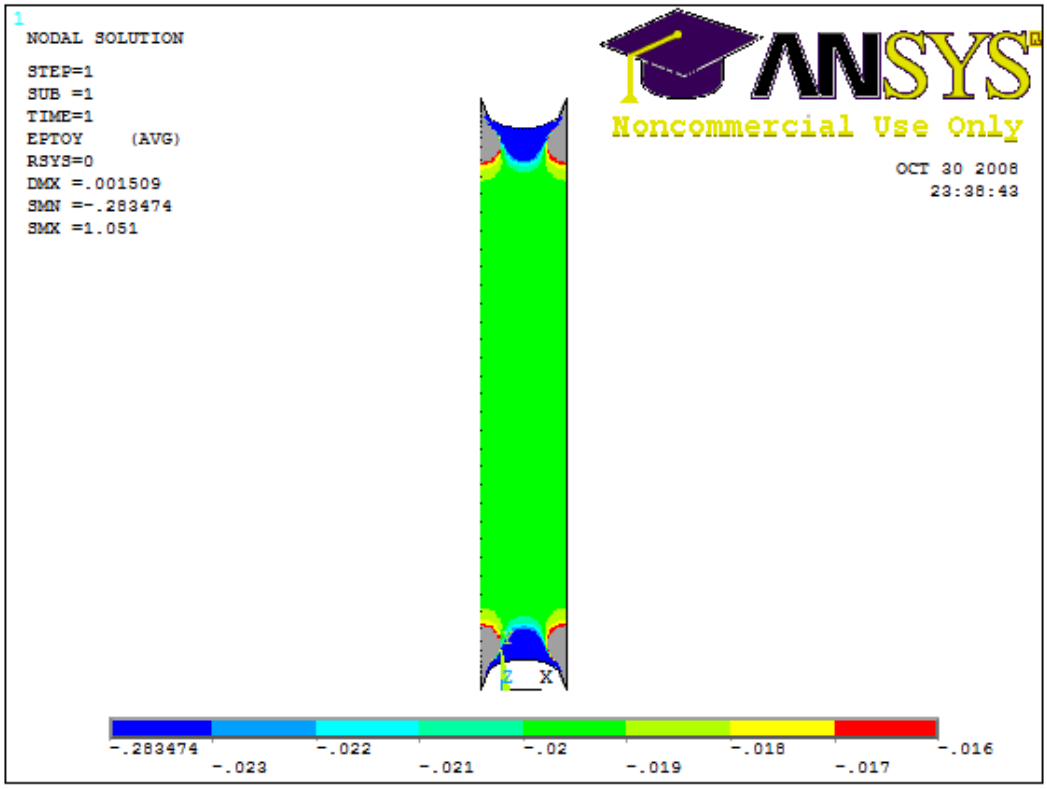


4:1 UY-Strain

Ratio 13:1

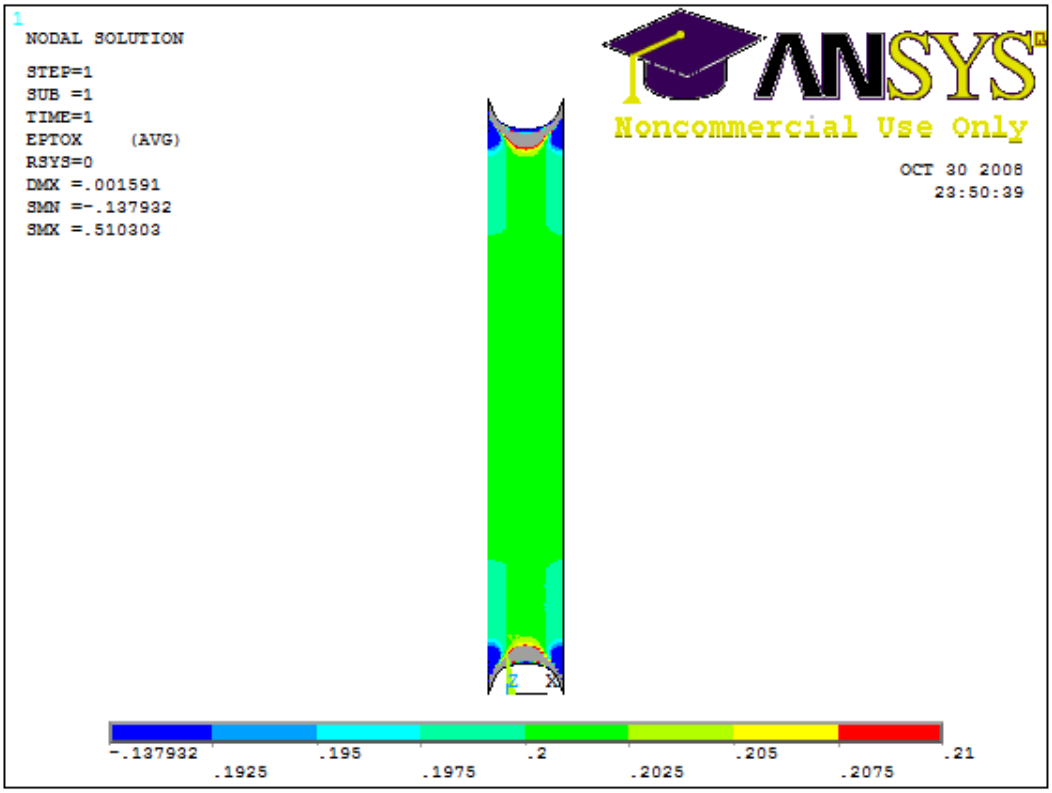


13:1 UX-Strain

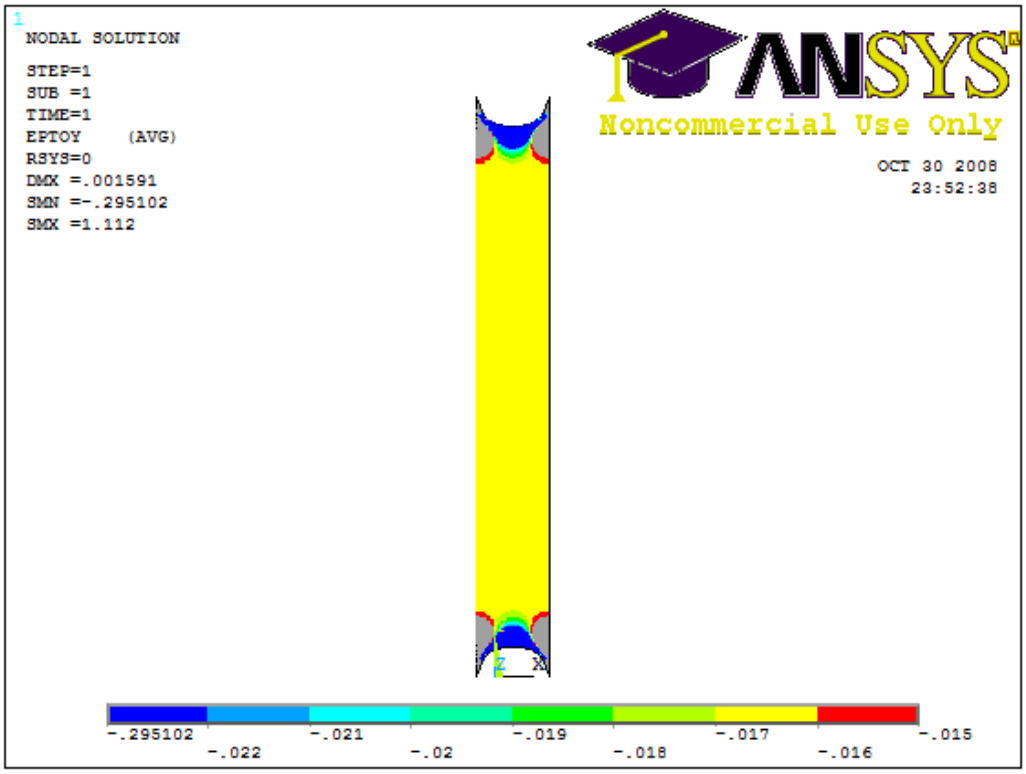


13:1 UY-Strain

Ratio 16:1



16:1 UX-Strain



16:1 UY-Strain

1/2 x [ ] (cm)	Usable Area* (cm^2)	Usable Area (%)	Center ε_y (%)	Norm. Width
Data 0.5	0.1135	45%	-6.9%	1
1	0.1105	22%	-5.5%	2
1.5	0.3575	48%	-4.3%	3
2	0.465	47%	-3.8%	4
2.5	0.93	74%	-3.4%	5
3	1.2	80%	-3.1%	6
3.5	1.5	86%	-2.9%	7
4	1.8	90%	-2.7%	8
4.5	2.07	92%	-2.5%	9
5	2.37	95%	-2.3%	10
1 x [ ] (cm)				
1	0.39	39%	-6.8%	1
2	0.6	30%	-5.3%	2
3	1.62	54%	-4.7%	3
4	2.64	66%	-3.9%	4
5	3.72	74%	-3.5%	5
6	4.8	80%	-3.2%	6
7	6	86%	-2.9%	7
8	7.2	90%	-2.7%	8
9	8.4	93%	-2.5%	9
10	9.6	96%	-2.3%	10
2 x [ ] (cm)				
2	1.5	38%	-6.9%	1
