# A NOVEL IN VITRO STRETCH DEVICE FOR SIMULATING IN VIVO CONDITIONS

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To God Almighty, Mom and Dad, and my little Sister.

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## ABSTRACT

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Biological cells are constantly subjected to mechanical forces such as tension, compression and shear. The importance of these forces in mediating cell signals, maintenance of lineages, promoting embryonic cell differentiation and tissue engineering is only now coming into focus [1]. It has been shown that stretch stimulus can influence growth, differentiation, as well as tissue strength and integrity [2]. Most stretch systems built to understand more of these phenomena suffer from shortcomings, as accurately replicating the *in vivo* environment is quite challenging [3]. Many of the devices currently available are very expensive as well as limited to a single application. The objective of this thesis is to design, manufacture, test, and validate a novel uniaxial cyclic cell stretch device that overcomes most of the major limitations of existing systems, and to experimentally demostrate that uniaxial cyclic stretch causes a shift towards *in vivo* characteristics of smooth muscle cells. The stretch mechanism is driven by a single servo motor which makes its operation simple and straight forward. Coolworks Lite, a proprietary software of the servo motor supplier, is used to control the motor and LabVIEW is used to obtain feedback from the sensors. Validation for the stretch machine was done by evaluating the performance of the device against engineering requirements. Methods were suggested to improve shortcomings that were encountered. Also, the machine's unique design allows its extension to a biaxial stretch unit while keeping the same driver platform, a concept for which has been discussed and illustrated.

# 1. INTRODUCTION

## 1.1 Background

Biological cells are subjected to a variety of mechanical stimulations and many studies have demonstrated the importance of mechanical forces in regulating cell specific markers *in vivo*. Identifying the mechanisms that regulate these expressions is critical for understanding the cell genetic and physical properties in both normal and pathological conditions. However, due to the complexity of the *in vivo* environment, cells could not be accurately studied *in vitro*. Some of the preliminary studies show a difference in the expression level of specific genes when the cells are cultured in stationary conditions- they acquire a generic gene expression profile with a slight change in physical structure. For example, smooth muscle cells (SMCs), which are a major component of blood vessels, are constantly under cyclic tension due to pulsating blood flow. Vascular SMCs (VSMCs) have significant plasticity and can show reversible changes in phenotype in response to the local environment. The complexity of the *in vivo* environment has made the study of these cells *in vitro* very difficult. Some of the preliminary studies conducted show changes in the expression levels of smooth muscle specific genes when the cells are cultured in stationary conditions- they acquire a generic gene expression profile with a slight change in physical structure [13, 14]. It was therefore thought that if cells are cultured at *in vivo* conditions *in vitro*, and subjected to controlled mechanical loading (cyclic strains at various frequencies and time durations), they will retain the same level of gene expression as *in vivo* [4–12].

The aim of this thesis is to design and develop a novel uniaxial stretch device that will simulate *in vivo* environmental conditions *in vitro*. The device must stretch cells through direct mechanical loading, and must allows both culturing and stretching

of SMCs on the same unit, effectively reducing working time needed by researchers to complete each run. The petri dishes and membranes used must be commercially available, reducing costs per experiment. The type of substrate used must be determined by the end user. This can vary from non-biological substrates like silicone and PDMS to biological ones such as collagen. The size of the substrate must also be chosen by the user according to his/her cell plating needs.

## 1.2 Literature Review

Most biological cells are constantly subjected to mechanical forces such as tension, compression and shear, and its importance in mediating cell signals, maintenance of lineages, promoting embryonic cell differentiation and tissue engineering is only now coming into focus. Different cells in different parts of a living organism are sensitive to different mechanical stimuli that occur in their immediate environments. These mechanical stimuli influence cells in various ways, such as defining its function. Changes in these stimuli may cause a diseased state. Hence the study of mechanical forces on cells has become very important [15]. Cyclic uniaxial stress is one such dominant force, which has been shown to influence growth, differentiation, as well as tissue strength and integrity in a variety of cells, either by itself, or in addition to other forces and chemical factors. For example, vascular cells are continuously subjected to cyclic strain among others. As a result of these stimuli, during fetal development, vascular remodelling and angiogenesis, cells automatically align to form the most efficient configuration functionally, based on its environmental dynamics [16], while other studies have shown that cyclic stretch also affects cell proliferation [17], and in some cases may show results that replicate *in vivo* conditions exactly. Cells show that their phenotype, or genotype, or both may change when acted upon by mechanical stimulus similar to the ones found *in vivo*.In order to study these phenomena, devices were created to maintain *in vivo* conditions *in vitro*. And since the *in vivo* environment is a highly complex one to mimic, devices often fall short

of accurately reproducing these conditions. Many custom-made devices have been designed and built to stretch cells to observe these phenomena. Simple uniaxial deformation has been one of the most popular ways of studying cell biomechanics, as it is the easiest to replicate in a controlled environment. Some of the first devices used for longitudinal stretching of cells were based on a single cycle, static type stretch rather than cyclic [18, 19]. In time, additional parameters such as cyclic loading, duration of stretch were later added to gain as much control over the environment as possible. Today, almost all the devices made are PC based programmable units [3]. The three major types of driving units in these devices are motor-based, vacuum based and pressure based [20, 21]. Flexcell, EMS Cell Stretcher and StrexCell are three commercially available devices for *in vitro* uniaxial cell stretch applications, of which flexcell is the most popular and is widely used. Flexcell is a vacuum based stretch unit which utilizes a base plate with sealed cylindrical well-like units that house rectangular strips of stretchable membranes. Vacuum is applied from the bottom end of these cylinders, which in turn causes the membranes to stretch. A solid cylindrical object, whose diameter is less than that of the cylindrical housing, is placed just under the elastic membrane. This prevents the membrane from forming a downward U-shape when vacuum is applied and will deform along the top surface of the solid cylinder in a uniaxial direction. Strexcell is a motor based stretch device that uses a thick silicone membrane (40mm x 25mm x 10mm) that is secured on both sides. A rectangular cavity (20mm x 20mm) is made at the centre of the membrane, leaving a small thickness of 0.1 mm at the bottom of the well. This well is used to hold the cells and the biological fluid, while it is stretched using a motor. The stretching mechanism, driver and the silicone chamber are enclosed in a box that can be placed in an incubator in order to maintain necessary environmental conditions. The EMS cell stretch unit is another stretch device that is commercially available. This device is unique as it is mainly designed to be operated under a microscope for real time observation. It is a motor based device that stretches an elastic membrane on both sides in a uniaxial fashion, so that the center of the membrane does not move relative

to the lens of the microscope. The membrane used is bent and held in a U-shape so as to hold cells and media at the bottom of the channel. The clamps used to stretch the membrane are attached externally to a driver unit, which can be placed in an incubation chamber. Many successful experiments have been performed with these devices.

These systems, while offering very precise displacements, have a few drawbacks. Flexcell has certain areas where does not deliver or limits the user in terms of functionality and uniaxial stretch [22]. Substrate dimensions used are fixed. This restricts the user in terms of the number of cells he/she would want to use per experiment. The type of substrate is also fixed- only one type of substrate (with different biological coating options) is available. Since the substrates come pre- attached to the stretch plates, there is no other alternative for the end user. Strexcell has only 64 pre-set values that control strain ratio and strain frequency. Only one material, silicone, is available to the user for plating cells. The EMS cell stretcher has only one stretch chamber, as opposed to the more desirable multiple chambers, which will minimize experiments failing completely. The drawbacks mentioned above create a necessity for a novel stretch device that is able to address these issues. This can be accomplished by creating a device in which the user has the ability choose the substrate size and material, and can control the displacement, frequency and duration of stretch, atleast within a given range.

### 1.3 Deficiencies in Capstone Design Project

The uniaxial stretch device was first designed by a group of students as a part of their undergraduate capstone design project. The project is explained briefly in appendix A. From the suggestions made by the capstone design group, it is apparent that the deficiencies of design and working of the uniaxial stretch device must be addressed before it can function reliably. Since the recommendations are to change the most important parts of the device such as the clamping mechanism, the stretch

plate design, materials etc., it was deemed best to note down all deficiencies, establish customer needs, and re-design and evaluate the uniaxial stretch device using established design and evaluation practices. The capstone design project has three major deficiencies in terms of design, and other issues related to the device overall. Addressing these issues is very critical for coming up with a good design. The issues are as follows:

1. The clip design is not practical. Both hands are needed to fix the clips. This means that the user must need help with holding the membranes in place. Another concern is that all the assembled membranes may not have the same tension. Due to the nature of clamping, there are more chances of the membranes having a slack.

2. The design of the stretch plates is another issue. The number of membranes planned for this design is eight. This makes each membrane very small, which will be very cumbersome to handle while assembling them.

3. Mount sub frame is another part that needs to be redesigned. For the device to function properly, it is important that the stretch plates and petri be together. This is because the membranes need to be immersed in a biological fluid at all times. In the current design, the parts have to be separated in order to be assembled into the frame.

4. User should have the ability to control the driver electronically via programmable input parameters. The device must also be able to display control and feedback data to the user for monitoring.

## 1.4 Goal and Objectives of Current Thesis

The goal of this thesis is to redesign the uniaxial stretch device, manufacture a prototype and test to see if it works as intended. The device should utilize an elastic membrane chosen by the user as a substrate on which cells are plated. The membranes

should be assembled into a stretch mechanism connected to a driver, which stretches them uniaxially at a given stretch magnitude, frequency and duration. The device should be kept in an incubation chamber that imitates environmental conditions found *in vivo*. Following are the objectives of this thesis:

1. Design a uniaxial stretch device that utilizes a stretchable membrane of the customer's choice to cyclically stretch biological cells at a desired magnitude, frequency and duration of stretch.

2. Manufacture a prototype of the machine that meets all design criteria.

3. Determine if the device is working as intended.

4. Determine if the device functions as desired in a real world experimental setup.

# 2. DESIGN AND DEVELOPMENT OF THE NOVEL UNIAXIAL STRETCH MACHINE

The design of uniaxial stretch machine is based on the process developed by author David G. Ullman, from his book "The Mechanical Design Process". The process is explained briefly in appendix B.

## 2.1 Generating Customer Requirements and Engineering Specifications

### 2.1.1 Identifying the Customers

In order to generate customer requirements and engineering specifications, it is important to identify who the customers are, who will be buying the device, and who will be using it. Are they the same person/ institution? This will help understand in the establishment of customer base, which will, in turn, help identify and understand the design problem better. In this case, the customers are primarily research institutes and researchers who work on biological cells. Dr. Omar El- Mounayri, post doctorate fellow at the McEwen center for regenerative medicine, Ontario, Canada, had played the role of a researcher/ customer for gathering priliminary information for identifying and understanding the design problem. Dr. El- Mounayri works primarily on cell research, and has an interest in understanding the effect of various mechanical stimuli on biological cells. There are two major customers for the product: the buyer (research facility/ university) and the end user (researcher/ lab technician). Broadly, the general aspects that these customers will look for when buying are price, durability, ease of use, accurate repeatability of experiments, and adaptability of the device to the lab environment.

### 2.1.2 Understanding the Problem

The steps required for cell culture must be taken into consideration, as it will be an integral part of the process of cyclically stretching biological cells. Cell culture methods will have an influence on the design of, and materials used for, the stretch device. For example, cell plating on membranes is done only inside a standard commercial fume hood in order to provide a sterile environment. A commercially available pipette is used to pour cells onto the membrane, and the cells are observed under the microscope that can only accommodate limited sizes. These dimensions are the constraints around which the dimensions of the components of the stretch device depend. To identify which steps of the cell culture process affect component dimensions and other functions, an understanding of the process is important. Following is a brief step by step process for culturing cells:

1. A set of cells cultured in a flask that have reached confluency is taken for subculture in order to plate a specific number of cells for experimentation.

2. The biological media in the flask is removed by aspiration.

3. Dead cells and other debris is washed off by pipetting in a buffer solution (Phosphate Buffered Saline, or PBS), and later aspirating it.

4. The cells, currently adhered to the flask wall, are loosened with the help of a known quantity of cell dissociation reagent, namely trypsin. The volume is usually 3ml.

5. A known quantity of biological medium (Dulbeccos Modified Eagles Medium, or DMEM), whose volume is usually 4ml, is added to the flask. The solution is mixed thoroughly. The total volume of the fluids in the flask is now 7ml.

6. Out of this volume, a 10 $\mu L$ sample is taken for counting cells. The rest of the mixture is centrifuged at 2100 rpm for five minutes.

7. The 10 $\mu L$ sample is taken for cell counting using a hemocytometer. The number of cells in each grid is counted and averaged. This is the number of cells per 100nL. It is multiplied by 10000 and then again by 7 (total volume of the fluids present before centrifuging the cells), to get the number of cells in 7ml of fluids. Say the researcher, by trial and error, has previously established that the desired concentration is 1000000 cells in 1 ml of media, the following formula is used to calculate the volume of medium to be added to the cell palette, so that the density of the cells is at the desired concentration:

$$\frac{cell\ count}{resuspension\ volume} = \frac{1000000\ cells}{1\ mL\ media} \tag{2.1}$$

8. The number of cells per milliliter of media is now at the desired number of 1000000. The user may transfer this into a flask, or in the case of this particular application, a silicone membrane attached to a stretch device.

The idea is to seamlessly integrate the stretch device into the cell culture process, so that the user may use familiar methods and equipment. The customer has also given his idea of the overall design of the device and how the process of cyclically stretching cells may be implemented. These guidelines are listed below:

1. The stretch machine must be designed as two integrated units: unit 1 must be a portable assembly of membrane stretching mechanism and petri dish. This unit is hand held, and is compact enough to fit in a fume hood and an incubator. The second unit, unit 2, is an assembly of the base frame to hold unit 1 and a driver to provide uniaxial cyclic motion.

2. Membranes are prepared and assembled into unit 1 in a fume hood.

3. Cultured cells are plated onto the membranes with a pipette. The stretch mechanism must accommodate a pipette in order to do this. The petri dish holds biological medium below. The membranes must be submerged in the medium at all times.

4. Unit 1 is placed in an incubator for the cells to reach confluency. After 24 hours, unit 1 is placed under an inverted microscope for checking cell proliferation and death.

5. Unit 1 is assembled into unit 2 which is already setup in an incubator. The driver is connected to the stretch mechanism.

6. The desired stretch magnitude, frequency and duration are fed into the driver via a control software. Sensors are used to gain feedback on these parameters. It is more desirable if the sensor and driver data can be displayed in real time.

Based on these guidelines, customer requirements were developed for the stretch device, which are listed below:

1. Design unit 1 of the stretch device, which consists of a stretch mechanism holding the membranes, that will sit on top of a commercially available four- well petri dish. A support should hold the mechanism and petri dish together.

2. User must be able to sterilize components of the device that are in direct contact with the cells and media.

3. Device must be able to stretch the membrane at a given magnitude of stretch, frequency and duration.

4. An environment imitating biological conditions, i.e. *in vivo* conditions, must be provided for the cells at all times.

5. The elastic membrane must be held tightly so as to not cause any slip during cyclic stretch process.

6. Plating cells on the membranes should be done in a sterile environment.

7. The user must be able place the membrane and petri dish unit on an inverted microscope in order to view cells on the membrane.

8. Membrane and petri dish unit must be assembled into the driver unit as soon as possible.

9. User must be able to cyclically stretch the membrane for a desired amount of time.

10. Total number of steps to assemble the membrane unit into the driver unit and to start the experiment should be as low as possible to avoid death of cells.

11. Verify if the displacement across the membrane, for a given magnitude of stretch, is distributed evenly.

12. Total number of steps to disassemble the membranes for collecting cells must be as low as possible.

13. Shear stress experienced by the cells must be negligible to ensure that cyclic tension is the only major force in action.

### 2.1.3 Comparing Currently Available Devices to Customer Requirements

In order to develop quality engineering requirements, the designer must look at currently available devices in the market for the same application. The devices must be evaluated against the customer needs developed, which will indicate if there are any more features that need to be added. This evaluation will also help identify the pros and cons of each device, which may be used as benchmarks for improvement. Areas where each of the devices need improvement are as follows:

*Flexcell*

- Substrate material and size cannot be customized.
- There is no built in environmental control. An external incubator must be used.
- Cells cannot be viewed under a microscope.

*EMS Cell Stretcher*

- Does not have a built in environment controller. An external incubator must be used.

*Strexcell*

- Substrate material and size cannot be customized. Only one material is available.
- Only one of 64 preset combinations of stretch magnitude, frequency and duration can be used.
- Does not have a built in environment controller. An external incubator must be used.

It may be observed that the most popular device on the list, Flexcell, has the least overall score, but is consistent with the scores for individual tasks. This shows that in order to build a successful device, all aspects of its working must be satisfactory. The evaluation is shown in the table below:

Table 2.1. : Current Devices in the Market- A Comparison to Customer Needs

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Choosing a substrate of choice | Substrate material cannot be customized. Only one material available with different coatings | 2 | The membrane can be customized depending on the user's needs | 4 | Substrate material cannot be customized. Only one material available | 1 |
| Sterilize components | Components can be sterilized by steam | 5 | Components can be sterilized by steam | 5 | Components can be sterilized by steam | 5 |
| Specify magnitude, frequency and duration of stretch | All three parameters can be used to control stretch | 5 | All three parameters can be used to control stretch | 5 | Only 64 preset combinations of magnitude, frequency and duration can be used | 2 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Provide *in vivo* conditions for cells at all times | Does not have a built in environment control. Must be placed in an incubator | 1 | Does not have a built in environment control. Must be placed in an incubator | 1 | Does not have a built in environment control. Must be placed in an incubator | 1 |
| There must be no slip of membrane at the clamps during stretch | Literature reviews do not mention any slip of membrane during experiments | 5 | No literature found, but membrane is clamped between two metal blocks secured with screws, so it safe to assume there will be no slip | 4 | Membranes have holes on four sides that are inserted into pins. No slip by design | 5 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| All components needed for cell plating and for assembling stretch unit must fit in a fume hood | All components are portable enough to work with in a fume hood | 4 | All components are portable enough to work with in a fume hood | 4 | All components are portable enough to work with in a fume hood | 4 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Stretch assembly must fit on a commercially available inverted microscope stage | Cells cannot be viewed under an inverted microscope after assembling the stretch unit | 1 | Cells can be viewed under an inverted microscope after assembling the stretch unit | 5 | Cells can be viewed under an inverted microscope after assembling the stretch plate | 5 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Stretch unit must be assembled into the driver unit as fast as possible | Very easy to connect stretch unit to driver | 5 | Very easy to connect stretch unit to driver | 5 | Very easy to connect stretch unit to driver | 5 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| The stretch unit must be assembled into the driver and the experiment must be started as soon as possible | Only few steps are required to assembled stretch unit to driver | 4 | Only few steps are required to assembled stretch unit to driver | 4 | Only few steps are required to assembled stretch unit to driver | 4 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Verify if stretch across the membrane is distributed evenly during operation | Stretch is distributed evenly across the length of the membrane, and lateral compression also exists along the edges | 4 | Stretch is distributed evenly across the length of the membrane | 5 | Stretch is distributed evenly across the length of the membrane | 5 |

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Table 2.1. : *continued*

| Customer Need | Flexcell | Score | EMS Cell Stretcher | Score | Strexcell | Score |
|---|---|---|---|---|---|---|
| Membranes must be disassembled after the experiment as soon as possible | Very few steps are needed to remove membranes | 4 | Very few steps are needed to remove membranes | 4 | Very few steps are needed to remove membranes | 4 |
| Shear stress experienced by the cells must be negligible | No information available | - | No information available | - | No information available | - |
| Total Score | | 40 | | 46 | - | 41 |

### 2.1.4 Developing Engineering Specifications

Engineering specifications are developed based on customer requirements. While customer requirements indicate 'what' needs to be done, engineering specifications indicate 'how' the customer requirements are going to be met. Engineering specifications are only measurable end goals that have units, and must not include specific methods and concepts. Table below shows the engineering specifications developed for each customer requirement.

Table 2.2. : Generating Engineering Specifications from Customer Requirements

| Customer Requirements | Engineering Specifications |
|---|---|
| 1. User must be able to use an elastic membrane as a substrate, the dimensions and material of which, is of his/ her choosing | 1. Dimensions of membrane relative to the dimensions of the petri dish well |
| | 2. Elasticity of the membrane |
| | 3. Thickness of the membrane |
| 2. User must be able to sterilize components of the device that are in direct contact with the cells and media | 4. Melting point of materials used |
| 3. Device must be able to stretch membrane at a given magnitude of stretch, frequency and duration | 5. Stretch magnitude range |
| | 6. Frequency range of cyclic stretch |
| | 7. Duration of stretch range |
| 4. An environment imitating biological conditions, i.e. *in vivo* conditions, must be provided for the cells at all times | 8. Temperature, atmospheric gases and humidity levels |

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Table 2.2. : *continued*

| Customer Requirements | Engineering Specifications |
|---|---|
| 5. The elastic membrane must be held tightly so as to not cause any slip during cyclic stretch process | 9. Slip, in relation to % strain |
| 6. Plating cells on the membranes should be done in a sterile environment | 10. Worksurface area needed |
| 7. The user must be able place the membrane and petri dish unit on an inverted microscope in order to view cells on the membrane | 11. Dimensions of the membrane and petri dish unit in relation to microscope stage |
| 8. Membrane and petri dish unit must be assembled into the driver unit as soon as possible | 12. Steps to assemble stretch unit into the driver unit |
| 9. Total number of steps to assemble the membrane unit into the driver unit and to start the experiment should be as low as possible to avoid death of cells | 13. Number of steps to complete assembly and start experiment |
| 10. Verify if the displacement across the membrane, for a given magnitude of stretch, is distributed evenly | 14. Difference in actual displacement across the membrane in relation to displacement applied |
| 11. Total number of steps to disassemble the membranes for collecting cells must be as low as possible | 15. Number of steps to disassemble membranes after experiment |

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Table 2.2. : *continued*

| Customer Requirements | Engineering Specifications |
|---|---|
| 12. Shear stress experienced by the cells must be negligible to ensure that cyclic tension is the only major force in action | 16. Shear stress on cells |

Table 2.3. : Engineering Specifications- Explanations, Units and Target Values

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Dimensions of membrane relative to the dimensions of the petri dish well. | Optimal length and width of the membrane relative to a petri dish well is determined by trial and error taking into consideration the ease of handling by the user. The lesser the better. | % | 50 / 70 |
| Elasticity of the membrane | Membrane must be able to stretch atleast 20% of its length without any permanent deformation. The lesser the better. | MPa | 0.1 / 5 |
| Thickness of the membranes | Maximum allowable thickness of the membrane that can fit in the clamping mechanism designed | mm | 0.05 / 2 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Melting point of materials used for stretch unit | Material property data sheet from manufacturer. The higher the better. | degrees C | 120 / 150 |
| Stretch magnitude range | The device must be able to stretch a 50mm long membrane for atleast 20% of its length. The higher the better. | mm | 6 / 20 |
| Frequency range of cyclic stretch | The device must be able to provide the general levels of frequency found *in vivo*, i.e. 1 - 5 Hz. The higher the better. | Hz | 1 / 5 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Duration of stretch | The device must be able to provide stretch for ten hours as most cells stop changing orientation after 6 to 8 hours of stretch. The higher the better. | Hours | 6 / 10 |
| Temperature, atmospheric gases and humidity levels | A commercial incubator is used to maintain atmospheric conditions | degrees C, % carbon di oxide, % humidity | 120, 5, 90 |
| Slip, in relation to displacement applied | Membrane must be held without any slip. A tolerance may be fixed at 5% of displacement applied. The lower the better. | % | 0 / 5 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Work surface area needed | The parts needed to assemble membranes into the stretch unit and plate cells must fit on the work surface of the smallest commercially available fume hood. The lower the better. | in x in | 20x20 / 30x30 |
| Dimensions of stretch unit in relation to area of an inverted microscope stage | Dimensions of the stretch unit must be smaller than the microscope stage for stability. The lower the better. | % of area | 70 / 100 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Steps to assemble the stretch unit into the driver unit | This must be done in the least number of steps theoretically possible in order to prevent cell death as they are not in an incubator during this period. The lower the better. | Number (N) | 3 / 8 |
| Number of steps to complete assembly and start the experiment | This must also be done in the least number of steps theoretically possible. The lower the better. | Number (N) | 5 / 10 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Diffference in displacement across the membrane | It is ideal that all cells experience the same magnitude of displacement applied, all across the membrane. Difference may be measured as a % of % stretch applied to the membrane. The lower the better | % | 0 / 5 |
| Number of steps to disassemble the membrane after experimentation | This step must be completed in as few steps as possible as cells must be collected for further analysis. The lower the better. | Number (N) | 6 / 11 |

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Table 2.3. : *continued*

| Engineering Specifications | Explanation | Unit | Min/ Max Target Value |
|---|---|---|---|
| Shear stress on cells | During stretch, the biological media is pushed around on the membrane, causing the cells to experience shear stress. According to literature, for shear stress to be negligible, it must be less that 1 Dyne-s/sq.cm | Dyne-s/sq.cm | 0 / 1 |

### 2.1.5 The House of Quality

The house of quality is an arrangement of all currently available information after developing engineering specifications. This arrangement allows the designer to evaluate various relationships between the customer requirements, engineering specifications and current competition in the market. He/ She will be able to compare and prioritize which aspects to value most. To start with, customer requirements are listed down, and their relative importances against each other (which should add upto 100) is noted beside them. Engineering specifiactions are written in a single row as shown in figure 2.1. Each customer requirement is now compared to all engineering specifications, and any relation between them strong = 9, medium = 3, and weak = 1 is noted. The relation values below each engineering specification is multiplied to its corresponding customer requirement value, and the totals are noted. After calculating the individual totals for all engineering specifications, they are added, and each individual total is divided by the total sum. This gives the importance of each engineering specification, enabling the designer to be able to prioritize which specification to spend more time on. The available competition is listed to the right side of the table, and are rated from 1 to 5 against customer requirements. Also, the values for each engineering requirement are noted. Based on the available data, target (customer is delighted) and threshold (customer is disgusted) values are generated. The last step in the house of quality is to see if any of the engineering specifications are related to each other. This is important for two reasons: It tells the designer which specifications to work on first, and if a specification has a detrimental/incremental effect on another specification/s. From figures 2.1 and 2.2, the following may be concluded:

- Slip occuring in the membrane, maintaining desired atmospheric conditions, stretch magnitude and frequency ranges are the most important engineering specifications, in that order.