4	2.4	30%	-5.4%	2
6	6.48	54%	-4.7%	3
8	10.8	68%	-4.2%	4
10	15.36	77%	-3.8%	5
12	19.2	80%	-2.6%	6
14	24	86%	-2.4%	7
16	28.8	90%	-2.2%	8
18	33.6	93%	-2.0%	9
20	38.4	96%	-1.8%	10

Table 8. FEM Data

- Boxes highlighted red indicate a usable are < $1cm^2$
- Boxes highlighted green indicate approximate strip biaxial behavior.

Results

Formula Variable Definitions

- $W = Normalized\ Width$
- $\epsilon_y = Y\ Strain\ in\ Center$
- $A_u = Usable\ Area$
- $A_i = Initial\ Area$
- $\nu_{eff} = Effective\ Poisson\ Ratio$

0.5 cm Unit Length

$$\epsilon_y = 0.0183 \ln(\mathrm{W}) - 0.0647;\ R^2 = 0.992$$
$$A_u = 0.3359 A_i \ln(W) + 0.1218;\ R^2 = 0.8862$$
$$A_u = 0.3085W - 0.6765;\ R^2 = 0.9994$$
$$\nu_{eff} = -0.092 \ln(W) + 0.3235;\ R^2 = 0.992$$

1 cm Unit Length