- There are limitations in all of the currently available devices.

- An increase in frequency of stretch will increase shear stress experienced due to movement of media by the cells.
- Work surface area needed to assemble parts will increase if there is an increase in the dimensions of the membrane.
- If length of the membrane is increased, stretch magnitude range available will decrease, as there is limited space.

| Θ = 9 Δ = 1 O = 3 | Cell Researcher | Dimensions of the membrane relative to the dimensions of the petri dish | Elasticity of the membrane | Thickness of the membrane | Melting point of the materials used | Stretch magnitude range | Frequency range of cyclic stretch | Duration of stretch range | Temperature, atmospheric gases and humidity levels | Slip, in relation to % strain | Work surface area needed for assembling membrane | Dimensions of the membrane and petri dish unit in relation to microscope stage | Steps to assemble stretch unit into driver unit | Number of steps to complete assembly and start experiment | Difference in actual strain across the membrane in relation to % strain applied | Number of steps to disassemble the membrane after the experiment | Shear stress on cells | Very Bad = 1 Very good = 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Direction of Improvement | ↑ | ↓ | ↓ | ↑ | ↑ | ↑ | ↑ | ! | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | Flexcell | EMS Cell Stretch | Strexcell |
| | Unit of measure | mm | GPa | mm | °C | % | Hz | s | °C, %, % | % | sq. in | % | N | N | % | N | Dyne-s/ sq.cm | | | |
| | Impotance | | | | | | | | | | | | | | | | | | | |
| Choose elastic substrate of choice | 8 | Θ | Θ | Θ | O | | | | | | | | | | | | | 2 | 3 | 1 |
| Sterilize components of the device | 8 | | | | Θ | | | | | | | | | | | | | 5 | 5 | 5 |
| Device must stretch at a given magnitude of stretch, frequency and duration | 12 | O | | | | Θ | Θ | Θ | | Δ | | | | | Δ | | | 5 | 5 | 1 |
| Environment imitating *in vivo* conditions | 12 | | | | | | | | Θ | | | | | | | | | 1 | 1 | 1 |
| No slip of elastic membrane during the stretch | 10 | | Δ | Δ | | O | | | | Θ | | | | | | | | 5 | 4 | 5 |
| Plating cells on to the membranes should be done in a sterile environment | 7 | | | | | | | | O | | Θ | | Δ | Δ | | | | 5 | 5 | 5 |
| Stretch unit must fit on to an inverted microscope stage | 3 | O | | | | | | | | | | Θ | | | | | | 1 | 5 | 4 |
| Stretch unit must be assembled into the driver unit as soon as possible | 8 | | | | | | | | Δ | | | | Θ | O | | | | 4 | 4 | 4 |
| Experimentation must be started as soon as possible | 8 | | | | | | | | Δ | | | | O | Θ | | | | 3 | 3 | 4 |
| Verify if the strain across the membrane is distributed evenly during stretch | 11 | | | | | | | | | O | | | | | Θ | | | 2 | 2 | 2 |
| Number of steps to disassemble the membranes must be as low as possible | 6 | | | | | | | | | | | | O | O | | Θ | | 2 | 3 | 5 |
| Shear stress experienced by the cells must be negligible | 7 | | | | | | O | | | Δ | | | | | | | Θ | N/A | N/A | N/A |
| Importance | | 7.1 | 5.5 | 5.5 | 6.5 | 9.3 | 8.7 | 7.3 | 9.8 | 10.4 | 4.2 | 1.8 | 8.1 | 8.1 | 7.5 | 3.6 | 4.2 | | | |
| Flexcell | | 38.3 | 0.05 | 0.254 | -106 | 12 | 5 | 18.2 | N/A | 0 | 27 | N/A | 6 | 10 | 0 | 6 | N/A | | | |
| EMS Cell Stretcher | | 100 | 0.8 | 0.127 | -40 | 80 | N/A | N/A | N/A | 0 | 6 | 185 | 4 | 7 | 0 | 4 | N/A | | | |
| Strexcell | | 40 | 0.05 | 0.1 | -106 | 20 | 1 | 336 | N/A | 0 | 6 | 38 | 2 | 5 | 0 | 3 | N/A | | | |
| Target (Delighted) | | 70 | 0.01 | 0.01 | 150 | 20 | 5 | 24 | N/A | 0 | 20 | 70 | 3 | 5 | 0 | 3 | 0 | | | |
| Threshold (Disgusted) | | 50 | 4 | 2 | 120 | 5 | 0 | 6 | N/A | 5 | 30 | 100 | 5 | 7 | 10 | 5 | 1 | | | |

Figure 2.1. : This figure shows the main body of the house of quality

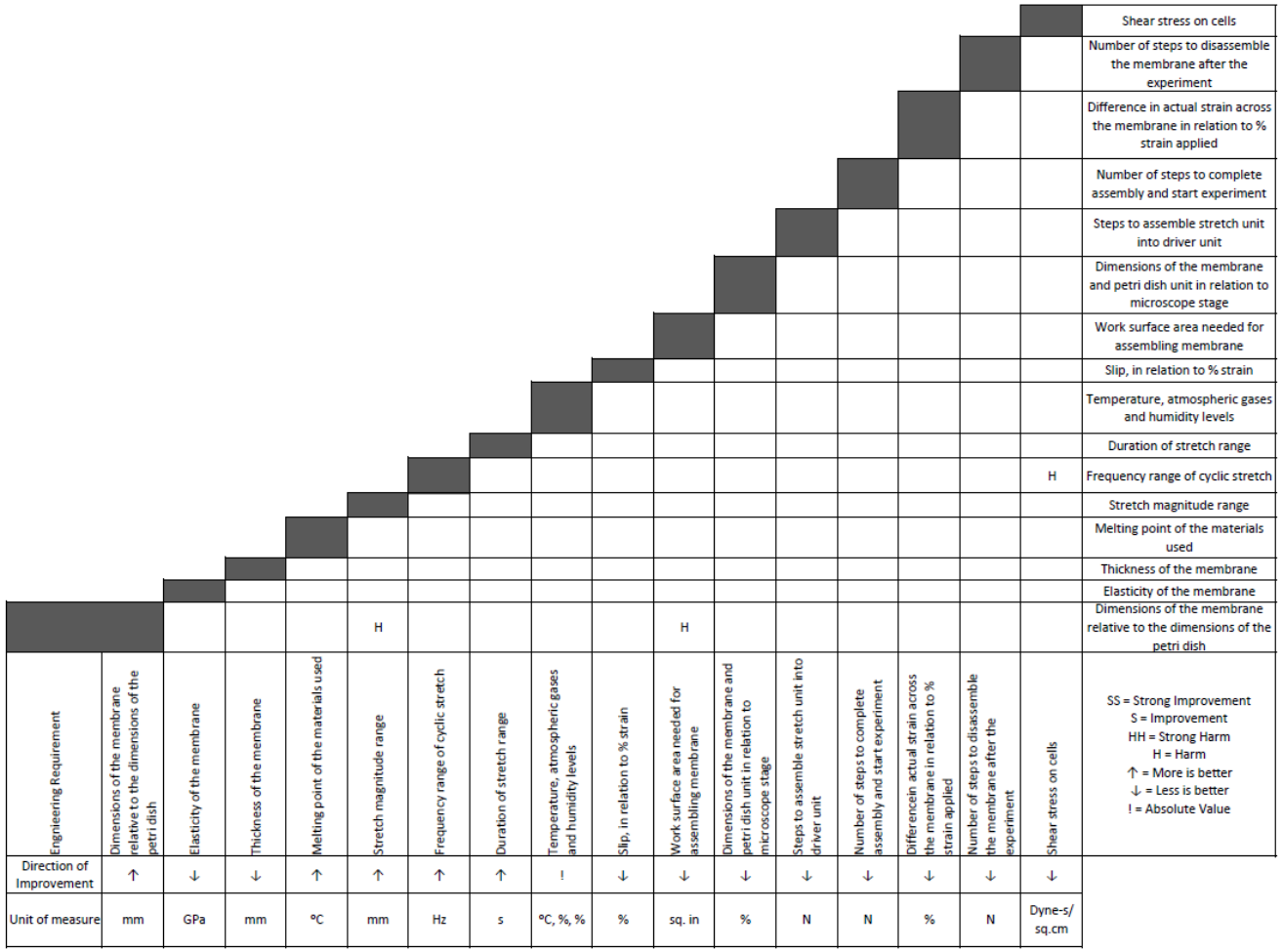



Figure 2.2. : This figure shows the roof of the house of quality

## 2.2 Concept Generation and Evaluation

### 2.2.1 Developing Functional Decomposition

Functional decomposition is describing the working of the product being designed in terms of its functionality. A single function is first developed, and then this function is further divided into sub functions, sub-sub functions and so on, until a function can no longer be split into smaller functions. For the uniaxial stretch device, the main function is to *cyclically stretch elastic membrane in a uniaxial direction.* This main function may be further divided into 11 sub-functions, as illustrated in figure 2.3, and as listed below:

1. Assemble stretch unit.
2. Transfer cells and media to membrane.
3. Transport stretch unit to incubator.
4. Supply *in vivo* environment.
5. Transport stretch unit to inverted microscope.
6. Observe cells for confluency.
7. Transport stretch unit to driver base.
8. Assemble stretch unit to driver base.
9. Supply *in vivo* environment.
10. Move membrane uniaxially.
11. Supply feedback to user.

Each of the sub- functions are now considered for further division into sub- sub- functions. The first sub function, assembling the unit, can be divided further into four sub- sub- functions, and two of these sub- sub- functions can be further divided

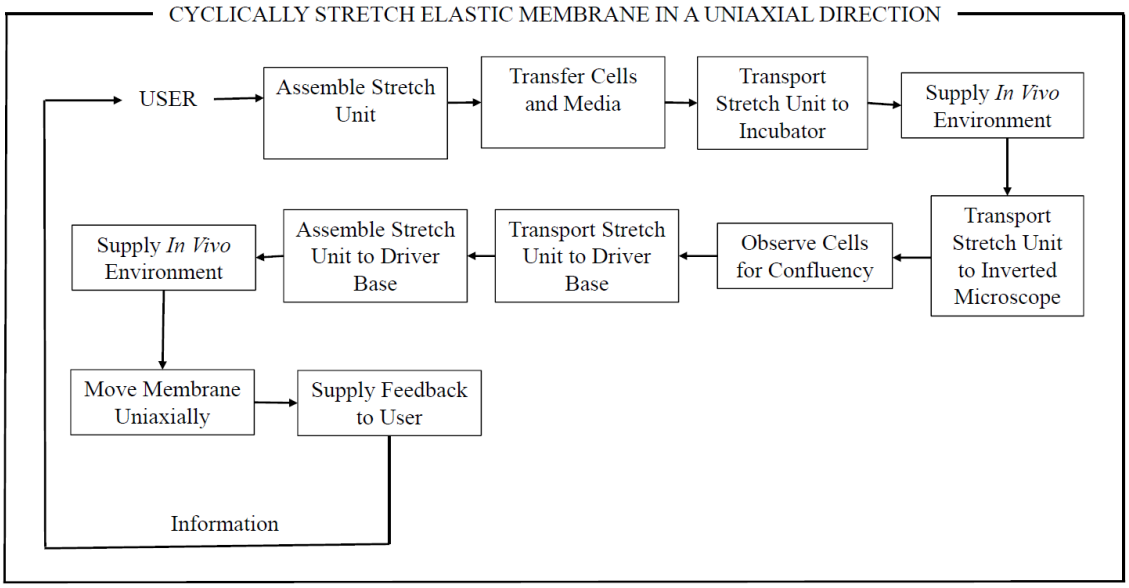



Figure 2.3. : This figure shows the functional decomposition for the uniaxial stretch machine. The overall and individual fucnctions in order of execution are described. This method helps the designer to better understand the individual tasks needed to accomplish the overall function. Each individual function is then analyzed and concepts are generated to implement the function.

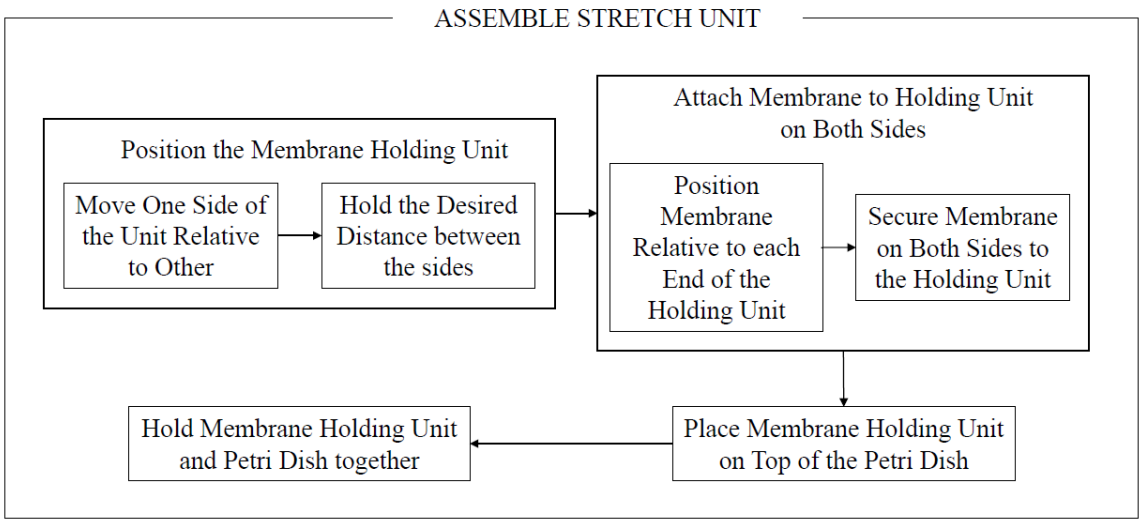



Figure 2.4. : This figure shows the first sub function further split into four smaller functions. The first two sub sub functions are further split into two smaller functions each.

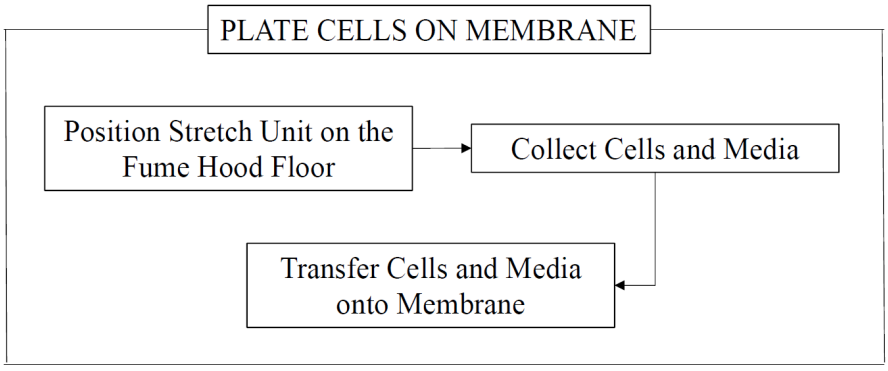



Figure 2.5. : This figure shows further divisions of sub- function 2, split into three smaller functions.

as shown in figure 2.4. In a similar fashion, sub- functions 2, 6, 8, 10, and 11 can also be divided into smaller functions, which are illustrated in figures 2.5, 2.6, 2.7, 2.8, 2.9.

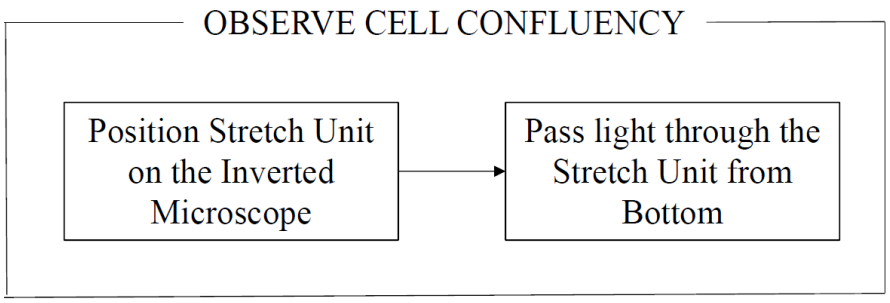



Figure 2.6. : This figure shows further divisions of sub- function 6, split into two smaller functions.

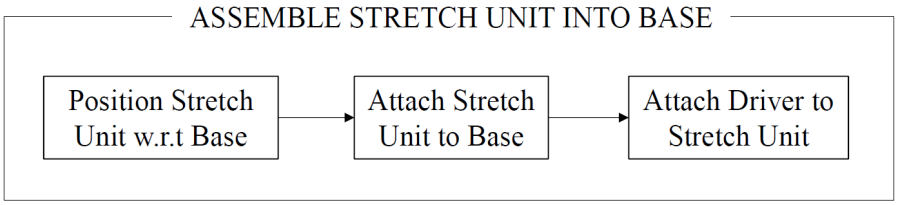



Figure 2.7. : This figure shows further divisions of sub- function 8, split into three smaller functions.

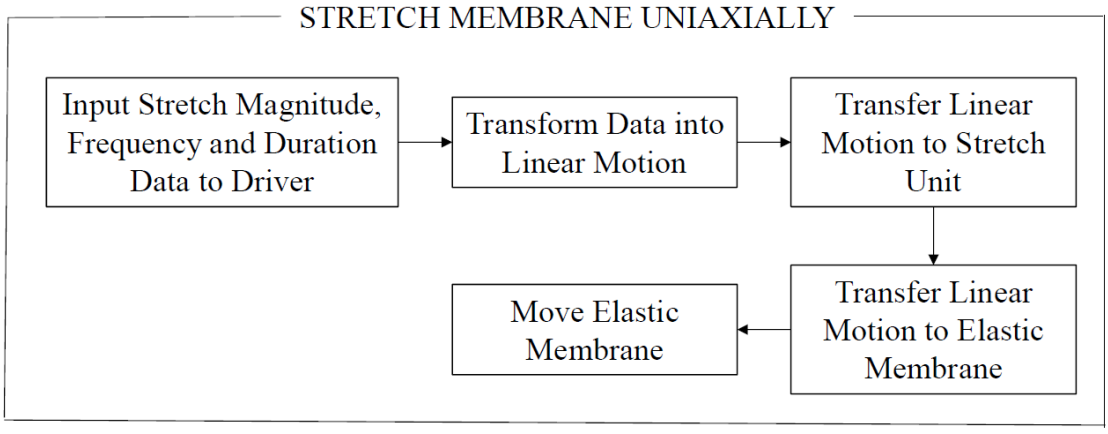



Figure 2.8. : This figure shows further divisions of sub- function 10, split into four smaller functions.

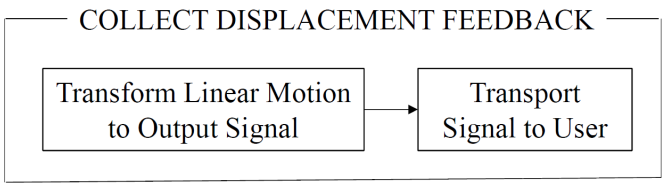



Figure 2.9. : This figure shows further divisions of sub- function 11, split into two smaller functions.

### 2.2.2 Function Concept Mapping

In this section, each function is listed in the order in which they are executed. As many ideas as possible are generated for each function using methods such as brainstorming. The best possible concepts only for each function are selected. Table 2.4 shows the details.