$$\epsilon_y = 0.0184 \ln(\mathrm{W}) - 0.0655;\ R^2 = 0.9964$$
$$A_u = 0.2856 A_i \ln(W) + 0.2498;\ R^2 = 0.8977$$
$$A_u = 1.1814W - 2.1314;\ R^2 = 0.9998$$
$$\nu_{eff} = -0.092 \ln(W) + 0.3235;\ R^2 = 0.9964$$

2 cm Unit Length

$$\epsilon_y = 0.0186 \ln(\mathrm{W}) - 0.0644;\ R^2 = 0.8875$$
$$A_u = 0.3132 A_i \ln(W) + 0.2091;\ R^2 = 0.9144$$
$$A_u = 4.6904W - 8.825;\ R^2 = 0.9996$$
$$\nu_{eff} = -0.093 \ln(W) + 0.3219;\ R^2 = 0.8875$$

Critical Values

- $W_c = Area\ Critical\ Normalized\ Width$
  - Theoretical Normalized Width that yields 1cm^2 of Usable Area
- $W_{S1} = Threshold\ Strain\ Normalized\ Width$
  - Theoretical Normalized Width where ε_y = -2%
- $W_{S2} = Absolute\ Strain\ Normalized\ Width$
  - Theoretical Normalized Width where ε_y = 0

	W_c	W_{S1}	W_{S2}
0.5	5.31	11.27	32.85
1	2.49	11.44	32.7
2	1.81	8.94	20.51

Appendix F: Motor Controller Set Up and Programming

Set Up

The first step in interfacing with the controller is to download two programs. The first is the Allmotion EZcommander program. This can be found at http://www.allmotion.com/software_windows/software_windows.html. This program is very user friendly and easy to learn. The second is the USB driver in order to interface with the controller which can be found at http://www.allmotion.com/support.htm. A useful tool to have as well is the guide for programming the controller. This is titled the Command Set for the EZHR17EN, which can be found at the same site.Once these are downloaded and installed open the EZcommander program.

Programming

The best way the team found to understand the coding for the controller was to break down sample codes.

/1gP50000M1000D50000M1000G10R<CR>

All the codes for this controller must start with a /. Then after that the lowercase g means that the program will repeat, and that this is the beginning of the repeating code. The P50000 means that the motor will move 50,000 microsteps in the positive direction. The M1000 means that after moving in the positive direction it will pause for 1000 milliseconds, or 1 second before doing the next action. The D50000 means it will move the motor negative 50,000 microsteps. The G10R means that the code is over and that it should repeat 10 times before stopping.

/1s0gV20000AP12480D12480G10R<CR>

Below are some sample codes that the team put together throughout testing the controller. With each one is a basic explanation of what it does

Rotate back and forth with pause
/1gP50000M1000D50000M1000G10R<CR>

Save to controller when turned on rotate back and forth no pause
/1s0gP50000D50000G10R<CR>

Velocity control and movement
/1V50000P50000D50000R

Send Home
/1Z0R

Go .6 mm out
/1AD5249R

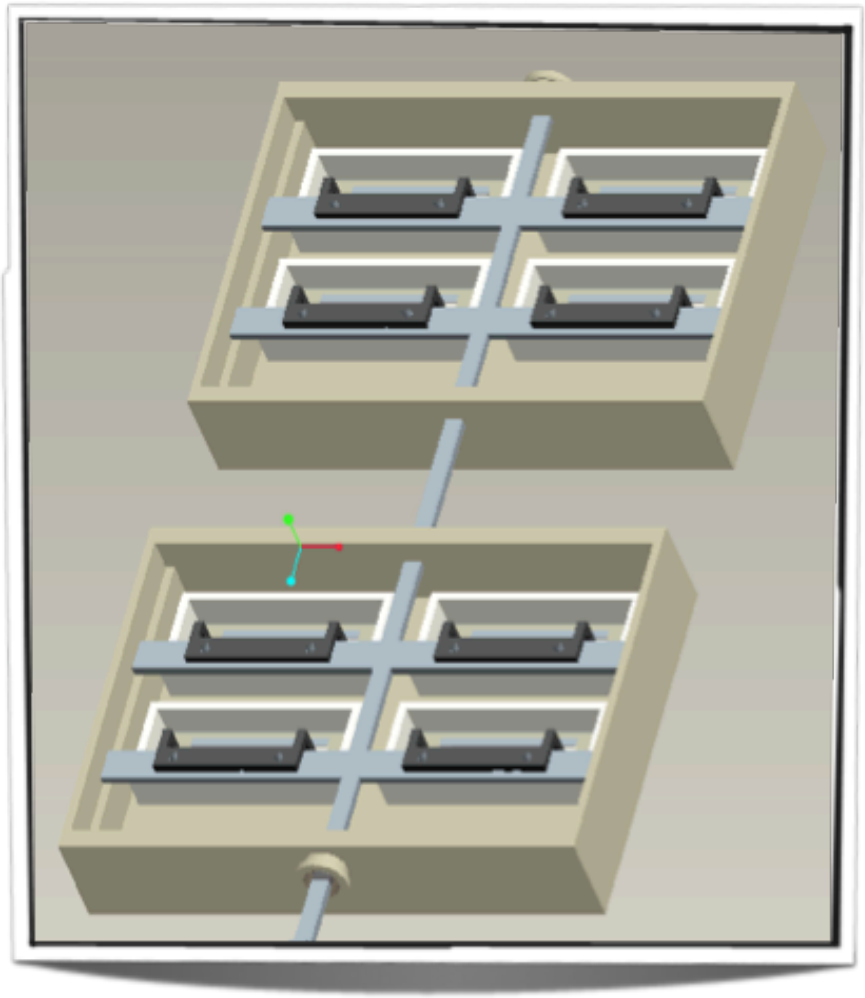
1 hz stretch 20% backward first repeat 10 times
/1V20000AP12480D12480G10R<CR>