Table 2.4. : This table shows the functional morphology and function concept mapping for the uniaxial stretch machine. The individual fucnctions in order of execution are listed, and ideas for possible concepts that may be used for implementing these functions are generated. The final concept for each function is emphasized.

| Function | Concept 1 | Concept 2 | Concept 3 |
|---|---|---|---|
| Move one side of the unit relative to the other | *One side slides on other* | One side rotates about a hinge | Two sides slide independently |
| Hold the desired distance between the sides | *Screw* | Wedge | Hole and pin |
| Position membrane relative to each end of the holding unit | *Insert membrane into clamps containing slots on both sides* | Lay on top of the holding unit | Use separate frame to temporarily position membrane with foldable clamps |
| Attach both ends of the membrane to holding unit | *Insert clamps into slots on holding unit* | Staple both sides to holding unit | - |

*continued on next page*

Table 2.4. : *continued*

| Function | Concept 1 | Concept 2 | Concept 3 |
|---|---|---|---|
| Place membrane holding unit on top of petri dish | *No fixture cover design* | - | - |
| Hold membrane and petri dish together | Adhesive | *Slide both into a sleeve together* | Screw |
| Position stretch unit on the fume hood floor | *Orient membranes parallel to the ground* | Place wedge like plate under the stretch unit | - |
| Collect cells and media | *With a pipette* | - | - |
| Transfer cells and media onto membrane | *Make a slot large enough to use pipette directly* | Use tube through an opening to access the membrane | - |
| Transport stretch unit to Incubator | *By hand only* | Hand held stabilizer | - |
| Supply *in vivo* environment | *Commercial incubator* | Custom chamber | - |
| Attach driver to stretch unit | Hole and pin | Screw | Spring latch |
| Supply *in vivo* environment | Commercial incubator | Custom chamber | - |

*continued on next page*

Table 2.4. : *continued*

| Function | Concept 1 | Concept 2 | Concept 3 |
|---|---|---|---|
| Transport stretch unit to Incubator | *By hand only* | Hand held stabilizer | - |
| Position stretch unit on the inverted microscope | *Make stretch unit dimensions smaller than standard microscope stage* | Make custom adapter to fit stretch unit on microscope | - |
| Pass light through stretch unit from bottom | *No obstacles above and below the membrane* | Stretch unit made entirely of transparent materials | - |
| Transport stretch unit to driver base | *By hand only* | Hand held stabilizer | - |
| Position stretch unit w.r.t base | *Slide into place* | Place in a cavity | - |
| Attach stretch unit to base | *insert slide lock bars* | Pin and hole | - |
| Attach driver to stretch unit | *Pin and hole* | - | - |
| Input stretch magnitude, frequency and duration data to driver | *Data via PC* | Adjustable manual mechanism control | - |

*continued on next page*

Table 2.4. : *continued*

| Function | Concept 1 | Concept 2 | Concept 3 |
|---|---|---|---|
| Transform input data into linear motion | *Convert input data into electrical signal to actuate driver* | Manually actuate driver via a mechanism | - |
| Transfer linear motion to stretch unit | *Attach membrane holding unit to driver directly* | Attach via an adapter to manipulate input parameters | - |
| Transfer linear motion to elastic membrane | *Transfer directly* | Transfer via an adapter to manipulate magnitude | - |
| Move elastic membrane | *Stretch membrane only on one side* | Stretch membrane on both sides | - |
| Transform linear motion to output signal | *Electrical signal* | Mechanical signal | - |
| Transport signal to user | *Electrical signal to PC* | Mechanical signal to dial | - |

Table 2.5. : This table shows two possible overall concepts developed from the various concepts generated previously for each function.

| Function | Overall Concept 1 | Overall Concept 2 |
|---|---|---|
| Move one side of the unit relative to the other | One side slides on other | Two sides slide independently |
| Hold the desired distance between the sides | Screw | Hole and pin |
| Position membrane relative to each end of the holding unit | Insert membrane into clamps containing slots on both sides | Use separate frame to temporarily position membrane with foldable clamps |
| Attach both ends of the membrane to holding unit | Insert clamps into slots on holding unit | Staple both sides to holding unit |
| Place membrane holding unit on top of petri dish | Place as a lid design | - |
| Hold membrane and petri dish together | Slide both into a sleeve together | Screw |
| Position stretch unit on the fume hood floor | Orient membranes from left to right | Orient membranes with the two ends towards and away from user |
| Collect cells and media | With a pipette | With a pipette |
| Transfer cells and media onto membrane | Make a slot large enough to use pipette directly | Use tube through an opening to access the membrane |

*continued on next page*

Table 2.5. : *continued*

| Function | Overall Concept 1 | Overall Concept 2 |
|---|---|---|
| Transport stretch unit to Incubator | By hand only | Hand held stabilizer |
| Supply *in vivo* environment | Commercial incubator | Custom chamber |
| Attach driver to stretch unit | Hole and pin | Screw |
| Supply *in vivo* environment | Commercial incubator | Custom chamber |
| Transport stretch unit to Incubator | By hand only | Hand held stabilizer |
| Position stretch unit on the inverted microscope | Make stretch unit dimensions smaller than standard microscope stage | Make custom adapter to fit stretch unit on microscope |
| Pass light through stretch unit from bottom | No obstacles above and below the membrane | Stretch unit made entirely of transparent materials |
| Transport stretch unit to driver base | By hand only | Hand held stabilizer |
| Position stretch unit w.r.t base | Slide into place | Place in a cavity |
| Attach stretch unit to base | insert slide lock bars | Pin and hole |
| Attach driver to stretch unit | Pin and hole | Screw |

*continued on next page*

Table 2.5. : *continued*

| Function | Overall Concept 1 | Overall Concept 2 |
|---|---|---|
| Input stretch magnitude, frequency and duration data to driver | Data via PC | Adjustable manual mechanism control |
| Transform input data into linear motion | Convert input data into electrical signal to actuate driver | Manually actuate driver via a mechanism |
| Transfer linear motion to stretch unit | Attach membrane holding unit to driver directly | Attach via an adapter to manipulate input parameters |
| Transfer linear motion to elastic membrane | Transfer directly | Transfer via an adapter to manipulate magnitude |
| Move elastic membrane | Stretch membrane only on one side | Stretch membrane on both sides |
| Transform linear motion to output signal | Electrical signal | Mechanical signal |
| Transport signal to user | Electrical signal to PC | Mechanical signal to dial |

### 2.2.3 Concept Evaluation

Concept evaluation deals with comparing the concepts generated with the customer requirements, as well as any previous designs that may have been developed. In this case, the new concepts are compared to the capstone design product, which is taken as datum. As can be seen from the totals, overall concept 1 has been evaluaed

as the better concept and will be used to generate the product. Table 2.6 shows the comparison in detail.

Table 2.6. : This table shows the decision matrix for concept evaluation- a comparison between the two overall concepts generated and the previous capstone design of the stretch machine (datum), evaluated w.r.t the customer requirements developed earlier.

| Criteria | Importance | Capstone Design (Datum) | Overall Concept 1 | Overall Concept 2 |
|---|---|---|---|---|
| User must be able to use an elastic membrane as a substrate, the dimensions and material of which, is of his/ her choosing | 8 | 0 | 0 | 0 |
| Hold the desired distance between the sides | 8 | 0 | 0 | 0 |
| Device must be able to stretch membrane at a given magnitude of stretch, frequency and duration | 12 | 0 | N/A | N/A |

*continued on next page*

Table 2.6. : *continued*

| Criteria | Importance | Capstone Design (Datum) | Overall Concept 1 | Overall Concept 2 |
|---|---|---|---|---|
| An environment imitating biological conditions, i.e. *in vivo* conditions, must be provided for the cells at all times | 12 | 0 | 0 | 0 |
| The elastic membrane must be held tightly so as to not cause any slip during cyclic stretch process | 10 | 0 | 0 | +1 |
| Plating cells on the membranes should be done in a sterile environment | 7 | 0 | +1 | -1 |

*continued on next page*

Table 2.6. : *continued*

| Criteria | Importance | Capstone Design (Datum) | Overall Concept 1 | Overall Concept 2 |
|---|---|---|---|---|
| The user must be able place the membrane and petri dish unit on an inverted microscope in order to view cells on the membrane | 3 | 0 | +1 | -1 |
| Membrane and petri dish unit must be assembled into the driver unit as soon as possible | 8 | 0 | +1 | +1 |
| Total number of steps to assemble the membrane unit into the driver unit and to start the experiment should be as low as possible to avoid death of cells | 8 | 0 | +1 | 0 |

*continued on next page*

Table 2.6. : *continued*

| Criteria | Importance | Capstone Design (Datum) | Overall Concept 1 | Overall Concept 2 |
|---|---|---|---|---|
| Verify if the displacement across the membrane, for a given magnitude of stretch, is distributed evenly | 11 | 0 | 0 | 0 |
| Total number of steps to disassemble the membranes for collecting cells must be as low as possible | 6 | 0 | +1 | -1 |
| Shear stress experienced by the cells must be negligible to ensure that cyclic tension is the only major force in action | 7 | 0 | N/A | N/A |
| Total | 100 | 0 | 32 | 2 |

### 2.2.4 Generating Options for the Driver

*Criteria for a Good Driver*

1. Compactness: The driver should be small enough to fit into a commercial sized incubator, while being connected to the rest of the assembly. Keeping these factors in mind, the ideal dimensions of the driver should be less than or equal to 150mm x 150mm x 150mm.

2. Power: By using the equation $F = (\Delta l.E.A)/L$ (where $\Delta l$ is change in length of membrane when a force F is applied, E is the elastic modulus of the material, and A is the area of cross- section), it was calculated that the force required to stretch a single membrane of silicone rubber (The exact model used is SILASTIC Biomedical Grade Liquid Silicone Rubber Q7- 4840, Post Cure, 8hours, for which the modulus of elasticity, = 4.01 MPa and Tensile Strength, = 7.45 MPa) of dimensions 50mm x 14mm x 0.254mm by 10% of its length, i.e. by 5mm was 1.426 N. Since the maximum number of membranes that can be used is 4, the total force needed to stretch the four membranes is 4 x 1.426N = 5.704N. Therefore, a good driver must be able to produce unidirectional motion while providing at least a force of 5.704 N.

3. Speed: This machine is being designed to stretch membranes from 5% to 20% of their length, at a frequency of 1 Hz to a maximum of 2 Hz. From these parameters, the minimum and maximum speeds with which the driver must move the stretch plate can be calculated to be 2.5 mm/s and 20 mm/s. To avoid operating at full load, a driver that can generate a maximum speed of 50mm/s or more may be considered as ideal.

4. Accuracy: Accuracy may be defined as the ability of a driver to impart a movement of exactly 10mm, when 10mm is desired. As this machine will be used in stretching membranes by a few mm, accuracy must be high. Positioning accuracy equal to or less than 0.05mm can be considered ideal.

5. Stroke Range: It is important that the driver must be able to move the stretch plate a minimum of 10mm, as that represents 20% stretch on a 50mm membrane.

*Potential Driver 1: Pneumatic Air Cylinder*

Pneumatic air cylinders use compressed air to move a piston back and forth. Stroke lengths available for these drivers range from 0.5 to 36, depending on the size of the model selected. Force produced at 100psi ranges from 7lbs to 700lbs. Due to the nature of this driver, only one stroke length is produced per driver. If a different length is necessary, another driver must be purchased. Disadvantages of this driver include air leaks, and bending and buckling of piston rod during operation.

*Potential Driver 2: Adjustable Stroke Length Air Cylinder*

These drivers have an adjustable shaft collar that can be moved to fine tune the length of the stroke. So the user may change length of the stroke but it can only be done manually after pausing the experiment. The rest of the operations are the same as the single stroke length air cylinder.

*Potential Driver 3: Servo Pneumatic Systems*

These systems use a servo mechanism to deliver desired stroke length, acceleration and frequency. These systems require compressed air, and may not produce absolute precision and speed. Stroke range varies from 10mm to 2000mm, while the force applied may vary from 153 N to 48,255 N. Servo pneumatics systems are generally used for industrial applications such as pressing, filling, gripping and labelling.

*Potential Driver 4: Air Powered Motors*

Air powered motors include an output shaft connected to a rotating vane run by compressed air. They are considered safer in harsh environments compared to electric motors. They remain cool even after continuous load for long durations. The user must use a rack and pinion to convert the rotation to linear motion. Power for these motors range from 0.18 hp to 5 hp.

*Potential Driver 5: Hydraulic Actuators*

Hydraulic cylinders work in the same way as a pneumatic cylinder, except for the

air, which is replaced by a liquid, usually an oil. For the specific application of cyclic stretch, double acting hydraulic cylinders are necessary. However, additional equipment is necessary in order to control and monitor the cylinder. Stroke and force ranges are shown in table 3 below:

Table 2.7. : Stroke and Force Ranges for Air Cylinders

| Description | Max. Pull Force | Max. Push Force | Stroke |
|---|---|---|---|
| Smallest Cylinder | 1209 lbs | 1762 lbs | 1” |
| Largest Cylinder | 25400 lbs | 31425 lbs | 12” |

*Potential Driver 6: Hydraulic Motor*

Works exactly as an air motor, except for the fluid, which in this case is an oil. Power generated in these motors are in the range of 6.2 hp to 9.6 hp.

*Potential Driver 7: Piezo Electric Actuators*

Piezo electric actuators use materials that expand or contract when a potential is applied to them, to push/ pull an object. Piezo electric actuators are extremely precise and may be a good option for cyclic stretch applications, the only drawback being their low force generation, at about 2.5 N

*Potential Driver 8: Electric Actuators*

These are fixed stroke actuators that reply on an electric motor to transfer power to a screw type linear moving rod. Stroke lengths are in the range of 2 to 6. Maximum pull/ push force is 25lbs.

*Potential Driver 9: Solenoid*

A solenoid works just like a pneumatic air cylinder, but instead of air, magnetic field is used to generate forces necessary to push a shaft out, while a returning spring brings the output shaft back to zero after the magnetic field is taken off. Typical stroke lengths of a solenoid range between 0.18 to 0.7, and power generated by the output shaft ranges between 8 oz. to 50 oz.

*Potential Driver 10: Position Control Motor with Integrated Driver and Controller*
These units are integrated servo systems that put together a stepper motor with an encoder, a driver and a controller. These systems produce a maximum stroke length of 500mm, maximum speed of 200 mm/s and a maximum axial load of 90 N, with a repetition accuracy of 0.025mm. Table 4 below shows specific models of each type of potential driver their specifications based on criteria specified above.

Table 2.8. : Specifications of the Potential Drivers for the Stretch Device

| Driver Type | Model Details | Dimensions | Force/ Torque | Speed | Accuracy | Stroke Range |
|---|---|---|---|---|---|---|
| Pneumatic Air Cylinder | McMaster sensor ready round body air cylinder: 4952K701 | 114.3mm x 15.7mm | 112.05N | N/A | N/A | 25.4mm |
| Adjustable Stroke Air Cylinder | McMaster adjustable stroke length air slide: 6350T211 | 172.2mm x 104.6mm x 31.75mm | 197.2N | N/A | N/A | 25.4mm |
| Servo Pneumatic Systems | Festo pneumatic system with standard cylinder and encoder: DNCI | 142 mm x 45mm x 45mm | 441N | N/A | ± 0.5mm | 100mm-750mm |
| Air Powered Motors | Mc Master air powered motor: Hub mount | 120.6 mm x 63.5 mm x 127 mm | 339 N mm | 10,000 rpm @max. psi | N/A | Depends on length of rack used |

*continued on next page*

Table 2.8. : *continued*

| Driver Type | Model Details | Dimensions | Force/ Torque | Speed | Accuracy | Stroke Range |
|---|---|---|---|---|---|---|
| Hydraulic Actuator | MTS 201 series hydraulic actuator, closed loop. Model: 201.17 | 300 mm | 32 KN | N/A | N/A | 254 mm |
| Hydraulic Motor | Hydroleduc micro hydraulic motor: MH450DS | 119 mm x 31.7 mm | 2300 N- mm | 350 6500 rpm | N/A | Depends on length of rack |
| Electrical Actuator | Cool Muscle cylindrical actuator RD35T1215017L30 | 298.73 mm x 53 mm x 42 mm | 103 N | 600 mm/s | ± 0.02mm | 0 -150 mm |
| Solenoid | Mc Master compact linear solenoid (push/ pull): 6873K6 | 120.65 mm x 69.85 mm | 12.5 N | N/A | N/A | 12.7 mm |

*continued on next page*

Table 2.8. : *continued*

| Driver Type | Model Details | Dimensions | Force/ Torque | Speed | Accuracy | Stroke Range |
|---|---|---|---|---|---|---|
| Piezo Electric Actuator | PI Piezo drive M664.164 | 90 mm x 60 mm x 15 mm | 2.5 N | 400 mm/s | ± 1 m | 25 mm |
| Position Control Motor | Cool Muscle RRA23 + CM123L20 | 125 mm x 71 mm x 56 mm | 90 N | 200 mm/s | ± 0.025 mm | 0- 500 mm |

### 2.2.5 Decision Matrix for the Selection of Driver

Table 2.9 shows various potential drivers and evaluates them based on the criteria defined. From the totals, a position control motor with integrated driver and controller is calculated to be the best suited for the project.

Table 2.9. : Decision Matrix for Selecting Driver

| Issue | Relative Importance | Pneumatic Air Cylinder | Adjustable Stroke Length Cylinder | Servo Pneumatic System | Air Powered Motor | Hydraulic Actuator | Hydraulic Motor | Piezo Electric Actuator | Electric Actuator | Solenoid | PCI Servo Motor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compactness | 20 | 5 | 3 | 5 | 5 | 2 | 5 | 5 | 1 | 5 | 5 |
| Power | 20 | 5 | 5 | 5 | 5 | 5 | 5 | 2 | 5 | 5 | 5 |
| Speed | 15 | | | | | | 5 | 5 | 5 | | 5 |
| Accuracy | 25 | | | 3 | | | | 5 | 5 | | 5 |
| Stroke Range | 20 | 1 | 2 | 2 | 5 | 5 | 5 | 5 | 5 | 1 | 5 |
| Weighted Total | 100 | 220 | 200 | 315 | 300 | 240 | 375 | 440 | 420 | 220 | 500 |

### 2.2.6 Generating Options for Proximity Sensor

There are four types of proximity sensors in general. They are induction, capacitive, photoelectric and ultrasonic sensors. Of these, photo electric type of proximity

sensors are not feasible for this particular application as they cannot interpret the distance at which the stretch plates are at a given point of time. Therefore, only three concept ideas will be generated.

*Criteria for a Good Proximity Sensor*

1. Compactness: The proximity sensor must be small enough to fit on to the stretch machine, exactly parallel to the stretch plate movement. It is also desirable if the emitter and receiver are on the same unit, instead of separate ones. Maximum desirable dimensions of the sensor may be set to 75mm x 50mm x 50mm.

2. Sensing Range: Based on the smallest and largest lengths of the membranes and % strains, the sensing range for a good proximity sensor may be calculated as 1mm to 10mm.

3. Operating Temperature: The sensor will be kept in an environment maintained at a temperature of 37o C and 90% humidity. The sensor must be able to function in these conditions.

*Potential Proximity Sensor 1: Inductive Sensor*

The model chosen here is a DC metallic object proximity switch, with model number 7674K833, from McMaster CARR. The dimensions of this sensor are 18mm x 51mm, while the sensing range is 0mm to 10mm. Operating temperature is $-10°F$ to $155°F$.

*Potential Proximity Sensor 2: Capacitive Sensor*

This model is also chosen from McMaster CARR. It is a DC universal object proximity switch, with model number 7675K86. The dimensions are 30mm x 80mm, while the sensing range is from 0mm to 15mm. Operating temperature is $-10°F$ to $155°F$.

*Potential Proximity Sensor 3: Ultrasonic Sensor*

This model is also chosen from McMaster CARR. It is a digital ultrasonic switch, with model number 6565K31. The dimensions are 30mm x 100mm, while the sensing range is from 4 inches to 40 inches. Operating temperature is from $35°F$ to $140°F$.

### 2.2.7 Decision Matrix for Selection of Proximity Sensor

Table 2.10 shows various potential sensors and evaluates them based on the criteria defined. From the totals, it can be decided that an induction sensor is the right choice for this application.

Table 2.10. : Decision Matrix for best Proximity Sensor

| Issue | Relative Importance | Concept 1 | Concept 2 | Concept 3 |
|---|---|---|---|---|
| Compactness | 30 | 30 | 20 | 15 |
| Sensing Range | 35 | 35 | 35 | 35 |
| Operating Temperature | 35 | 35 | 35 | 35 |
| Total | 100 | 3350 | 3050 | 2900 |

## 2.3 Product Generation

### 2.3.1 Form Generation

Product generation deals with generating details of the form, materials and processes required for manufacturing of the product, and manufacturing the product itself. These operations are inter-dependent on functions, as well as each other.

Form generation requires information on four sub-categories: constraints, configuration, connections, and components. Each of the sub-categories are explained and information is generated for the uniaxial stretch devices.

**Spatial Constraints**

Spatial constraints may refer to the the space occupied by a product, space required by the product to function, or the space required for the interaction of two moving components in a product. Spatial constraints are important as the dimensions of a product are constrained by the space they work in.

For the uniaxial stretch machine, functions developed for the product in the earlier sections may be used to identify those functions that are dependent on spatial constrains. They are as follows:

- Assemble the stretch unit.
- Transfer cells and media to the membrane.
- Transport stretch unit to the incubator.
- Observe cells for confluency.
- Assemble stretch unit to driver base.
- Supply *in vivo* environment.
- Move membrane uniaxially.

From the concepts developed earlier from sub-functions, assembling the stretch unit will depend on the following dimensions:

1. Size of the single well petri dish
2. Size of the membrane
3. Size of clamp
4. Size of slot in the clamp
5. Size of the stretch plates

6. Size of the slot for pipette in the stretch plates

7. Size of sleeve to hold the stretch plate and petri dish assembly

8. Area needed to assemble the stretch plates

The spatial constraints for each of these components are explained in detail below:

*Size of the Four Well Petri Dish*

This is the most important component w.r.t defining spatial constraints, as the petri dish used is a commercially available one and cannot be customized. All the other components must be designed to fit around the petri dish. The overall dimensions of the petri dish are: L=127.8mm, W=85.5mm, H=14.5mm. Bottom thickness is 1.14mm. The internal dimensions of a single well are: L=78mm and W=27.9mm. These dimensions will influence the dimensions of the membrane, clamps, and the stretch mechanism.

*Size of the Stretch Plates*

The size of the stretch plates depend on the size of the petri dish. The thickness of the plates were established as 10mm in order to provide enough stiffness to resist bending. The idea here is to create two plates with slots for clamps. The bottom plate will sit on top of the petri dish like a lid while being fully constrained, the top plate rests on top of the bottom plate, and slides back and forth on the bottom plate. Adding material for handling, the outermost dimensions of the bottom plate were calculated to be 130 mm x 98 mm, and those of the top plate were calculated to be 180 mm x 118.4 mm. Details of all specific dimensions of the top and bottom plate are illustrated in figures below:

*Size of the Slots for the Pipette*

The user must be able to reach the membranes from the top of the stretch plates after assembling the assembly. For this, the width of the slot should be about the same size of the membrane, as the cells must be distributed as evenly as possible. The minimum size of the slot was determined to be 50mm x 14mm, the same size as the largest possible membrane. The dimensions of the slots are shown in figure .

*Size of the Clamp*

The size of the clamp depends on the internal dimensions of a single well of the petri dish as well as the thickness of the slot in which the clamp will sit. The internal height of the petri dish well is 13.36mm, which can be considered to be the minimum height of the clamp. Since outer and inner widths of the slot were calculated to be 24mm and 20mm respectively, the maximum width of the clamp can be calculated to be 20mm. A rectangular slot of length 15mm, width 4mm, and height 2mm is placed in the clamp in order to accommodate for the membranes.

*Size of the Sleeve to hold Petri Dish and Stretch Plate Together*

Size of the sleeve depends on the dimensions of the stretch plates and the petri dish assembled together. The length and width should be 130mm x 98mm, based on the bottom plate dimensions. One side of the box must be open so as to allow for the top plate to slide freely. The height of the sleeve depends on two factors:

- It must hold both the stretch plates and the petri dish together.
- Although the plates and the petri dish are inserted together, the sleeve must support the top and bottom plates independently. User must be able to move the petri dish up and down by a distance of 2mm.

A detailed drawing of the sleeve is shown in figure .

*Area Needed to Assemble the Stretch Unit*

All assembly of the components must be done in a sterile environment. A fume hood is the best option for this purpose. All components required for the asembly of the stretch unit must fit in the smallest fume hood commercially available. This constrains the area available to 30"x30".

*Transfer Stretch Unit by Hand to Microscope Stage to Observe Cells*

Transporting the stretch assembly was best done by hand, as was discussed previously. Hence, the dimensions of the stretch assembly is limited by the average user's hand size. In order to hold the assembly properly, it was decided that the height of the assembly should not exceed 100mm. The length and width of the assembly is

dependent on the size of the inverted microscope stage. Nikon TS-100f, a popular model was used as a reference for this purpose. The dimensions of the stage are as follows: 170mm x 225mm. The maximum height of an object that can be placed on the stage is 115mm, which is more than the required height of the assembly. Total height of the stretch assembly, which is also the height of the sleeve is illustrated in figure .

*Size of the Driver Base*

The size of the base is constrained by the dimensions of the following components:

- Driver and its accessories
- Proximity Sensor
- Stretch Plate Assembly

The base must be large enough for all components, i.e. the driver, proximity sensor and the stretch unit, to fit and function properly. Also, the base must be small enough to fit in a commercially available incubation chamber, and must have enough free space to allow for assembling the stretch unit. Considering all constraints, the total length and width were limited to 450mm x 250mm.

**Configuration**

Configuration deals with the development of individual components, their locations, and orientations. The driver is placed upright, and the rack is positioned and oriented in such a way that its axis alignes with the central axis of the stretch plates. This is done to ensure that the stretch plate moves in a straight line. The stretch plates, petri dish and the sleeve are assembled together and their centers align with the central axis of the rack. The proximity sensor is fixed at an offset and parallel to the rack.

## Developing Connections

A connection, also known as an interface, is a point that supports a component's function, and transfers forces, materials, etc. to the next component. Interfaces are classified into fixed, non adjustable, adjustable, separable, locator and hinged connections.

*Connection between the Clamp and the Stretch Plate*

This will an adjustable connection and only one degree of freedom is needed for the clamp to slide into the stretch plate slot, which is necessary to insert and remove membrane. The connection will lock in place due to friction.

*Connection between the Stretch Plates and Sleeve*

The connection between the stretch plates may be classified as a separable connection. This is needed in order to be able to assemble membranes. Only one degree of freedom is required in order for the top plate to slide back and forth on the bottom plate. The bottom plate is constrained in all directions to the sleeve, making it a separatable, non adjustable connection.

*Connection between the Top Stretch Plate and Driver Rack*

The most suitable and simple connection between the driver rack and the stretch plate is a fork joint. It transfers the forces to the plates without any losses, and is a separable connection, which is necessary in order to disassemble the components.

*Connection between the Top Stretch Plate Assembly and Base*

A separarble, non adjustable connection is used to secure the stretch assembly to the base. A dovetail slide protrusion on both sides of the sleeve, and slots on the base make the sliding joint complete, while a horizontal bar is used to lock the assembly in place.

*Connection between the Driver, and the Proximity Sensor, with the Base*

Both the driver and the sensor are fixed, non adjustable connections. They are held in place by screws and do not have any degree of freedom.

### 2.3.2 Developing and Manufacturing Components

*Uniaxial Stretch Device- The Final Product*

This section illustrates and summarizes each component of the newly designed and manufactured uniaxial stretch device.

*Overview*

As depicted in figure 2.10, the uniaxial stretch device consists of a PC, connected to a power and signal conditioning unit, used to control a servo motor at the other end. The servo motor, with the help of a rack and pinion attachment, is connected to one of the stretch plates, while the other plate remains fixed sitting above the petri dish containing biological media. A membrane is suspended in the media via clips attached to the stretch plates. As the servo motor is driven, the rack moves the top stretch plate, which in turn moves the clip holding the membrane, stretching it. This cycle is repeated as many times as desired. The inductive sensor is used to monitor the stretch magnitudes applied.

*The Controller - LABVIEW*

Labview is a data acquisition based control software. All programs are written not in terms of text but in terms of diagrams, which makes it interactive and easy to learn. The programs are run through another window called the front end, where commands to run are in the form of interactive buttons. Data collected from any devices connected may also be displayed in real time. Figures 2.11 and 2.12 show the front end and back end of the program written to control the uniaxial stretch machine.

*Individual Components of the Uniaxial Stretch Machine*

*Membrane Holding Clips*

The clips hold the silicone membrane suspended in the media, and are one of the key components of the stretch device. Figure 2.13 below shows the main dimensions of the clips.

*The Stretch Plates*

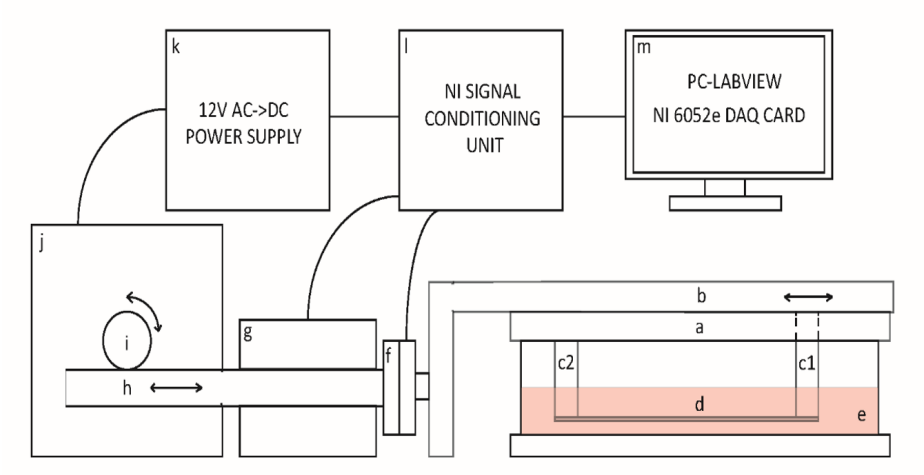



Figure 2.10. : Schematic of the stretch machine. Two plates with clips hold a membrane submerged in media. A servo motor with a rack is attached to the moving plate via a load cell. LabVIEW is used to monitor signals from the displacement and load sensors. a) Fixed plate. b) Moving plate. c1),c2) Clips on each plate with membrane assembled at desired length. d) Membrane onto which cells are plated. e) Petri dish with media. f) Inductive sensor for measuring cyclic displacement. g), h) RRA23 rack attachment for motor shaft. i) Motor output shaft. j) Myostat CM123L servo motor. k) 12V AC to DC converter. l) National Instruments signal conditioning unit. m) PC with National Instruments 6052e data acquisition card installed.

The stretch plates hold the membrane bearing clips, and one of them is also connected to a servo motor, while the other remains stationary on top of a petri dish. Each of the stretch plates has its own unique design, whose dimensions are shown in figures 2.14 and 2.15.

*The Sleeve*

The sleeve holds the stretch plate, clips, membranes and petri dish assembly together as a single unit. This allows the user to move it around by hand between the incubator, fume hood, and the microscope. Figure 2.16 below shows the dimensions of the sleeve design.

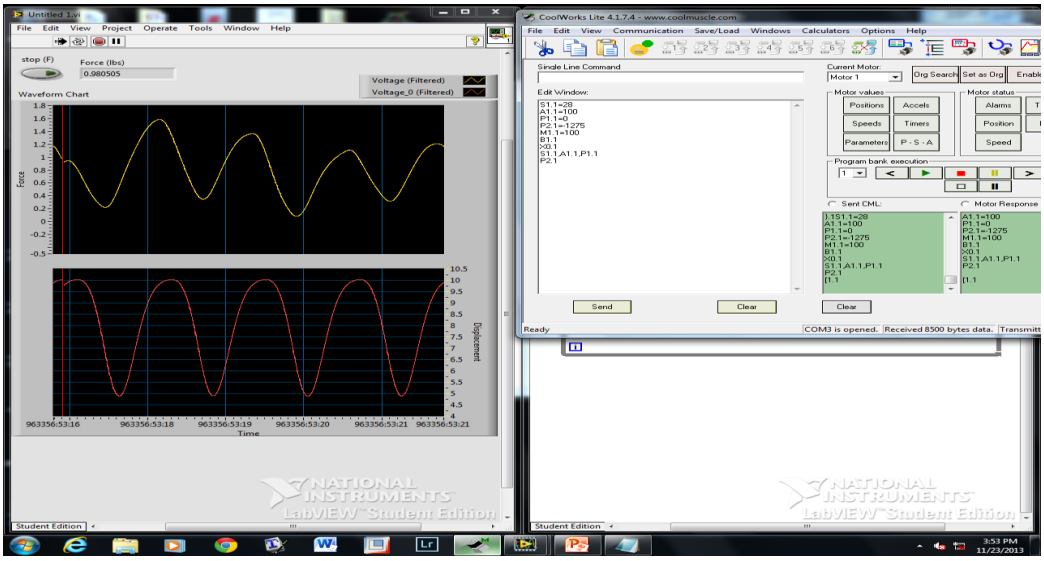


Figure 2.11. : Front End of the Labview Control Software

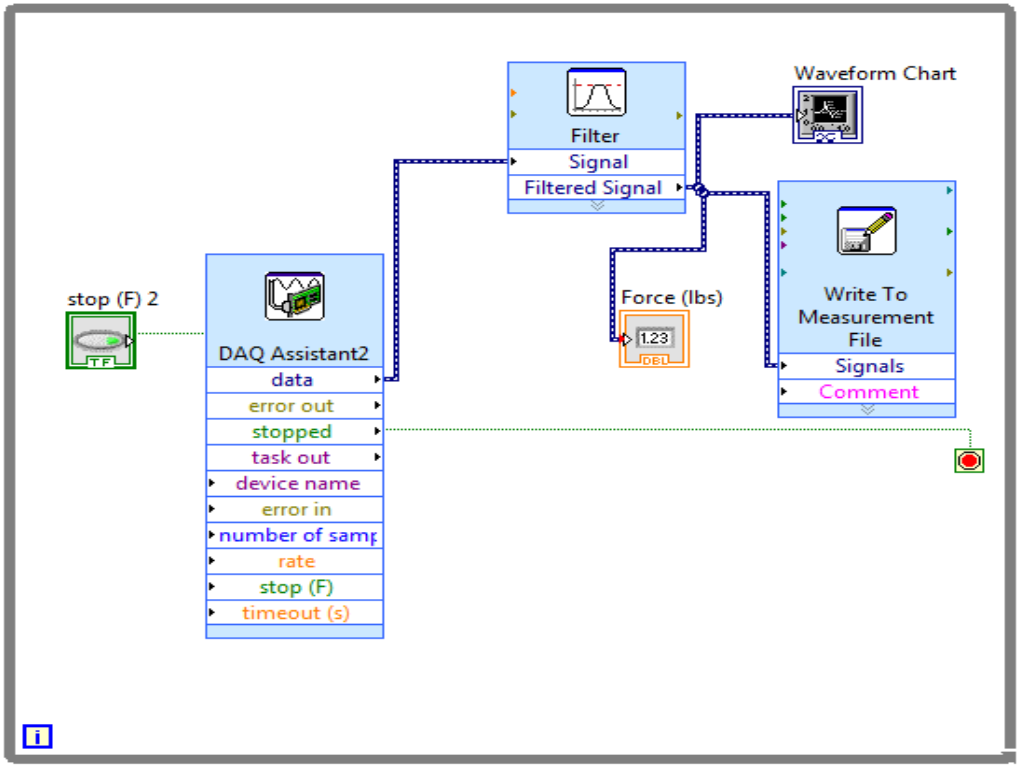



Figure 2.12. : Back End of the Labview Control Software

*The Plate Cover*

The plate cover, as the name suggests, sits on top of the top plate in order to stop contamination from the openings on the plate. The cover also has a provision for an external tube of carbon di oxide supply, which is channeled to the membranes below. Figure 2.17 shows the dimensions of the plate cover.

*The Base*

The base of the stretch machine was manufactured using polycarbonate sheets. It houses all components of the stretch device, and is fairly large. It must be assembled in an incubator where the experiments are done and cannot be moved from its place. Figure 2.18 shows the dimensions of the base.

*The Stretch Device - Full Assembly*

Figure shows the stretch device with all components assembled and its overall dimensions. Figure 2.21 shows the bill of materials.