Same as above w/ 3 second pause between each side
/1V20000AP10498M3000D10498M3000G10R<CR>

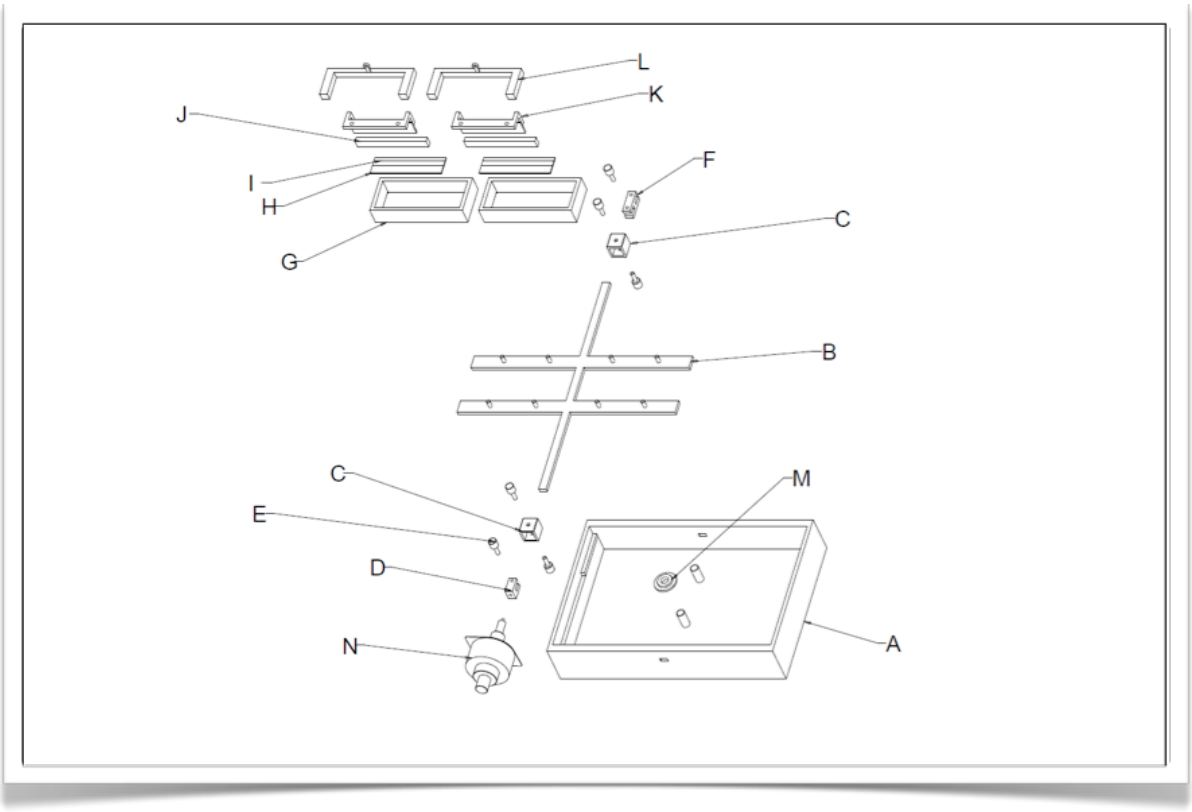
Appendix G: Polyacrylamide Curing Guide

Cyclic Stretch Device

User's Manual for Two Samples*



Andrew Kenoian
Derek Pepicelli
Ryan Rasmussen



Parts List

A. Device Case