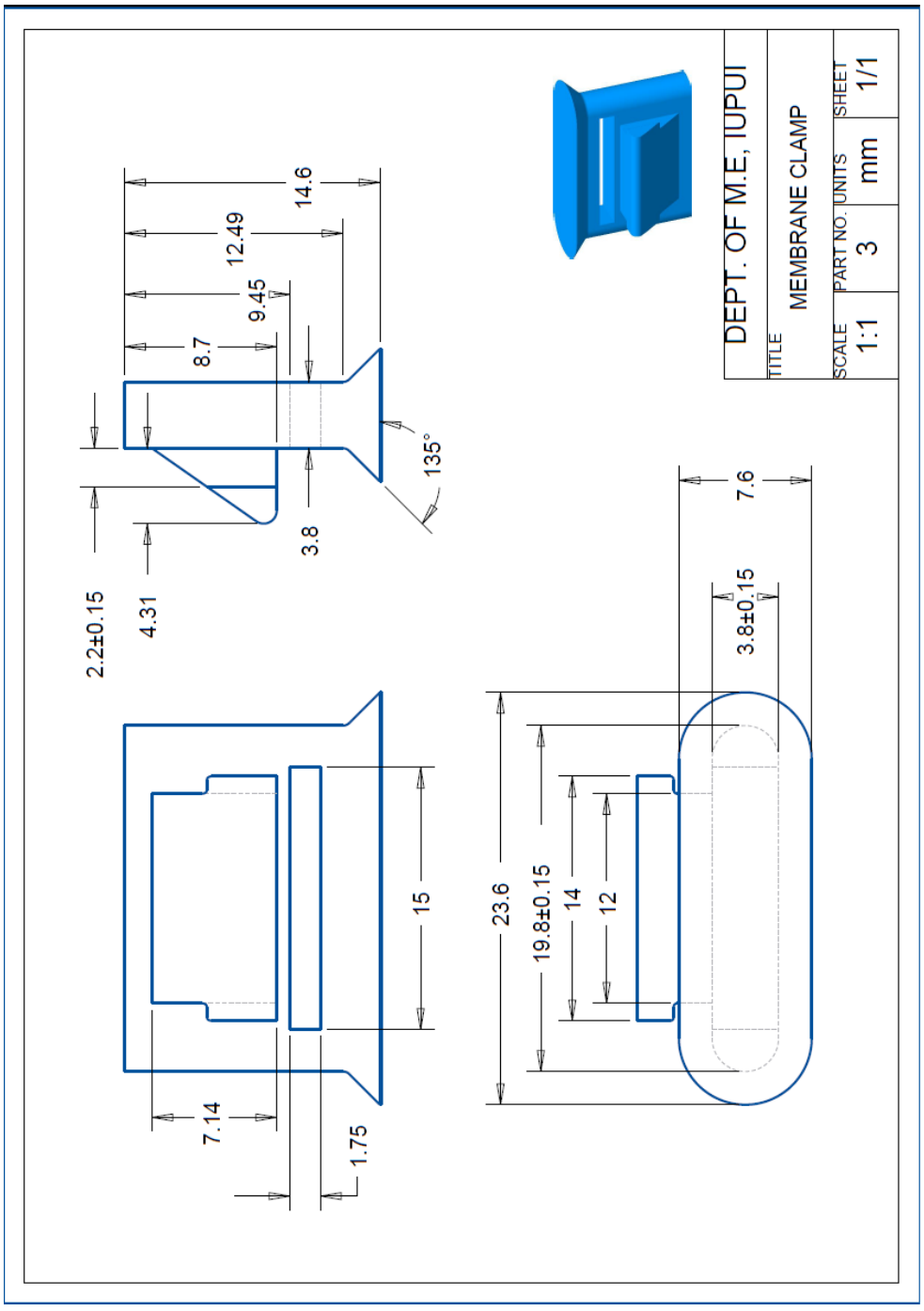



Figure 2.13. : Dimensions of the Clamp

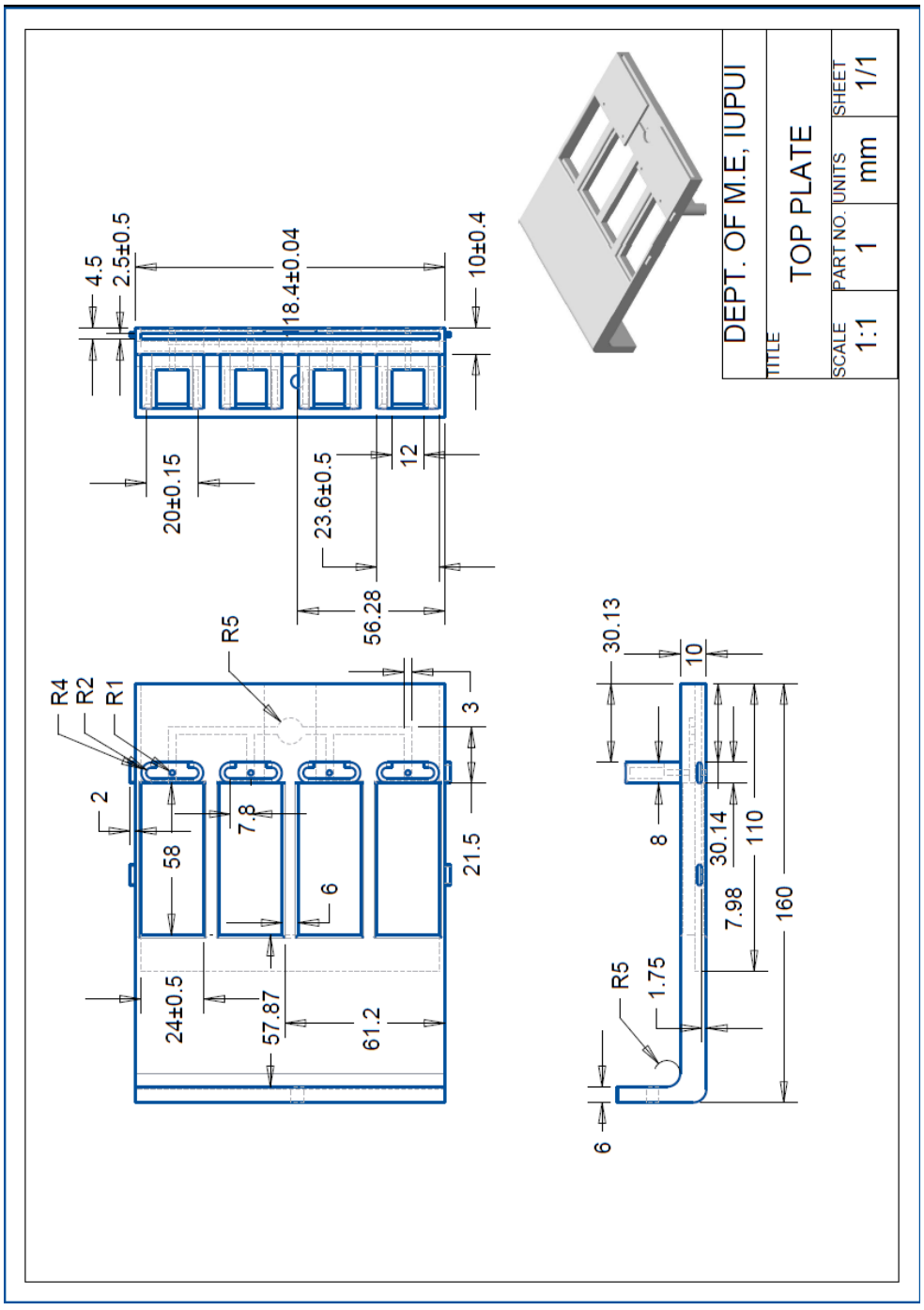



Figure 2.14. : Dimensions of the Top Plate

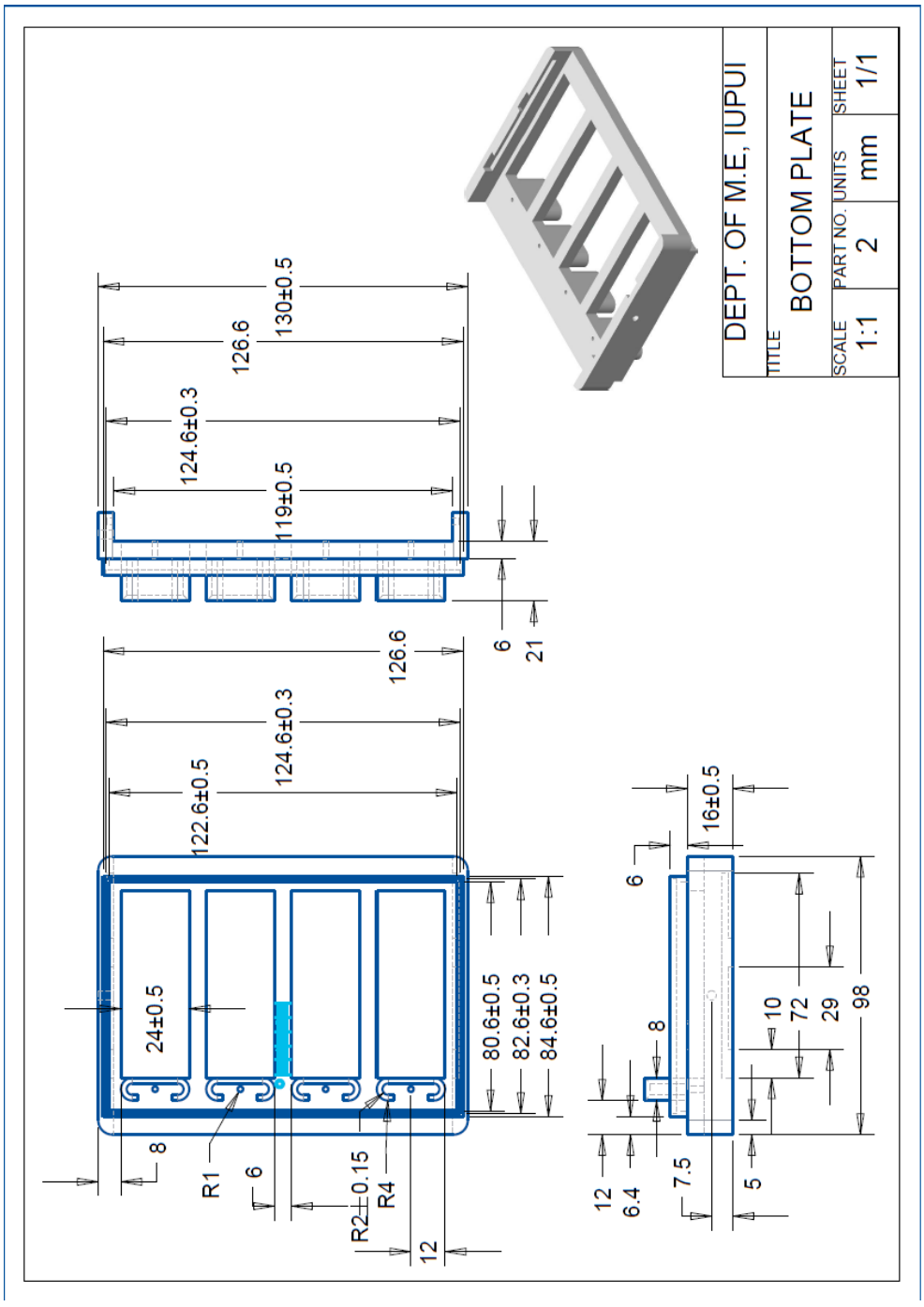



Figure 2.15. : Dimensions of the Bottom Plate

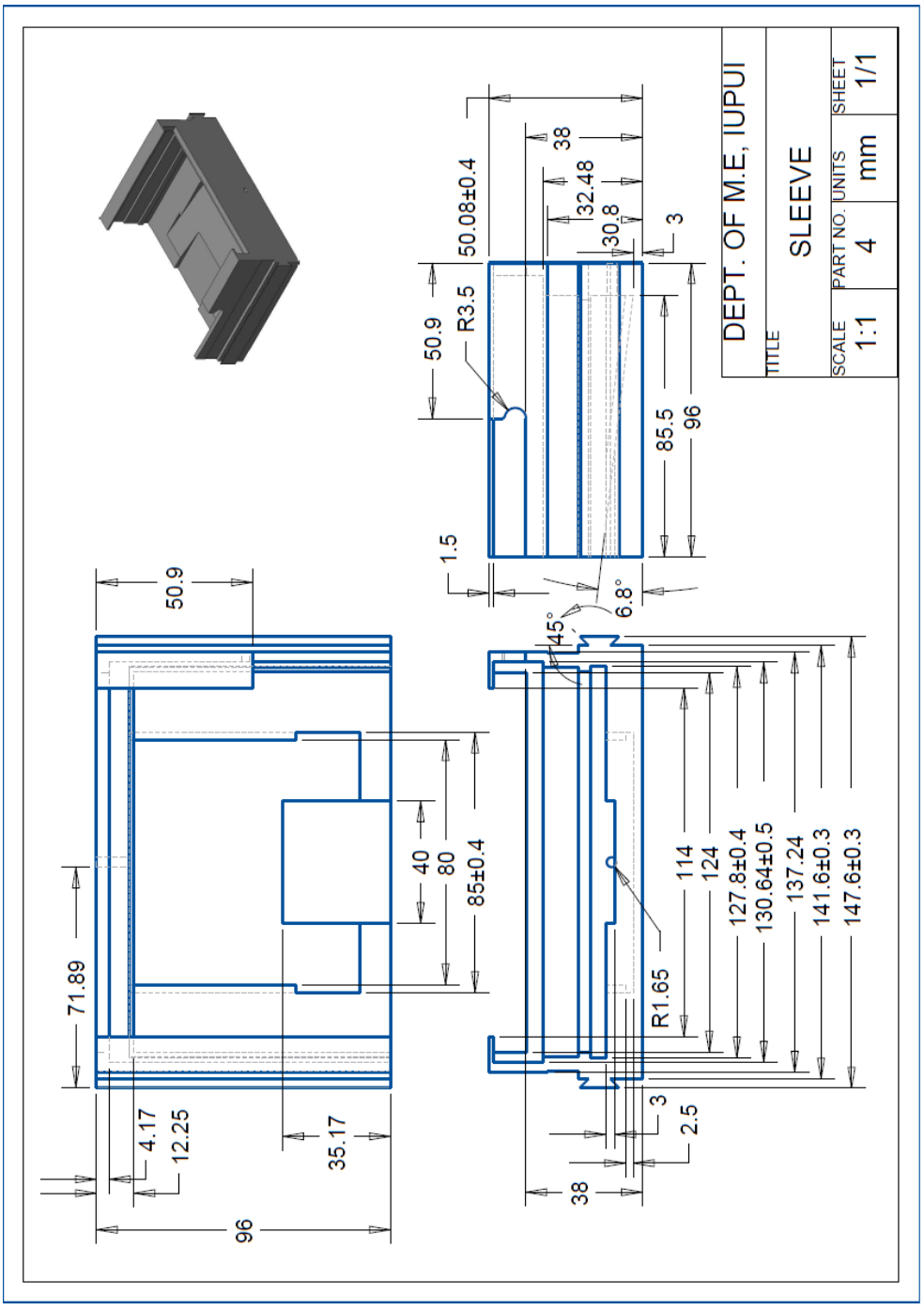



Figure 2.16. : Dimensions of the Sleeve

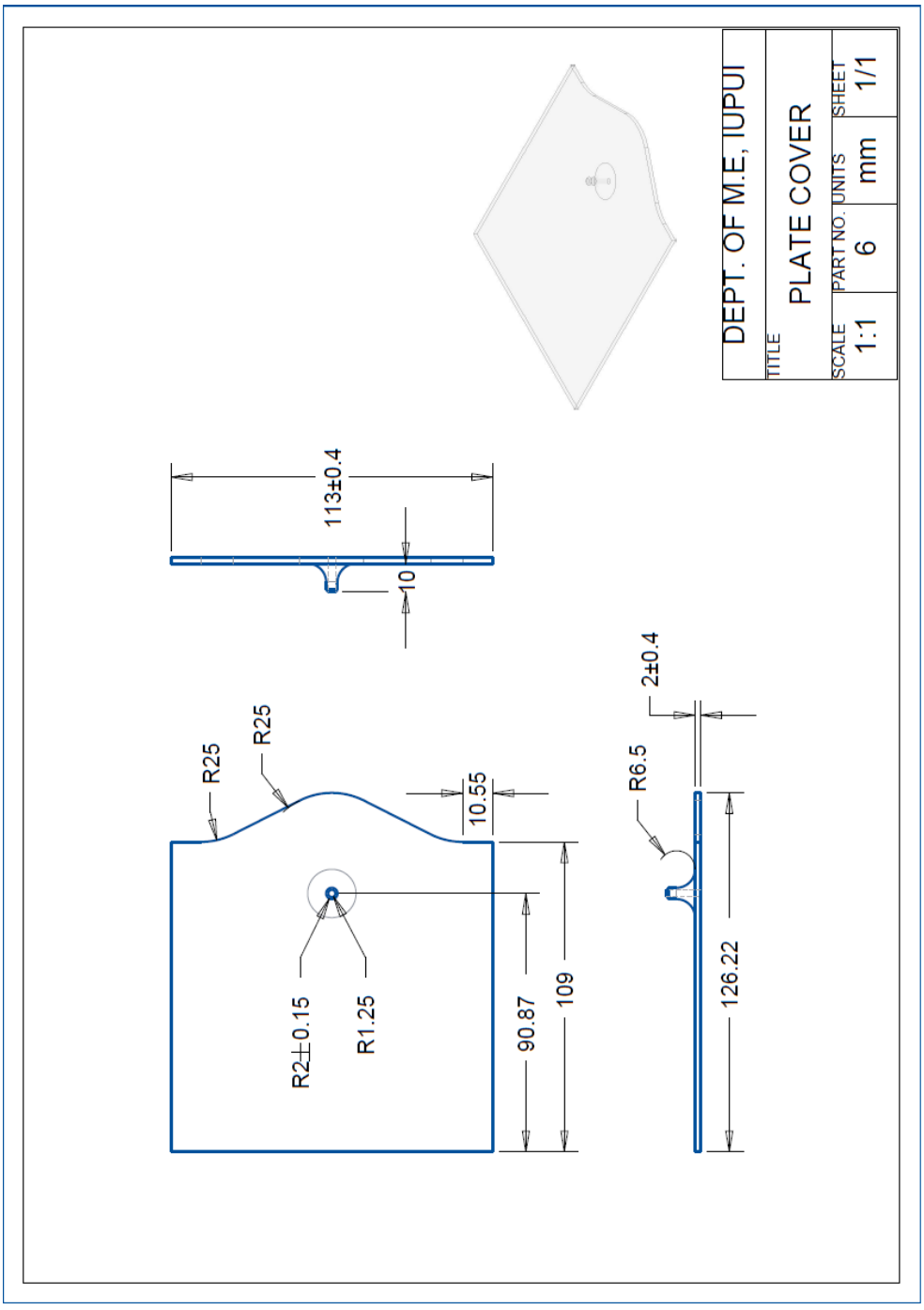



Figure 2.17. : Dimensions of the Plate Cover

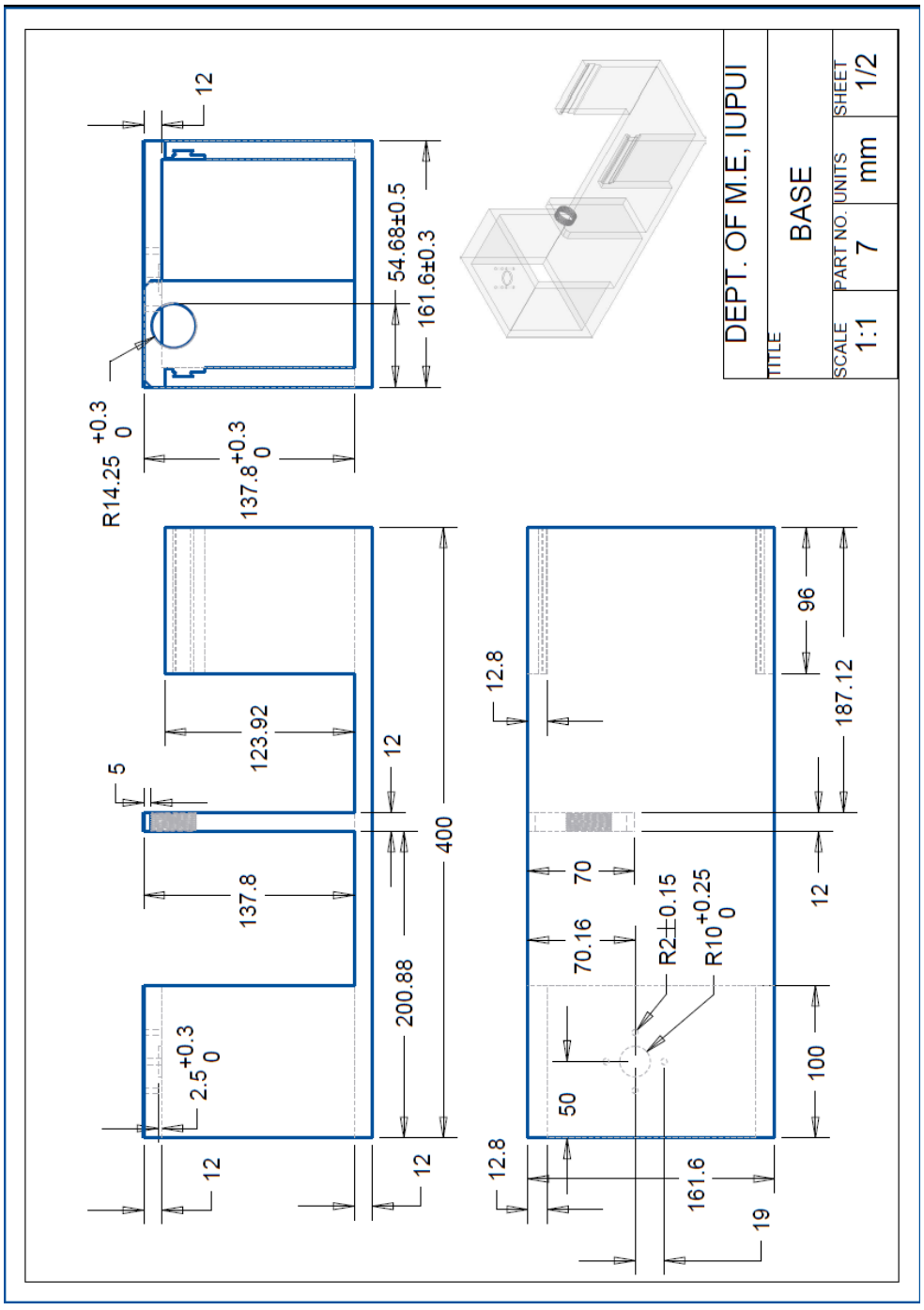



Figure 2.18. : Dimensions of the Base

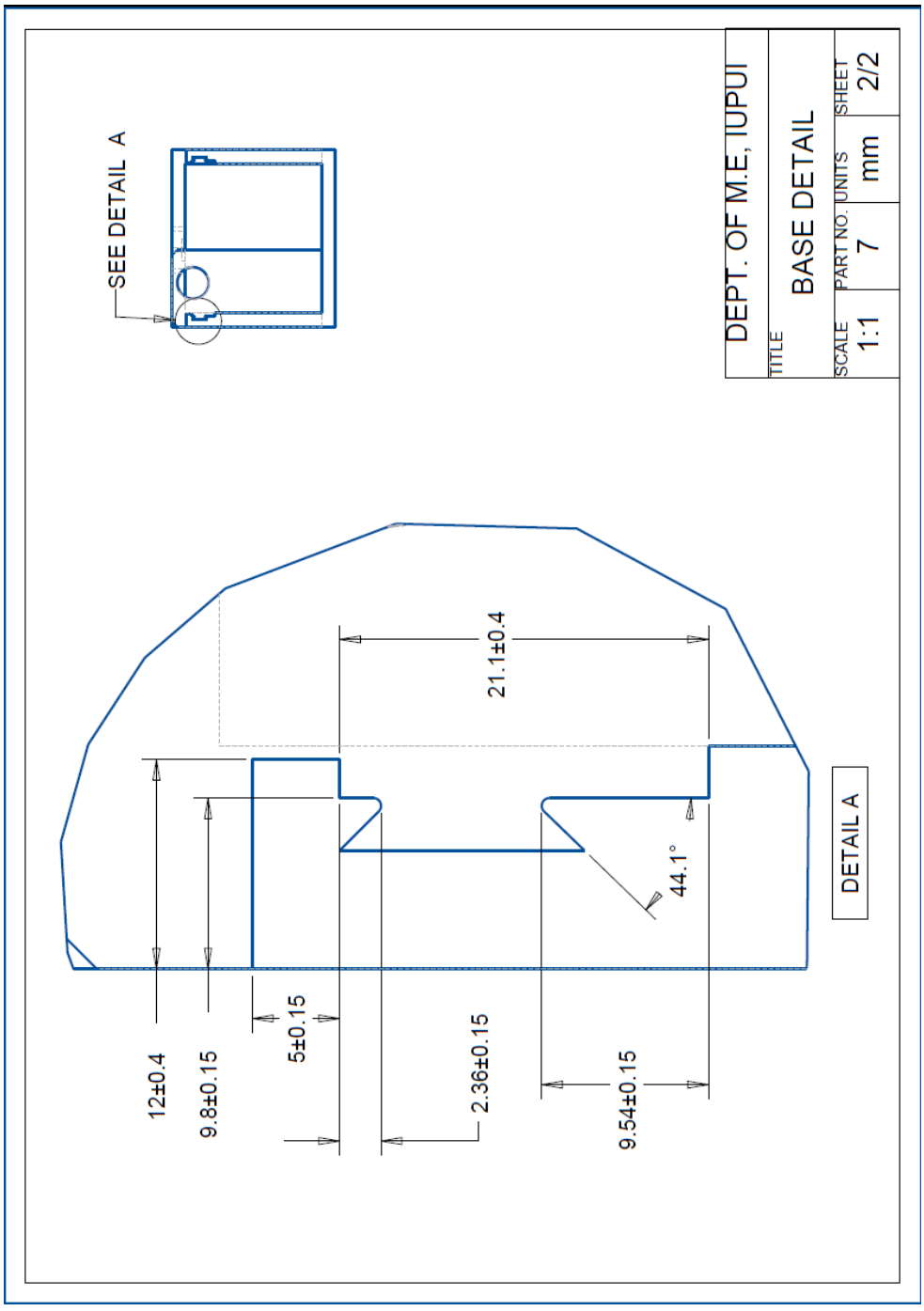



Figure 2.19. : Details of section A

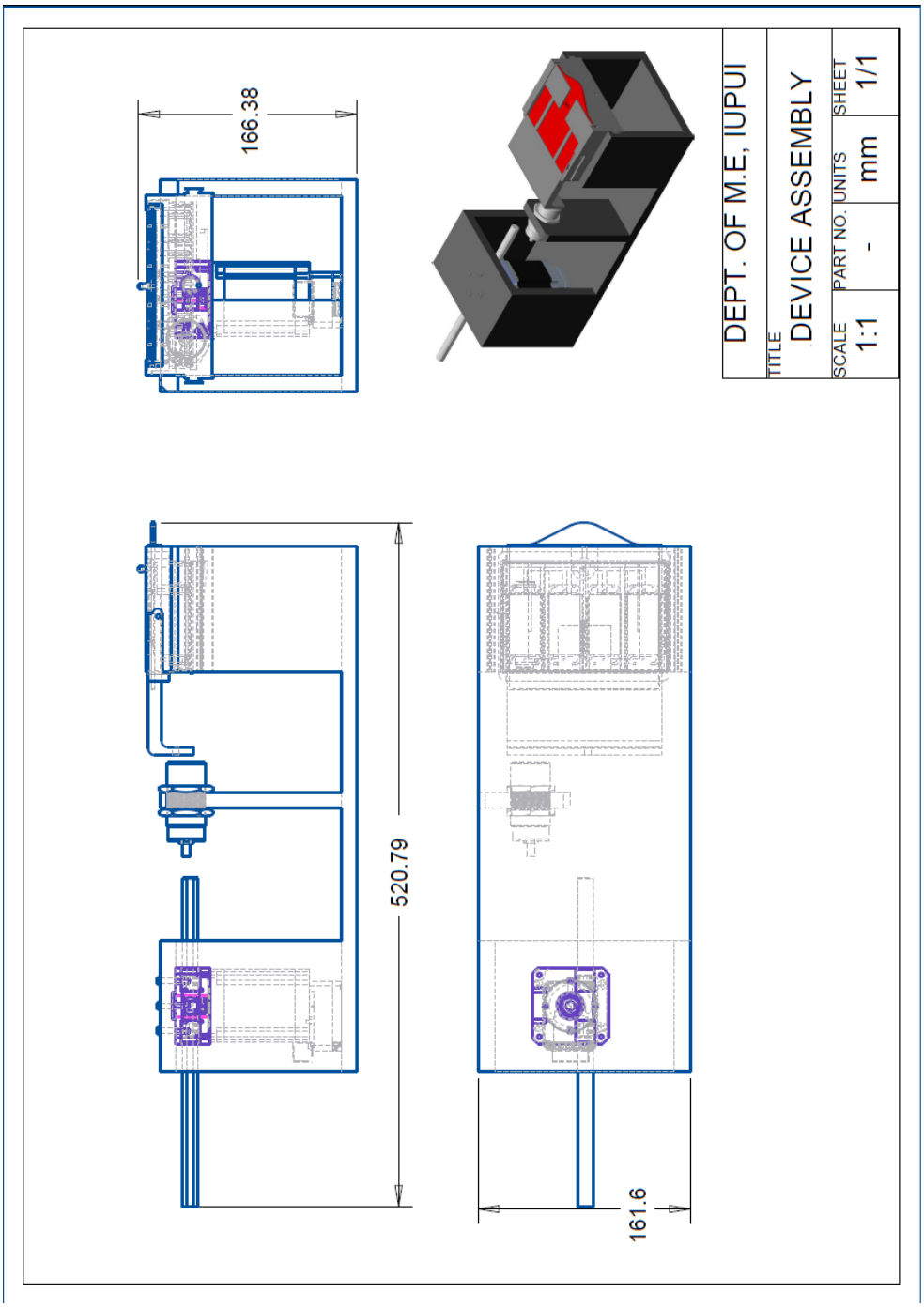



Figure 2.20. : Dimensions of the Stretch Device - Full Assembly

| Part No. | Part Name | Material | Quantity |
|---|---|---|---|
| 1. | Top Plate | ABS Plastic | 1 |
| 2. | Bottom Plate | ABS Plastic | 1 |
| 3. | Clips | ABS Plastic | 8 |
| 4. | Level Support | ABS Plastic | 1 |
| 5. | Petri Dish | Polystyrene | 1 |
| 6. | Plate Cover | ABS Plastic | 1 |
| 7. | Base | Polycarbonate | 1 |
| 8. | Induction Sensor | | 1 |
| 9. | RRA 23 rack and pinion | | 1 |
| 10. | Cool Muscle CM1-X23L20C Motor | | 1 |

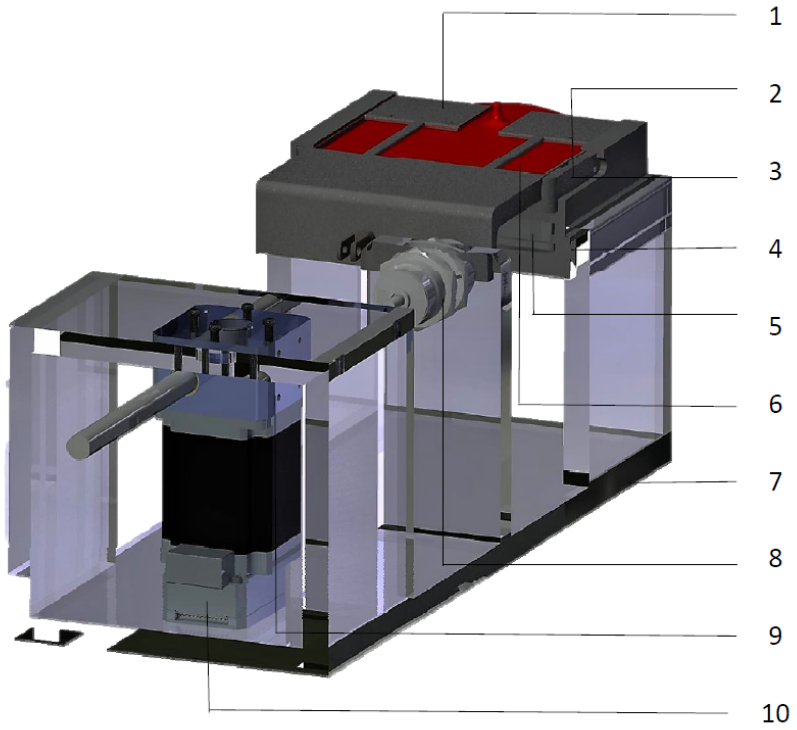



Figure 2.21. : Bill of Materials

### 2.3.3 Material and Process Selection

A few criteria were developed in order to select the best possible materials for the stretch device.

1. The material must be autoclave friendly (should be able to withstand steam @ 1200C for 15- 20 min).

2. The material must be non-porous and chemical resistant. since it will be submerged in biological media for long durations

3. It should be able to withstand high humidity, 5%CO2 and 370C for at least a week without any effect.

4. The material chosen must be rapid prototype friendly since conventional manufacturing methods are not capable of producing the complex geometries of parts.

5. FDA class VI compliance is an added advantage but not a necessity since the parts are not implants.

But these materials were very expensive to model a prototype with. Hence a standard material used with 3D printers, called Acrylonitrile Butadiene Styrene (commonly known as ABS plastic) was used. Of the five criteria stated above, ABS plastic does not fit criteria 1, 2 and 6, but is relatively much cheaper and can be easily replaced with new copies. Working prototypes of the stretch unit were manufactured with ABS polymer using a Dimension BST 1200es 3D printer (resolution: 1/1000 in). Table 2.11 shows various alternative materials that may be used for better performance.