B. Stretching Arm

C. Transition Chamber (x2)

D. Motor Coupling

E. Set Screw (x6)

F. Arm-to-Arm Coupling

G. Stretching Well (x2)

H. Porous Polyethylene, Wide (x2)

I. Porous Polyethylene, Narrow (x2)

J. Polyethylene Block

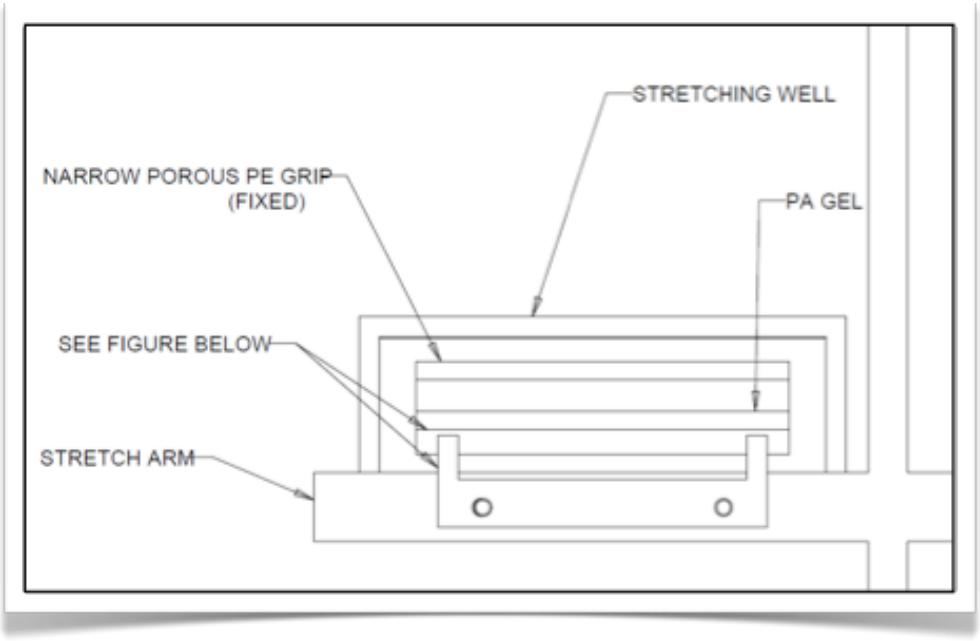
K. Glass-Backed Grip Arms (x2)