Table 2.11. : Material Options for the Stretch Device

| Company Name | Material | Concept 1 | Operating Temperature/s | Bio-Compatibility (USP Class VI) |
|---|---|---|---|---|
| 3rd Dimension | 17-4 Steel | 760 Mpa | 316°$C$ | YES |
| GPI Prototyping | GP1 Steel | 540+/-50 Mpa | | YES |
| GPI Prototyping | 17-4 Steel | 760 Mpa | | YES |
| GPI Prototyping | ABS M30i | 36Mpa | 108°$C$ | YES (EtO, UV, No steam) |
| Proto 3000 | GP1 Steel | 540 +/-50Mpa | | YES |
| GPI Prototyping | Ultem 9085 | 71.6MPa | 186°$C$ | NO |
| Cideas | Duraform PA Nylon | 44 Mpa | 177°$C$ | YES |
| 3D Systems | Polyphenyl Sulphone (PPSF) | 55.158Mpa | 189°$C$ | YES |
| 3D Systems | Polycarbonate ISO | 51.7Mpa | 126°$C$ | YES |

## 2.4 Product Evaluation

### 2.4.1 Criteria and Score Allotment for Product Evaluation

*Engineering Requirement 1: "Dimensions of the membrane relative to the dimensions of a single well of the petri dish"*

The internal dimensions of a single well of the petri dish are 78mm x 27.9mm. As the maximum length of the membrane that can be used was calculated to be 55mm, the effective available area for experimentation is 55mm x 27.9mm, which is 1534.5 sq.mm. In terms of percentage, this is 70.5% of the total internal area of a single well. Hence, 60% to 70% area is deemed to be the best case scenario, and will be allotted the maximum number of points, which is 5. The rest of the point distribution is shown in table 2.12 below:

Table 2.12. : Score Allotment for Product Evaluation- Engineering Specification 1

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 60% - 70% |
| 4 | Very Good | 50% - 60% |
| 3 | Good | 40% - 50% |
| 2 | Average | 30% - 40% |
| 1 | Bad | 20% - 30% |
| 0 | Worst | 10% - 20% |

*Engineering Specification 2: "Modulus of elasticity of substrate material."*

The material selected for the substrate must be a hyperelastic material, as it will need to sustain deformations ranging from 5% to 50%. Hence a substrate with an elastic modulus of less than 5MPa is deemed satisfactory, the lesser the value, the better. Table 2.13 below shows the distribution of scores:

*Engineering Specification 3: "Thickness of the membrane."*

Table 2.13. : Score Allotment for Product Evaluation- Engineering Specification 2

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 0 - 1 MPa |
| 4 | Very Good | 1 - 2 MPa |
| 3 | Good | 2 - 3 MPa |
| 2 | Average | 3 - 4 MPa |
| 1 | Bad | 4 - 5 MPa |
| 0 | Worst | $\geq$ 5 MPa |

Thickness of the membrane is critical to holding the membrane firmly. If it thicker than what the clamps can handle, there is a chance that the membrane might slip during the stretch process. By trial and error, a maximum thickness of 2mm was deemed acceptable. The lower the thickness of the membrane, the better it is to work with. Table 2.14 below shows the score distribution:

Table 2.14. : Score Allotment for Product Evaluation- Engineering Specification 3

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 0 - 0.4 mm |
| 4 | Very Good | 0.4 - 0.8 mm |
| 3 | Good | 0.8 - 1.2 mm |
| 2 | Average | 1.2 - 1.6 mm |
| 1 | Bad | 1.6 - 2 mm |
| 0 | Worst | $\geq$ 2 mm |

*Engineering Specification 4: "Melting point/ glass transition temperature of the materials used"*

All materials used for the stretch unit and sleeve must be sterilized before use with cells, as any contamination will affect cell growth. This is done by placing the items in a steam bath @ 120 degrees centigrade for 20 min. Hence all components must have a melting point or glass transition temperature of atleast 120 degrees centigrade. The higher this temperature, the better. The score distribution is shown in table 2.15

Table 2.15. : Score Allotment for Product Evaluation- Engineering Specification 4

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 140°C - 145°C |
| 4 | Very Good | 135°C - 140°C |
| 3 | Good | 130°C - 135°C |
| 2 | Average | 125°C - 130°C |
| 1 | Bad | 120°C - 125°C |
| 0 | Worst | $\leq$ 120°C |

*Engineering Specification 5: "Stretch magnitude range"*

Literature shows that the general magnitudes of stretch used for biological cells ranges from 0 to 15%. Hence, at the maximum length of substrate possible, an elongation of 20% is deemed excellent. The higher the % elongation, the better. The score distribution is given in table 2.16 below:

*Engineering Specification 6: "Frequency range of cyclic stretch"*

Most experiments in current literature do not exceed 2 Hz. Hence, a frequency of 5 Hz may be taken as excellent. The greater frequency that be achieved, the better. The score distribution is shown in the table 2.17 below:

*Engineering Specification 7: "Duration of stretch"*

Table 2.16. : Score Allotment for Product Evaluation- Engineering Specification 5

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | $\geq$ 20% |
| 4 | Very Good | 16% - 20% |
| 3 | Good | 12% - 16% |
| 2 | Average | 8% - 12% |
| 1 | Bad | 4% - 8% |
| 0 | Worst | 0% - 4% |

Table 2.17. : Score Allotment for Product Evaluation- Engineering Specification 6

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | $\geq$ 5 Hz |
| 4 | Very Good | 4 Hz - 5 Hz |
| 3 | Good | 3 Hz - 4 Hz |
| 2 | Average | 2 Hz - 3 Hz |
| 1 | Bad | 1 Hz - 2 Hz |
| 0 | Worst | 0 Hz - 1 Hz |

As seen in the literature review, most cells stop changing their orientation after 6 to 8 hours, so a stretch duration of 10 hours is deemed excellent. The greater this value, the better. The score distribution is shown in figure 2.18 below:

*Engineering Specification 8: "Temperature, atmospheric gases and humidity levels"*

In order for cells to sustain growth, an ideal environment must be provided. This environment must be maintained at 37°C, 5% carbon di oxide, and 90% humidity.

Table 2.18. : Score Allotment for Product Evaluation- Engineering Specification 7

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 9 hrs - 10 hrs |
| 4 | Very Good | 8 hrs - 9hrs |
| 3 | Good | 7 hrs - 8 hrs |
| 2 | Average | 6 hrs - 7 hrs |
| 1 | Bad | 5 hrs - 6 hrs |
| 0 | Worst | $\leq$ 5 hrs |

Hence there are only two scores for this specification: 5, if the environmental conditions are met, and 0, if they are not.

*Engineering Specification 9: "Slip, in relation to displacement of membrane"*

The membrane secured to the clamps, may sometimes slip, which causes undesired stretch magnitudes. Hence, the lower the slip, the better. The score distribution is shown in table 2.19 below:

Table 2.19. : Score Allotment for Product Evaluation- Engineering Specification 9

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 0% - 1% |
| 4 | Very Good | 1% - 2% |
| 3 | Good | 2% - 3% |
| 2 | Average | 3% - 4% |
| 1 | Bad | 4% - 5% |
| 0 | Worst | $\geq$ 5% |

*Engineering Specification 10: "Work surface area needed to assemble the stretch unit"*

All components of the stretch unit must be assembled in a fume hood, in order to keep them out of contamination. Hence, there is only a limited amount of area for the user to work with. The smallest commercially available fume hood has an area of 30"x30". If the user is able to fit all components within an area of 20"x20", it is deemed to be excellent. The score distribution is given below in table 2.20:

Table 2.20. : Score Allotment for Product Evaluation- Engineering Specification 10

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 22"x22" - 20"x20" |
| 4 | Very Good | 24"x24" - 22"x22" |
| 3 | Good | 26"x26" - 24"x24" |
| 2 | Average | 28"x28" - 26"x26" |
| 1 | Bad | 30"x 30" - 28"x28" |
| 0 | Worst | $\geq$ 30"x30" |

*Engineering Specification 11: "Dimensions of the membrane and petri dish in relation to the microscope stage dimensions"*

The dimensions of a popular model inverted microscope Nikon TS-100f was taken as a reference for this specification. The area of the stage is 170mm x 225mm. In order to observe the cells on the membranes, the size of the stretch unit must be less than or equal to the stage size. The lesser the size, the better. An area of 70% was deemed to be the best for handling and observation, while 100% was deemed the worst. The score distribution is shown in table 2.21 below:

*Engineering Specification 12: "Steps to assemble the stretch unit into the driver unit"*

The least number of steps logically possible to assemble the stretch unit into the driver unit is three: Slide stretch unit into base, secure stretch unit to base, and connect stretch unit to driver unit. Any steps added will make the process less

Table 2.21. : Score Allotment for Product Evaluation- Engineering Specification 11

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 70% - 75% |
| 4 | Very Good | 75% - 80% |
| 3 | Good | 80% - 85% |
| 2 | Average | 85% - 90% |
| 1 | Bad | 90% - 95% |
| 0 | Worst | $\geq$ 95% |

efficient. Hence, 3 steps is taken as excellent. The lesser the number of steps, the better. The score distribution is shown in the table 2.22 below:

Table 2.22. : Score Allotment for Product Evaluation- Engineering Specification 12

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 3 |
| 4 | Very Good | 4 |
| 3 | Good | 5 |
| 2 | Average | 6 |
| 1 | Bad | 7 |
| 0 | Worst | 8 |

*Engineering Specification 13: "Total number of steps to complete assembly and start experimentation"*

The minimum number of steps logically needed to complete assembly and run experiments are five. Three steps from the assembly of stretch unit into the base,

and two more steps- connecting the driver to PC, and open and running the stretch program. Hence, 5 steps is taken as excellent. The lesser the number of steps, the better. The score distribution is shown in the table 2.23 below:

Table 2.23. : Score Allotment for Product Evaluation- Engineering Specification 13

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 5 |
| 4 | Very Good | 6 |
| 3 | Good | 7 |
| 2 | Average | 8 |
| 1 | Bad | 9 |
| 0 | Worst | 10 |

*Engineering Specification 14: "Displacement across the membrane in relation to % stretch applied"*

It is important to ensure that the stretch applied to the membrane is being transmitted to it without losses, and if the stretch is the same at all points on the membrane. The difference between % displacement applied to the membrane and the % displacement achieved at any point on it, should not cross a threshold value. This was set as 5%. The lesser this value is, the better. The score distribution is shown in table 2.24 below:

*Engineering Specification 15: "Number of steps to disassemble the membranes after the experiment"*

The least number of steps logically needed to disassemble the membranes is six: disconnect driver from stretch unit, unlock stretch unit, remove stretch unit from base, remove sleeve, push clamps out of stretch plate slots, and place membranes in separate dish. Any steps added will result in increased chances of cells dying, hence six is deemed to be excellent. The score distribution is presented below in table 2.25:

Table 2.24. : Score Allotment for Product Evaluation- Engineering Specification 14

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 0% |
| 4 | Very Good | 1% |
| 3 | Good | 2% |
| 2 | Average | 3% |
| 1 | Bad | 4% |
| 0 | Worst | 5% |

Table 2.25. : Score Allotment for Product Evaluation- Engineering Specification 15

| Score | Description | Value Range |
|---|---|---|
| 5 | Excellent | 6 |
| 4 | Very Good | 7 |
| 3 | Good | 8 |
| 2 | Average | 9 |
| 1 | Bad | 10 |
| 0 | Worst | 11 |

*Engineering Specification 16: "Ensure that there are no shear stresses experienced by the cells, and if present, are negligible"*

As the cells are stretched under a biological fluid, there may be a small amount of shear produced due to the movement of the cells under the fluid. It must be ensured that these stresses are reduced to negligible levels in order to ensure that cyclic tension is the only dominant force acting on the cells. Literature states that a shear stress

that is less than 1 Dyne-s/sq.cm may be considered negligible. Hence any value less than 1 is given the highest points, 5 and any value greater than 1 is given 0.

### 2.4.2 Uniaxial Stretch in Membranes

From engineering specification 14, in order to verify if the strain distribution is even given a required magnitude of displacement, an FEA simulation was performed using ANSYS. All material properties of silicone elastomer (acquired from the manufacturer, table 2.26) were assigned to a 50mm 3D model of the membrane, one of the sides was fixed and a displacement of 5mm (10% stretch) was applied on the other side. Figure 2.22 illustrates this. After solving, the FEA results show that the desired magnitude of stretch is being transmitted as desired to the loaded side of the membrane. This is illustrated by figure 2.23. The membrane also experiences constant strain along the entire membrane, verifying that all points on the membrane are subjected to the same amount of stretch. This is illustrated by figure 2.24.

Table 2.26. : Properties of Silastic Biomedical Grade Silicone Rubber Q7-4840

| Property | Value |
|---|---|
| Relative Density | 1.12 |
| Tensile Strength | 9.4 MPa |
| Elongation, % | 540 % |
| Elastic Modulus, @ 200 % | 2.6 MPa |
| Tear Strength, Die B | 37 KN/m |
| Compression Set, % | 77.3 % |
| Poisson's Ratio | 0.47 |

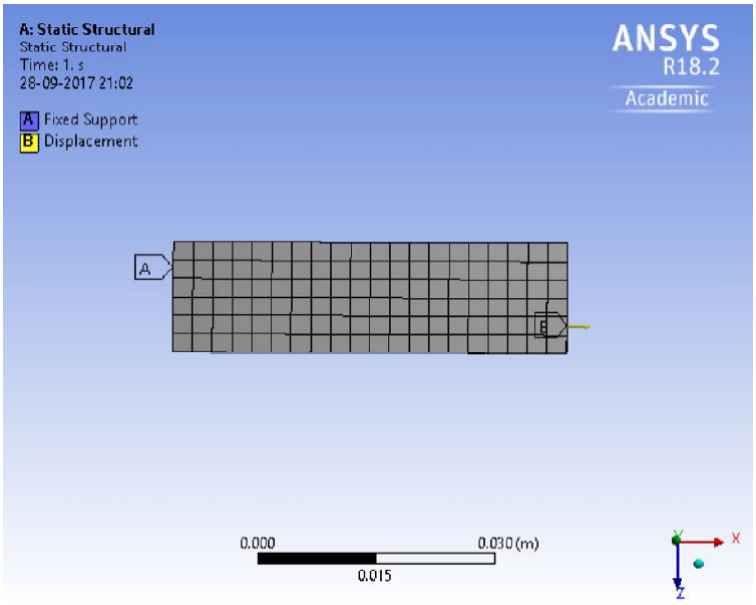



Figure 2.22. : Finite element 3D model to simulate 10% stretch in a 50mm long silicone elastomer membrane. In the figure, A represents the fixed side, and B represents the moving side, with a displacement of 5mm.

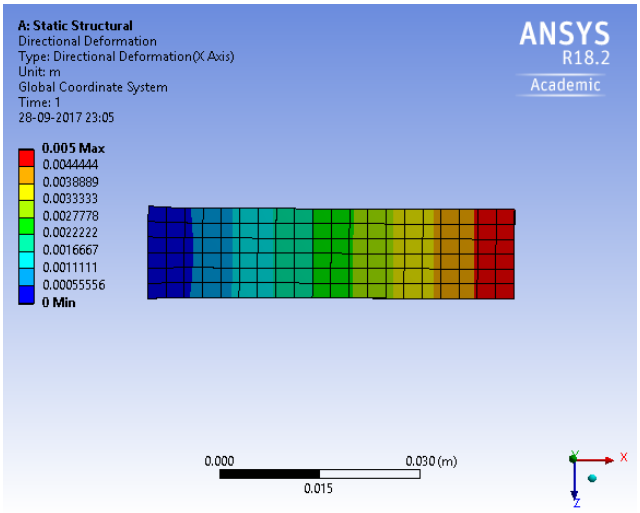



Figure 2.23. : Finite element analysis showing the required magnitude of stretch applied at one end of the membrane, 5mm in this case, and the consequent displacements along the length of the membrane

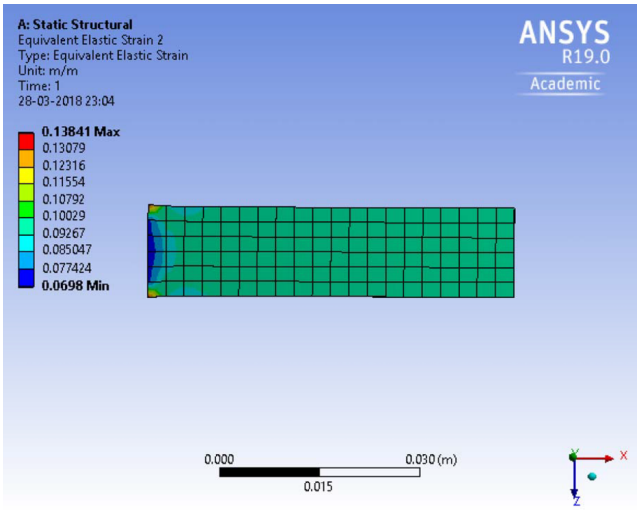



Figure 2.24. : This figure shows the distribution of strain along the membrane during stretch. As depicted, strain is distributed evenly across the membrane, causing every point on it to experience the same amount of stretch.

### 2.4.3 Slip Experienced by Membranes

To verify that there was no slip of the membrane at the clamps while stretching, two ink stains were placed using an alcohol based permanent marker at two random points along the 50mm long/ 14mm wide silicone membrane. It was assembled on to the stretch plates, which were then subjected to a strain of 10% (the membrane will be 55mm in length after application of strain). Before and after stretch pictures of the same stains were taken with a microscope. ImageJ software was used to measure length in pixels between two points placed in the x direction, along the opposite outer edges of the stain. The images before and after stretch, are shown in figures 2.25 through 2.28. The yellow lines represent the points where length was measured. To reduce measuring errors, each distance was measured 5 times and averaged. Theoretical stretch values were also calculated and the difference between these values and values obtained experimentally were compared to find the magnitude of slip. All the data obtained is shown in table 2.27. However, results obtained showed that there are differences between the theoretical and experimental values of distance between the

points after stretch. In a single stain, there is a difference in after stretch distances between two points at different locations. From table 2.27, these differences may be observed. However, since the same stain also shows almost no difference between theoretical and experimental distances at some points, it may be derived that there is no slip at the clamps, and the different values may be a result of the alcohol based stains peeling off at certain areas.

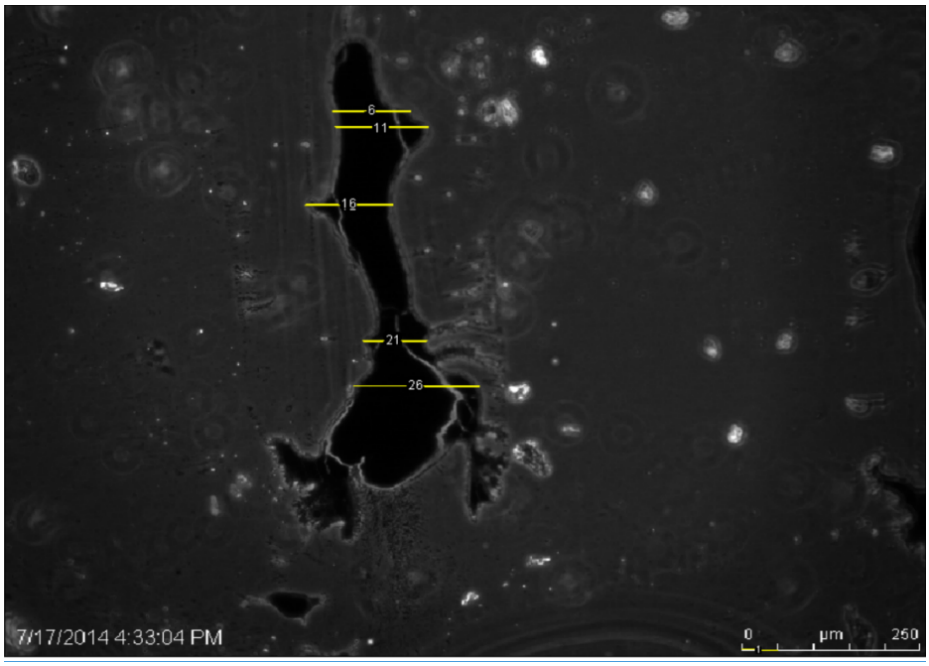



Figure 2.25. : Ink stains were placed on the membrane for the validation of 10% Stretch on a 50mm Silicone Membrane. Lengths of the lines 1 to 26 were measured in pixels using ImageJ software.

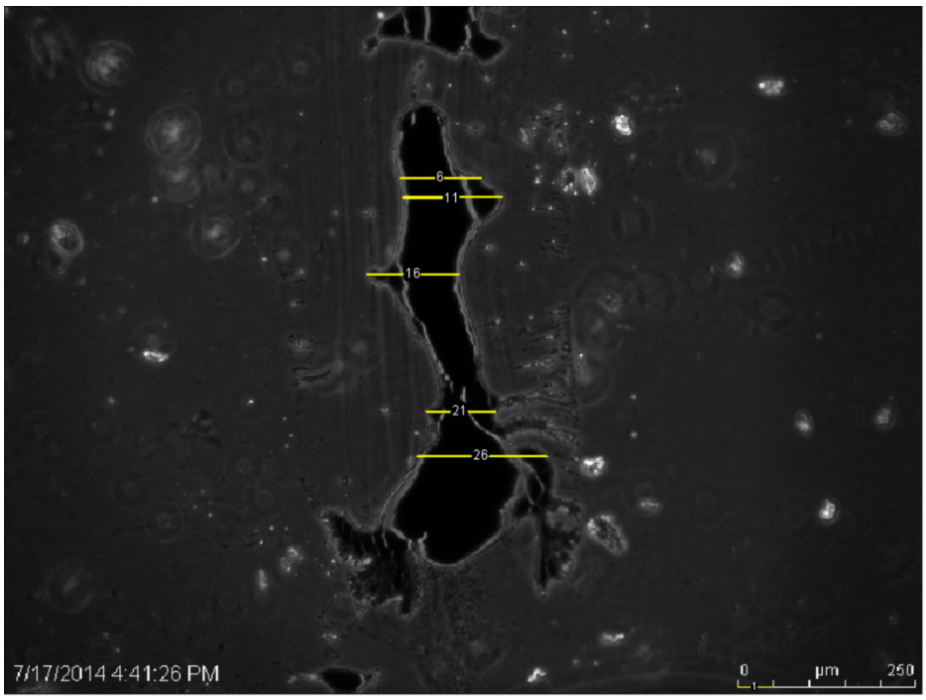



Figure 2.26. : Lines 1-26 were measured again after stretching the membrane by 10% longitudinally. All lines showed elongation.

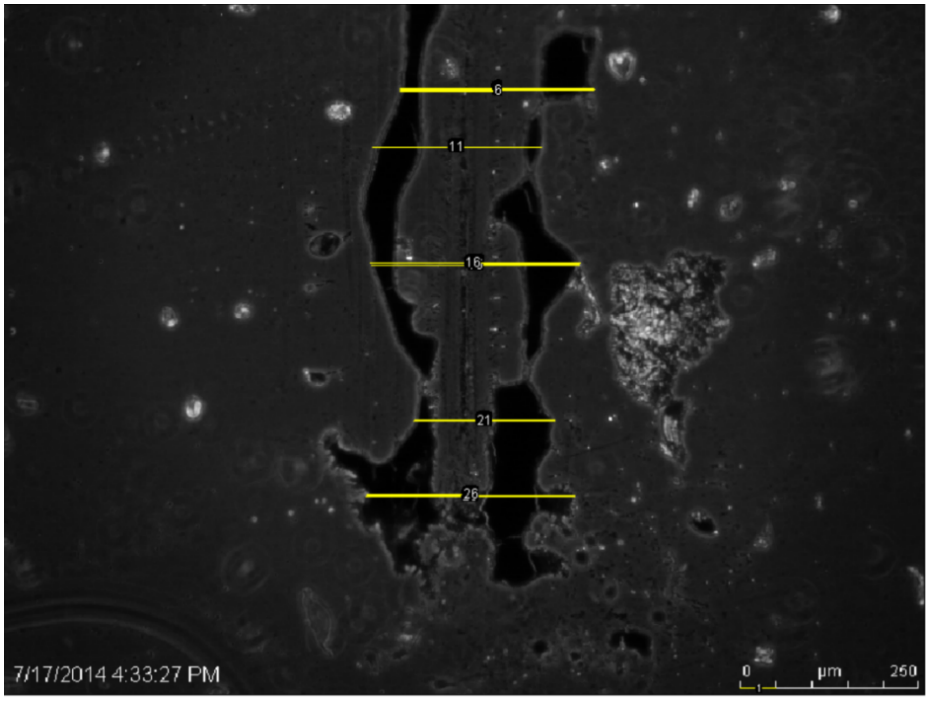



Figure 2.27. : Length of lines 1- 26 were measured in Pixels using ImageJ Software.