L. Casting Well (x2)

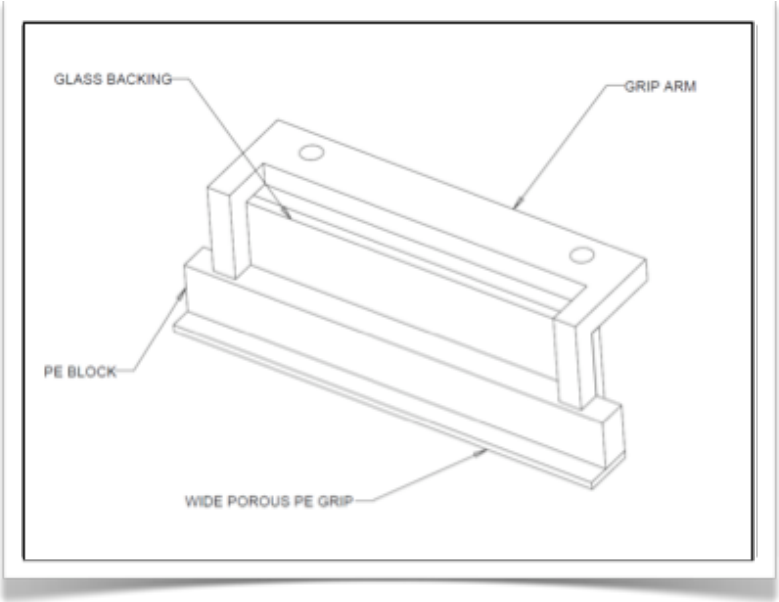
M. Locking Washer

Guide for Set Up

1. Autoclave necessary parts
    a. Case (A), Lid (N), Arm (B), 2 Chambers (C), and 2 Locking Washers (M) in one bag per case.
    b. Well kits of parts G-L in **one bag per well kit.**
2. Insert Casting Wells (L) into Stretching wells (G) so that they lie flush to one another.
3. Fix the narrow Polyethylene Grip (I) to the glass slide with a small amount of Silicone Adhesive. Position grip so that it is pressed against the long-glass wall of the Casting Well. Place into Well Slots in Case (A).



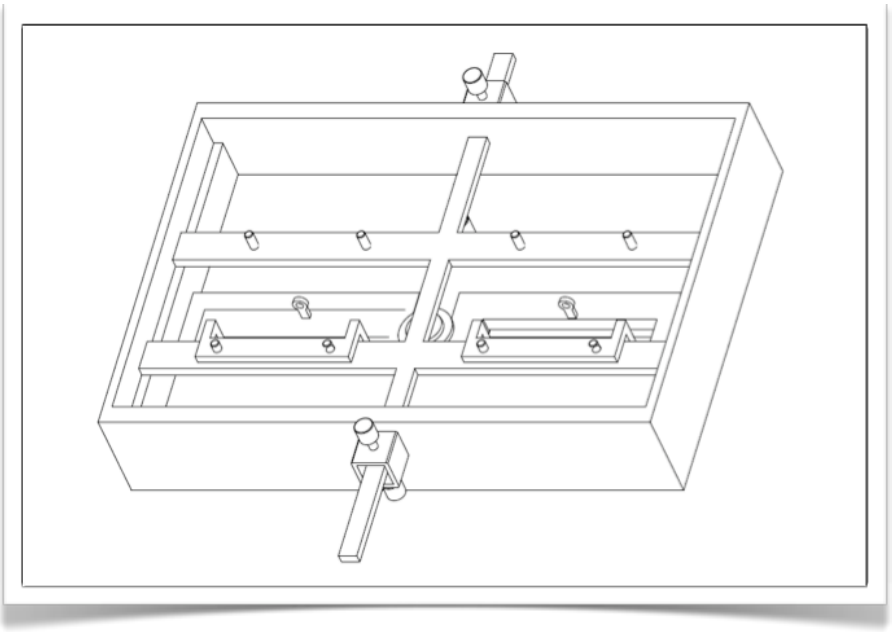
4. Tighten Locking Washer (M) down over every pair of wells.
5. Press PE Block (J) into the hooks in Grip Arm (K) until it touches the surface of the arm.
6. Glue wide Polyethylene Grip (H) to the bottom of Polyethylene Block (J).
7. Insert the end of Sliding Arm (B) through the holes in Case (A).
8. Tighten 4 Set Screws (E) through Transition Chambers (C) to lock Arm (B) in place.



9. Attach Grip Arms (K) to threaded standoffs on Arm (B) with hex-nuts.

10. Pre-soak Polyethylene Grips (H,I) with uncross-linked liquid PA and place entire unit in bell vacuum for five minutes. Discard excess fluid.

11. Add 2000 μL of cross-linked liquid PA and place in bell vacuum for ten minutes.
12. Remove Casting Well (L), glass cover slip, and any excess polyacrylamide.
13. Cells are to be seeded on the middle 2 cm of the gel.



14. Apply individual well lids and case lid. Tighten case clamps.

15. Couple Arm (B) to motor using Coupling and set screw (D, E).

16. Attach additional units to Arm (B) by Coupling and 2 set screws (F, E).

17. Carefully transport entire assembly to incubator.

18. Remove 4 Set Screws (E) from transition chambers and power the controller.