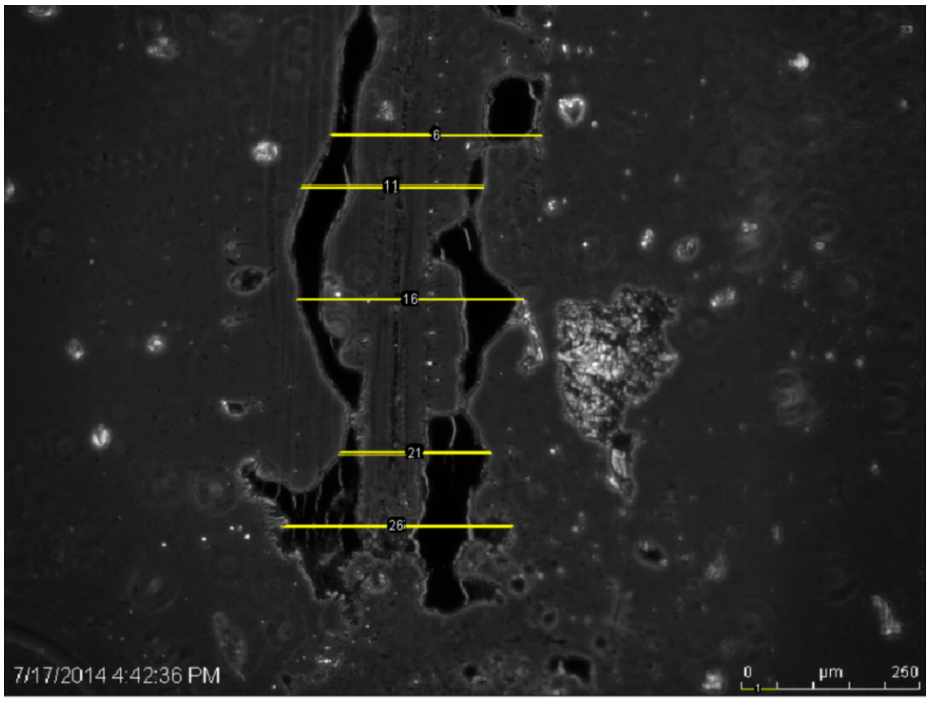



Figure 2.28. : Lines 1- 26 were measured in pixels after stretching the membrane by 10% longitudinally. All lines showed elongation.

Table 2.27. : Data for Ink Stains 1 and 2. Analysis of Theoretical and Experimental Values

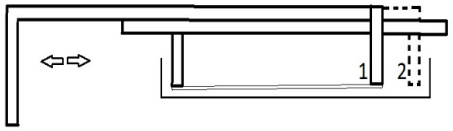
| Line No. | Length Before Stretch ($\mu$m) | Length After Stretch (Theoretical) ($\mu$m) | Length After Stretch (Experimental) ($\mu$m) | Error (Theoretical - Experimental) ($\mu$m) | % Error |
|---|---|---|---|---|---|
| Ink Stain 1 | | | | | |
| 2 to 6 | 106.662 | 117.328 | 112.769 | 4.559 | 3.8 |
| 7 to 11 | 127.946 | 140.741 | 138.786 | 1.955 | 1.38 |
| 12 to 16 | 120.24 | 132.264 | 127.86 | 4.404 | 3.32 |
| 17 to 21 | 86.969 | 95.666 | 95.734 | -0.068 | 0 |
| 22 to 26 | 172.837 | 190.121 | 181.931 | 8.19 | 4.3 |
| Ink Stain 2 | | | | | |
| 2 to 6 | 269.618 | 296.58 | 292.223 | 4.357 | 1.46 |
| 7 to 11 | 234.805 | 258.285 | 252.778 | 5.507 | 2.13 |
| 12 to 16 | 290.543 | 319.597 | 315.186 | 4.411 | 1.38 |
| 17 to 21 | 195.547 | 215.102 | 210 | 5.102 | 2.37 |
| 22 to 26 | 288.877 | 317.764 | 318.889 | -1.125 | 0.35 |

### 2.4.4 Shear Stress on Cells

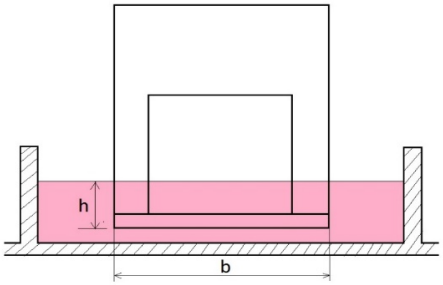
During cyclic stretch, as the membrane is stretched back and forth, the media remains stationary while the cells move along with the membrane. This causes shear stress on the cells. As seen in figure 2.29(a), when the membrane is stretched from position 1 to 2, the cells attached to the membrane move, causing shear stress between the cells and the media above the membrane. To calculate the approximate shear stress on the cells, a parallel plate flow chamber model may be used. A parallel plate flow chamber model can be visualized as a very thin rectangular hollow box, with an inlet and outlet on either side. These are connected to tubes, which are in turn connected to a two-way pump. Biological media can now be pumped through the inlet, causing a constant velocity flow of media inside the chamber. Cells are plated onto the underside of the ceiling of the rectangular chamber, so that when media passes through the chamber, they are adhered to the ceiling, and thus experience shear stress due to fluid flow. Shear stress on cells (calculated from the parameters media viscosity , volumetric flow rate Q, and cross sectional dimensions width b and height h (see figure 2.29(b)) of the flow channel), is said to be negligible if its value is less than or equal to 1 dyne-s/cm2 ($\tau \leq 1$) [23]. During uniaxial stretch, a membrane goes through two different phases. A stretch phase, where the membrane is stretched from position 1 to position 2 (figure 2.29(a)), and the return de-stretch phase, where the membrane is restored to its original position at 1. During each of these phases, cells on the membrane experience shear stress. As this case is approximated using the parallel plate shear stress model, the equation used was as follows:

$$\tau = \frac{6\mu Q}{bh^2} \tag{2.2}$$

[23]. Volumetric flow rate, Q, was taken as the rate at which the membrane was being stretched, which is 0.8 cm/s multiplied by the cross sectional area of the medium directly above the membrane (1.4 cm x 0.2 cm), which yields 0.224 cm3/s. Viscosity of DMEM media was taken to be 0.0078dyne.s/cm2 [24]. The dimensions, b and h were 1.4 cm and 0.2 cm respectively. Substituting these values in the equation above,




(a) Movement of Stretch Plates that induces Shear Stress




(b) Dimensions of the Clip submerged in Media

Figure 2.29. : The figures above show the movement of stretch plates that causes media to move back and forth, causing shear stress on the cells plated on the silicone membranes.

the approximate shear stress was calculated to be 0.1872 $dyne - s/cm^2$, proving that shear is negligible in this case. Also, in order to verify that the shear stress experienced by the cells would not increase with increase in the level of media above the membrane, a maximum value for h (1cm) was considered. Substituting h = 1cm in the equation above, the shear stress was calculated to be 0.03744 $dyne - s/cm^2$, verifying that any volume of biological media in the petri dish would only produce negligible shear stress on the cells.

### 2.4.5 Evaluation of the Unaxial Stretch Machine

This section deals with evaluating the unaxial stretch machine by comparing it to the engineering specifications generated. Criteria were developed for each specification, and points were given based on how well the device satisfied each specification. The device satisfies all engineering specifications successfully. Table 2.28 gives the details.

Table 2.28. : Product evaluation for the uniaxial stretch device, w.r.t. engineering specifications generated.

| Engineering Specification | Product Score | Does the Product meet the Specification? | Explanation |
|---|---|---|---|
| Dimensions of membrane relative to the dimensions of the petri dish well | 4 | Yes | Maximum dimensions of the substrate was arrived at 55mm x 14mm, which is 50.16% of the effective area available for experimentation |
| Elastic modulus of the membrane | 3 | Yes | Silastic biomedical grade silicone elastomer (Q7-4840) was selected, whose modulus of elasticity is 2.6 MPa |
| Thickness of membrane | 5 | Yes | The thickness of the silicone elastomer was 0.254 mm |

*continued on next page*

Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Melting point of materials used | N/A | Yes, with reservations | ABS plastic was used to manufacture the components as it was the cheapest material available. The glass transition temperature, however, is at 105F, making it incompatible for sterilization by steam. Bleaching powder and a lab grade detergent were used to clean the parts, and were exposed to UV light for sterilization. |

*continued on next page*

Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Stretch magnitude range | 5 | Yes | The rack attachment for the driver is more than 100mm, which is well above the 20% elongation needed at maximum length of membrane |
| Frequency range of cyclic stertch | 5 | Yes | The maximum speed of the rack and motor chosen, Cool Muscle RRA 23 with CM1-C-23L20, has a maximum speed of 200 mm/s |
| Duration of Stretch | 3 | Yes | Experiments upto 8 hours have been conducted successfully. The device has not been tested above 8 hours |

*continued on next page*

Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Temperature, atmospheric gases and humidity levels | 5 | Yes | A commercial incubator was used to maintain the environment |
| Slip, in relation to the displacement given to the membrane | 5 | Yes | Tests were conducted to show that there is no slip. However, tests must be done again with better adhesion stains as they may have peeled slightly during stretch. |

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Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Work surface area needed to assemble the stretch unit | 5 | Yes | The total surface area occupied by the top plate, bottom plate, 8 clamps, 4 membranes, plate cover, and sleeve is calculated to be 106.17 sq.in |
| Dimensions of the stretch unit in relation to the microscope stage | 5 | Yes | The largest dimensions in the assembly belong to the top plate, whose area is 21,312 sq.mm, which is 55.7% of the area of the stage,whose dimensions are 170 mm x 225mm |

*continued on next page*

Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Steps to assemble the stretch unit into the driver unit | 4 | Yes | Four steps are needed to assemble the stretch assembly to the base unit |
| Total number of steps to complete assembly and start experimentation | 3 | Yes | Eight steps are needed to finish assembly and start the experiment |
| Displacement across the membrane in relation to % stretch applied | 5 | Yes | The % stretch applied is distributed evenly across the entire membrane. |
| Number of steps to disassemble the membrane after the experiment | 4 | Yes | Seven steps are necessary to disassemble the stretch unit and remove membranes |

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Table 2.28. : *continued*

| Engineering Specification | Product Score | Engineering Specification Met? | Explanation |
|---|---|---|---|
| Ensure that there are no shear stresses experienced by the cells, and if present, are negligible | 5 | Yes | Shear stress was present, and was calculated to be 0.1872 dyne-s/sq.cm, which is less that 1 |

## 2.5 Suggestions for Improvement and Conclusions

The following may be concluded from the evaluation process:

- All engineering requirements have been satisfied. The device is capable of uni-axial cyclic stretch of certain types of substrates.
- Further testing must be done to verify if there is any slip of the membrane during stretch. Although the test has shown that fluctuations may be due to stains losing adhesion to the membrane, they must be redone with stains that have stronger adhesive strengths to absolutely verify that this conclusion is true.
- From the FEA analysis, it is observed that the stretch distribution across the membrane is even.
- The material used for the current product, ABS plastic, is incapable of resisting sterilization by steam. As this is a temporary material, it was cleaned using laboratory grade detergent, bleach and UV light exposure. A list of materials that may be useful has been presented.

# 3. CASE STUDY- CYCLIC STRETCH EXPERIMENT USING THE UNIAXIAL STRETCH DEVICE

## 3.1 Introduction

The experiment presented here were done under the IUPUI Multidisciplinary Undergraduate Research Institute (MURI) program, under mentors Dr. Hazim El-Mounayri, Dr. Julie Ji, and Dr. Omar El-Mounayri. The participating undergraduate students were Jessica Collins, Caleb Comoglio, Zahir Sheikh, Niraj Vipra, Joseph Yeoh, Caleb Comoglio, Jeffery Joll II, and Mai Khuu.

## 3.2 Design of the Experiment

SMCs, when cultured in stationary conditions acquire a generic gene expression profile. In order to prove that in vitro cyclic mechanical stretch can bring the concentration of these markers back to in vivo levels, the markers must show signs of increase in their concentration. An experiment was designed to test this. The following points briefly explain the order in which the experiment was designed to be executed:

1. Two types of SMCs, specifically coronary and bladder cells will be used for this experiment. Each type of the SMCs are plated onto a single silicone membrane, which are pre- assembled into a stretch plate unit, and are incubated.

2. Cyclic stretch is now applied on the SMCs, giving specific magnitude of stretch, frequency and duration.

3. After experimentation, cells are checked visually for density, morphology and dead cells. Proteins are extracted and frozen.

4. Protein analysis will be done to check concentrations of - actin, - actin, calponin and transgelin.

### 3.3 Experimentation

Silicone substrates of dimensions 75mm x 14mm x 0.254mm were used in this experiment. They were coated with fibronectin so as to improve cell adhesion. The membranes were assembled onto the stretch plate assembly and placed on top of the petri dish. Biological media DMEM containing a fixed number of cells are plated onto the membranes. This unit is placed in the incubator maintained at $37^oC$, 5% $CO_2$ and 90% humidity for 24 hours for cell proliferation. The cells are now checked for density and death. The stretch plate and petri dish unit is now slid into the level support and the unit is assembled into the stretch machine, which is placed in a biological incubator. The cells are now stretched cyclically causing percentile strains of 5% to 10% lengthwise, gradually increasing the stretch magnitude every two hours, for a total of 8 hours. The stretch plate is removed and cells are collected. Proteins are extracted and frozen for later analysis. Concentrations of - actin, - actin, calponin and transgelin are identified, with - actin as control. Western blot method is used for this. The results are then normalized and plotted.

### 3.4 Results and Discussion

Material selected for manufacturing various components was ABS plastic as it was a cheap option for testing, and also satisfied a criteria required. However, due to the nature of 3D printing, there were small gaps and imperfections in the components which aided in fungal growth. The components were cleaned with soap and bleached after every experiment as they could not withstand the temperature of the steam sterilization chamber. Alternative materials that satisfied all criteria were discussed. Stainless steel was found to be the best choice.

All components were evaluated for performance and scored above the acceptable 5 points. Table 2.28 shows the details of evaluation. The minimum value was 6.5, while the maximum was 8. If the material change is made, components would score better. Uniaxial stretch that was transferred from the driver to the membrane was verified to be without losses. This was done by simulating stretch on the membrane using ANSYS simulation software. As seen from figure 2.24, strain distribution was even throughout the membrane. There was also no slip calculated, but this must be verified using better methods.

Coronary and bladder SMCs were used to perform a stretch experiment to find out if the device works as intended. The aim of the experiment was to prove that the stretch device can increase concentration levels of proteins in cells cultured in a static environment. 5- 10% strain was provided to the cells for eight hours, raising the stretch levels every two hours. Concentrations of - actin, - actin, calponin and transgelin are identified using the western blot analysis method, with - actin as control. CSMC protein analysis did not follow any expected patterns. Each protein had a fairly erratic expression behavior as indicated by western blot. Alpha actin was barely present after four hours of stretch, peaked at 6 hours, and then fell below static levels again at 8 hours. Gamma actin peaked at 4 hours with an over 2.5 fold increase over static levels. After this it returned to below static levels for both 6 and 8 hours. Calponin followed the same erratic behavior, falling below static levels at 4 hours, drastically peaking at 6 hours, and back to below static levels at 8 hours. Transgelin initially dropped below static at 4 hours, and then gradually returned to base levels at 6 and 8 hour stretch. Protein analysis of bladder smooth muscle cells, however, showed more expected levels of expression for gamma actin and transgelin for static, 4 hour, and 6 hour time periods. Alpha actin was the only protein that exhibited unexpected expression. From its static sample, it fell below base level and remained there for 4 and 6 hours. Both gamma actin and transgelin had slight increases after 4 hours of stretch and at 6 hours they both experienced significant fold increases. This information is illustrated in figure 3.1. These unusual results are most likely explained

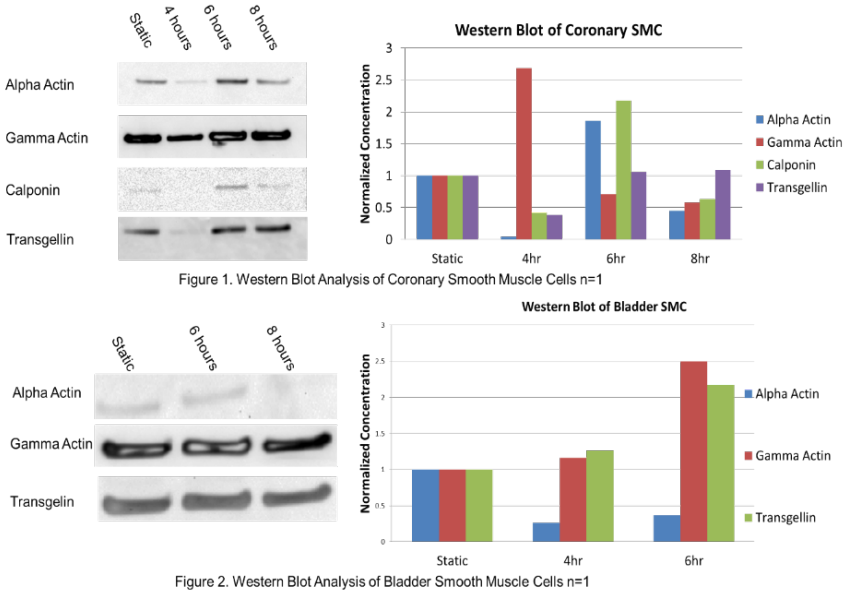



Figure 3.1. : Western Bolt Analysis for CSMCs and BSMCs

by experimental errors caused by researchers or machine errors. Several modifications were made to the machine throughout the research process, which may have altered expected results. Cell culturing, adhesion, and plating techniques all may have to be modified in order to prevent inaccurate protein expression. Because only one round of stretching was completed, it is difficult to make any definitive conclusions about the data. Future rounds of testing will serve to validate and regulate the results.

# 4. CONCLUSIONS AND FUTURE SCOPE

The Capstone design project report was summarized. Future improvements as suggested by the report were taken into account and implemented in the new design. Customer requirements and engineering objectives were re- written. Uniaxial stretch machine was designed based on the new engineering requirements, CAD models of the different parts were created and 3D printing was used to manufacture a working prototype. Testing and validation of the machine was done to verify that all design requirements were met. Experiment was conducted to verify if cyclically stretched cells show a shift towards contractile phenotype. Analysis of data shows that the device has the potential to carry out its intended function, although more tests should be run in order to make sure. A change in material of the manufactured components, most preferably to stainless steel, may result in a much better device, as this will allow for sterilization by steam and better handling.

A biaxial stretch unit is also being developed to fit in to the same driving unit, so the user can run either uniaxial or biaxial experiments. The biaxial system will use the same driving unit as the uniaxial system. The only difference will be in the stretch plate design. Circular membranes are placed on top of the bottom plate and the top plate will slide onto it, fixing the membranes. The plates are secured using thumb screws. This assembly is inverted onto a 6 circular well petri dish containing the indenter plate, against which the membranes are stretched. Springs stop the stretch plates right on top of the indenters. Cells are plated from the top using a pipette via media. This assembly is placed into the incubator for proliferation and adherence. The entire assembly is now placed onto the driving unit. A steel rope is used to connect the motor shaft to a cam mechanism located on top of the stretch plates. When the shaft moves, the cam is rotated back and forth in partial cycles,

which transmits the movement vertically via a cam follower on the stretch plates, stretching the membranes. The biaxial device is illustrated in figures 4.1 and 4.2.

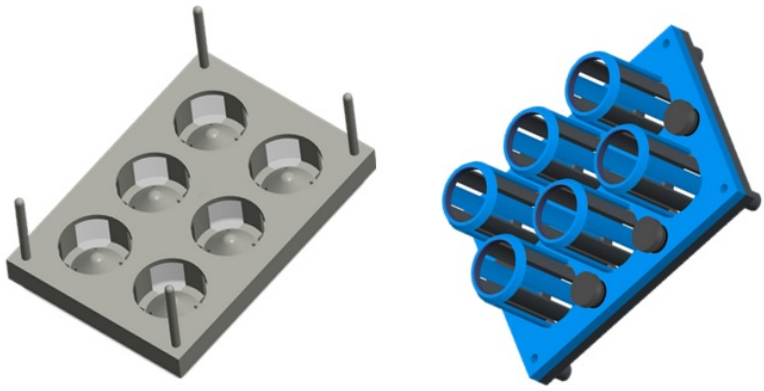


Figure 4.1. : Figure 4.1 illustrates the CAD model for the biaxial stretch unit plates and bottom dish. The membrane is secured between the rings of the two plates. The membrane is held in place by friction.

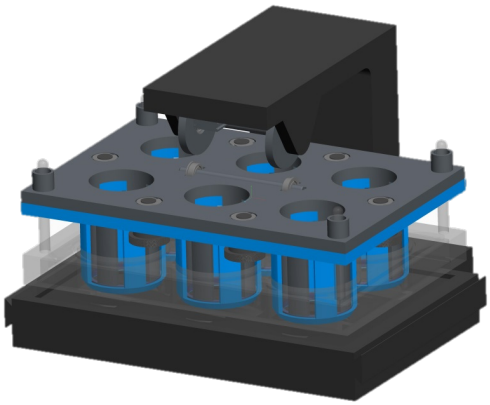


Figure 4.2. : Figure 4.2 shows the corresponding CAD model for the unit assembly. Assembled stretch plates are placed onto the bottom dish, and this unit is placed into the cam rocker holding unit.

# APPENDICES

# A. APPENDIX
# UNIAXIAL STRETCH DEVICE - SUMMARY OF CAPSTONE DESIGN 2008

## The Final Product

### The Clips

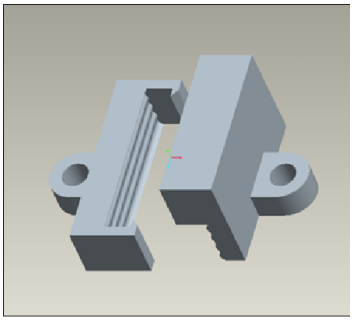
The design of the clips was treated as a completely separate design. The requirement here was that the clips should hold the membrane without any slip, and should be easy to handle. The idea was modeled in Pro-E and a prototype was manufactured using 3D printing methods. The material used was ABS plastic. Figure A.1 shows the clips modeled in Pro-E and the clips after being manufactured by 3D printing. For 10% stretch, these clips were very effective in holding the membranes without any slip.

### The Stretch Plates

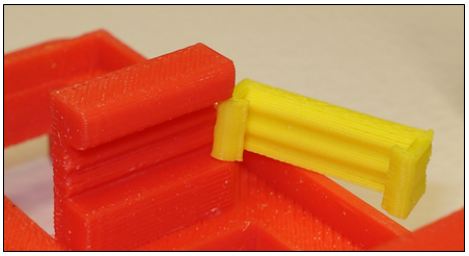
The stretch plates were designed to be able to slide on top of one another. Clips mount directly onto these plates, which in turn will hold the membranes. Figure A.2 shows the CAD model and the manufactured stretch plates using 3D printing methods.

### Mount Sub - Assembly

This is the part that is used to hold the petri dish and stretch plates together. The petri dish would slide onto and rest on the two sliding red bars, right below the

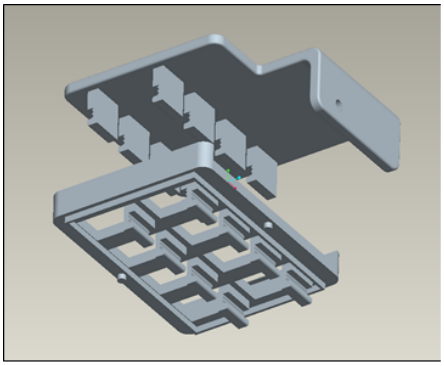


(a) Clip Design- CAD Model

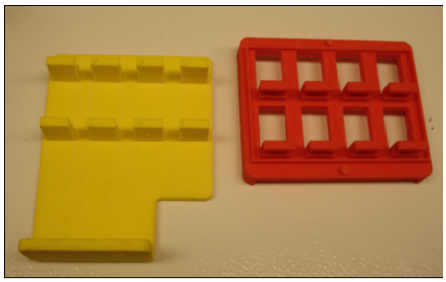


(b) Clip Design- 3D Printed Prototype

Figure A.1. : Figure A.1(a) illustrates the CAD model of the membrane clamping mechanism developed by the capstone design team. Figure A.1(b) shows the 3D printed model of the prototype. A flexible silicone membrane is sandwiched between the two clips and pressed together until they click into place, restraining the membrane.



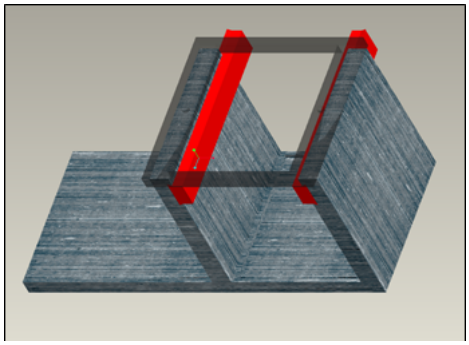
(a) Stretch Plate Design- CAD Model



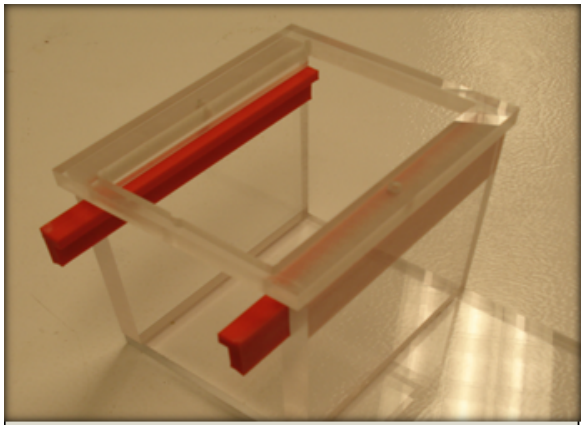
(b) Stretch Plate Design- 3D Printed Prototype

Figure A.2. : Figure A.2(a) illustrates the CAD model of the stretch plates developed by the capstone design team. Figure A.2(b) shows the prototype created using 3D printing methods. These plates are assembled onto each other so that they slide in a linear fashion. The unit is then turned upside down and the membranes are asembled.

rectangular form. The stretch plates are then assembled onto so that it is constrained completely in all directions. Figure A.3 illustrates this.



(a) Mount Sub Assembly- CAD Model

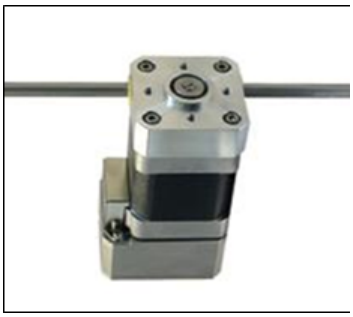


(b) Stretch Plate Design- Prototype made from Polycarbonate Sheet and 3D printing methods

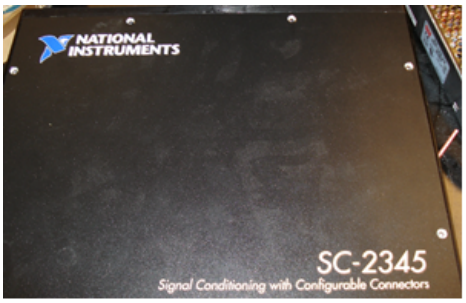
Figure A.3. : Figure A.3(a) illustrates the CAD model of the mount sub- assembly. Figure A.3(b) shows the manufactured parts. The red bars were developed by 3D printing methods and the transparent base was manufactured using a polycarbonate sheet. The petri dish was neamt to sit on top of the red bars just under the rectangular top, and the stretch plates were placed from the top of the unit. The assembled membranes would sit just above the bottom of the petri dish.

### The Driver

The driver chosen for this design was a cool muscle motor with an RRA linear actuator. The reason this motor was chosen was because it came preassembled, and the hardware driver and the encoder came with it. A National Instruments signal conditioner was used for signal conditioning. The driver was also suitable for lab environment as it could withstand a humidity of 90 % and temperatures of up to 40 degrees centigrade. Figure A.4 shows the driver and the signal conditioning unit.



(a) Cool Muscle Servo Motor with RRA Linear Actuator




(b) National Insruments Signal Conditioner

Figure A.4. : Figure A.4(a) illustrates the driver used to move the stretch plates cyclically. Figure A.4(b) shows the signal conditioner from the manufacturer National Instruments.

### Sensors

The design team decided on two sensors to collect real-time information on the magnitude of stretch and force exerted on the membranes for each cycle. These would be an inductive proximity sensor and a load cell. For the inductive sensor, the one made by the manufacturer Baumer was selected. It can measure up to 5mm of displacement. For the force sensor, a 50 lb load cell was selected from the company HTC- Load cell central. Figure A.5 shows the sensors.


Figure A.5. : This figure illustrates the sensors used to measure. Figure on the left shows the inductive proximity sensor from Baumer Inc, which measures the distance travelled by the stretch plate from each cycle, and on the right is a load cell from the manufacturer HTC Load Cell Central, which measures the amount of force transmitted from the driver for each cycle of stretch.

### Control Software

The software used to enter commands and control the motor is called Cool Works Lite, a proprietary software of the servo motor company Cool Muscle Corp. The software used to monitor and record the data from the devices is LabVIEW from National Instruments. This software was selected because of its ease of use, and excellent technical support from the company. Figure A.6 shows the LabVIEW software interface.

### Suggested Changes by the Capstone Design Team

The capstone design group suggested a few areas where changes may be made. The clips are one area where a change is necessary. They have also suggested that the dimensions of the stretch plates could be changed for better assembly of membranes. Another change recommended was to replace the induction sensor with a laser type sensor, so as to increase range and accuracy. The last recommendation was to change to material used for the 3D printed parts. Polymers commercially available through the firm Solvay, were suggested as an ideal material.

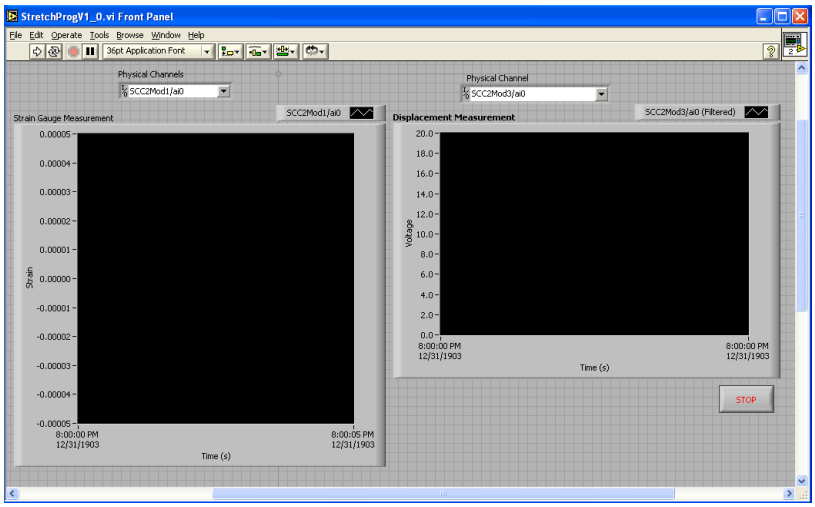



Figure A.6. : This figure illustrates the front end user interface of the LabVIEW software developed by National Instruments. LabVIEW may be used to control electronic devices using signal acquisition.

# B. APPENDIX
# THE DESIGN PROCESS

For successfully designing and manufacturing a product, a standardized design process has been put forth by author David G. Ullman. The process involves five major steps, which are further classified in to three phases. The first phase, also known as the product definition phase, involves the first step of the design process. The next two steps in the process is the second phase or the conceptual design phase, where concepts are developed and evaluated. The last two steps of the design process, the product generation and evaluation, form phase three. Each of these steps are discussed briefly below:

**Phase 1**

- **Step 1: Understanding and Analyzing the Problem**

  This is the first step in the design process. The designer must first identify the target group of customers. He must then gather information on customers requirements. This can be done by directly talking to them, or by taking surveys, or by taking feedback on existing devices. After establishing the customer needs, the relative importance of each need is determined. The devices currently available in the market are evaluated against these needs. Engineering specifications are now developed corresponding to the customer requirements. In order to develop engineering requirements, it is mandatory that the designer convert each customer need into a set of parameters that can be measured and evaluated. If a customer need cannot be represented by numbers, it must be re-done so that calculations can be made to evaluate the requirement. If parameters cannot be developed for evaluation, the customer need must be scrapped.

A relation must now be made between customer needs and engineering specifications. The inter-relation between engineering specifications, and the current competition evaluation are also noted. All this information will give the user a chance to prioritize his requirements. A popular tool to do this is called quality function deployment. The quality function deployment chart establishes a relation between the customer needs and engineering requirements. It also assigns importance to each engineering requirement so the engineer may prioritize among them. The flow chart shown in figure B.1 shows sequence in which a QFD is implemented. Figure B.2 shows the different sections (or rooms) in a house of quality diagram, which illustrates the QFD process.

**Phase 2**

- **Step 2: Developing Potential Concepts**

  According to author David Ulman, a concept is "*An idea that is sufficiently developed to evaluate the physical principles that govern its behavior*". A concept maybe a sketch, an idea or even a set of calculations. A concept must hold enough information so that the designer may be able to assess what technologies might be helpful in turning the concept into a product, how the product might look and work, and what methods may be used to manufacture it. The method to generate concepts maybe summarized briefly with the following steps:

  1. Understand the basic overall function of the intended product.
  2. Split the overall function into smaller, individual sub functions, sub- subfunctions, and so on. This is called functional decomposition until a function can no longer be split. Arrange all functions according to their order of execution.
  3. Use methods such as brainstorming, the 6-3-5 method, researching patents or the theory of inventive machines to develop ideas that can be used to

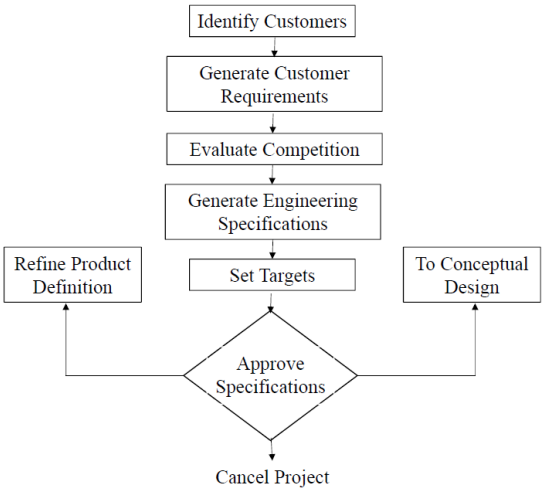



Figure B.1. : This figure illustrates the sequence of steps taken during the product definition phase. The information gathered during this process is illustrated using a house of quality diagram.

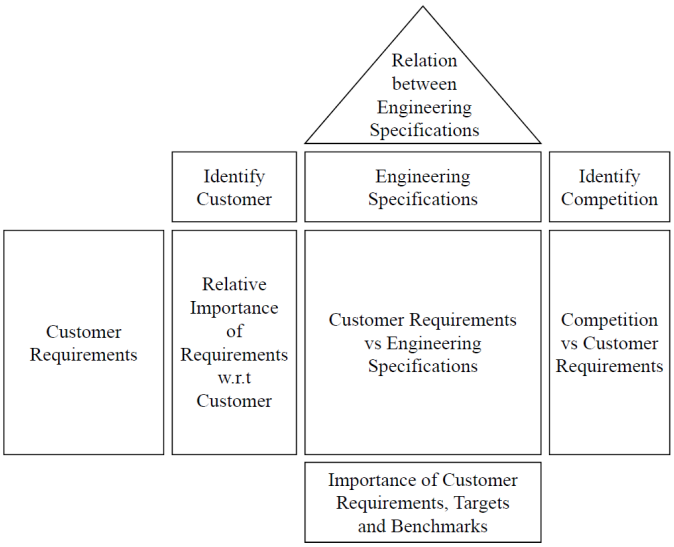



Figure B.2. : This figure illustrates the compilation of different steps taken during the project definition phase. It provides an easy reference and acts as a guide for the rest of the design process.

fulfill the individual functions while fulfilling the overall function. Add information on possible design, manufacturing methods and materials. This is a concept. Develop as many concepts as possible for each function. This is called a morphology.

4. Select the best concept for each individual function. Combine the best possible concepts of different functions to implement subfunctions, and eventually, the overall function. This is called function concept mapping, and will help the designer decide on the best concept for each as well as the overall function.

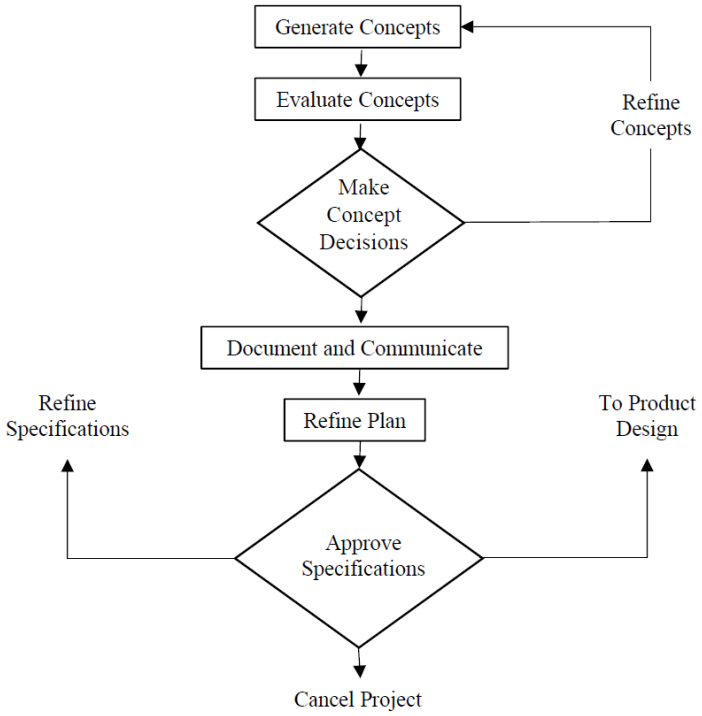



Figure B.3. : This figure illustrates the different steps taken during the conceptual design phase.

- **Step 3: Concept Evaluation**

  Every concept must be evaluated before they are compared against each other and a final selection is made. The first step is to gather all information that

is known about the concepts and refine it in such a way that it may be at the same level of abstraction as the customer requirements. Once the concepts are generated and have enough information, a decision matrix is built using the Pugh's method (developed by Stuart Pugh, a mechanical design engineer). In this method, all functions and engineering specifications that are related to each concept is listed out and their relative importance is determined. They are then given scores based on how well they satisfy each requirement. Scores are multiplied to their relative importance, and the total sum of each concept is noted. The concept with the highest score is best suited for the application at hand. Figure B.3 illustrates the steps involved in phase 2.

**Phase 3**

- **Step 4: Product Generation**

  Product generation deals with the materials and manufacturing methods used to generate products based on concepts developed earlier. The most common method is to list all constrains and relations between individual components, create CAD models to verify all parameters, and manufacture the components using the most feasible of the different methods available (CNC machining, molding, casting, 3D printing etc.).

- **Step 5: Product Evaluation**

  Similar to concept evaluation, product evaluation also aims at listing each parameter, determining their relative importance, creating a datum for evaluation and evaluating the product based on the functional parameters. Product evaluation is done after manufacturing and testing the prototype.

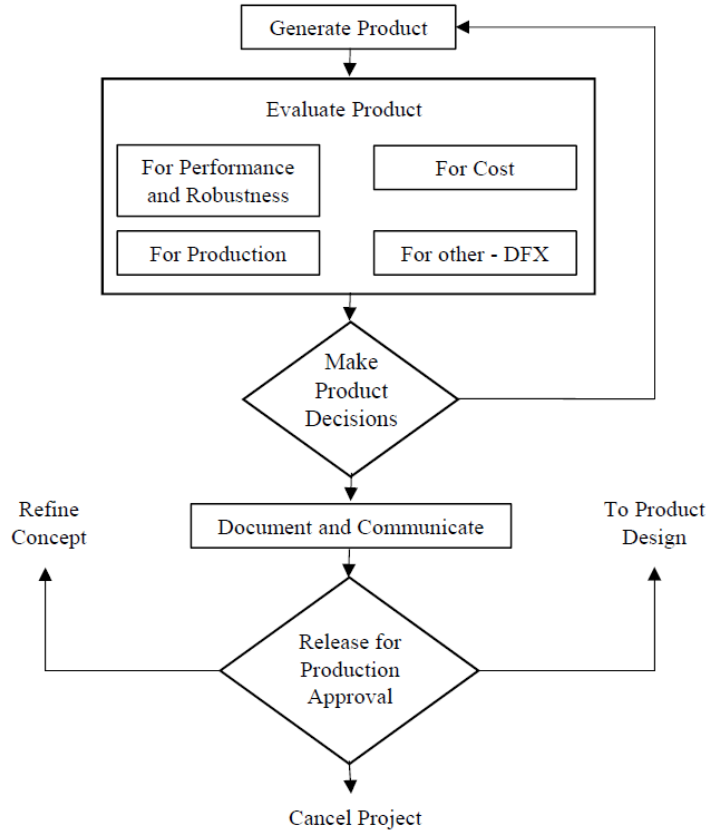



Figure B.4. : This figure illustrates the different steps taken during the product design phase. The steps include generating and evaluating the product, making decisions and approving/ cancelling the process, refining the concept, or proceeding to the next step.

# REFERENCES

## REFERENCES


[1] Mark W Majesky. Developmental basis of vascular smooth muscle diversity. *Arteriosclerosis, thrombosis, and vascular biology*, 27(6):1248–1258, 2007.

[2] PC Dartsch and H Hämmerle. Orientation response of arterial smooth muscle cells to mechanical stimulation. *European journal of cell biology*, 41(2):339–346, 1986.

[3] Thomas D Brown. Techniques for mechanical stimulation of cells in vitro: a review. *Journal of biomechanics*, 33(1):3–14, 2000.

[4] Kirit A Bhatt, Eric I Chang, Stephen M Warren, Shin-e Lin, Nicholas Bastidas, Shadi Ghali, Aurelia Thibboneir, Jennifer M Capla, Joseph G McCarthy, and Geoffrey C Gurtner. Uniaxial mechanical strain: an in vitro correlate to distraction osteogenesis. *Journal of Surgical Research*, 143(2):329–336, 2007.

[5] Douglas W Hamilton, Timothy M Maul, and David A Vorp. Characterization of the response of bone marrow-derived progenitor cells to cyclic strain: implications for vascular tissue-engineering applications. *Tissue engineering*, 10(3-4):361–369, 2004.

[6] Sung-Gon Kim, Toshihiro Akaike, Tadashi Sasagaw, Yoriko Atomi, and Hisashi Kurosawa. Gene expression of type i and type iii collagen by mechanical stretch in anterior cruciate ligament cells. *Cell structure and function*, 27(3):139–144, 2002.

[7] C Lee Wen-chi, Timothy M Maul, David A Vorp, JP Rubin, and Kacey G Marra. Effects of uniaxial cyclic strain on adipose-derived stem cell morphology, proliferation, and differentiation. *Biomechanics and modeling in mechanobiology*, 6(4):265, 2007.

[8] Deidre MacKenna, Sonya R Summerour, and Francisco J Villarreal. Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. *Cardiovascular research*, 46(2):257–263, 2000.

[9] Bryan J Pfister, Timothy P Weihs, Michael Betenbaugh, and Gang Bao. An in vitro uniaxial stretch model for axonal injury. *Annals of biomedical engineering*, 31(5):589–598, 2003.

[10] James H-C Wang, Pascal Goldschmidt-Clermont, Jeremiah Wille, and Frank C-P Yin. Specificity of endothelial cell reorientation in response to cyclic mechanical stretching. *Journal of biomechanics*, 34(12):1563–1572, 2001.

[11] James H-C Wang, Fengyan Jia, Guoguang Yang, Shaohua Yang, Brian H Campbell, David Stone, and Savio L-Y Woo. Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin e 2 and levels of cyclooxygenase expression: a novel in vitro model study. *Connective tissue research*, 44(3-4):128–133, 2003.

[12] Guoguang Yang, Richard C Crawford, and James HC Wang. Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. *Journal of biomechanics*, 37(10):1543–1550, 2004.

[13] *Biomechanics of Smooth Muscle Cell Differentiation: Development of and experimental. Study using an Innovative In Vitro Mechanical System - Phase I.* Indiana University, 2005.

[14] *Biomechanics of Smooth Muscle Cell Differentiation: Experimental Study Using an Innovative in Vitro Mechanical System- Phase II.* Indiana University, 2006.

[15] Gang Bao and Subra Suresh. Cell and molecular mechanics of biological materials. *Nature materials*, 2(11):715–725, 2003.

[16] Keiichi Kanda, Takehisa Matsuda, and Takahiro Oka. Two-dimensional orientational response of smooth muscle cells to cyclic stretching. *ASAIO journal (American Society for Artificial Internal Organs: 1992)*, 38(3):M382–5, 1991.

[17] Paul R Standley, Antonino Camaratta, Brian P Nolan, Christian T Purgason, and Melinda A Stanley. Cyclic stretch induces vascular smooth muscle cell alignment via no signaling. *American Journal of Physiology-Heart and Circulatory Physiology*, 283(5):H1907–H1914, 2002.

[18] Dalia Somjen, Itzhak Binderman, Esther Berger, and Arie Harell. Bone remodelling induced by physical stress is prostaglandin e2 mediated. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 627(1):91–100, 1980.

[19] Murray C Meikle, John J Reynolds, Anthony Sellers, and John T Dingle. Rabbit cranial sutures in vitro: a new experimental model for studying the response of fibrous joints to mechanical stress. *Calcified Tissue International*, 28(1):137–144, 1979.

[20] Emily Wilson, Krishnankutty Sudhir, and Harlan E Ives. Mechanical strain of rat vascular smooth muscle cells is sensed by specific extracellular matrix/integrin interactions. *Journal of Clinical Investigation*, 96(5):2364, 1995.

[21] Konstantin G Birukov, Nathalie Bardy, Stéphanie Lehoux, Régine Merval, Vladimir P Shirinsky, and Alain Tedgui. Intraluminal pressure is essential for the maintenance of smooth muscle caldesmon and filamin content in aortic organ culture. *Arteriosclerosis, thrombosis, and vascular biology*, 18(6):922–927, 1998.

[22] Loren A Matheson, N Jack Fairbank, Geoffrey N Maksym, J Paul Santerre, and Rosalind S Labow. Characterization of the flexcell uniflex cyclic strain culture system with u937 macrophage-like cells. *Biomaterials*, 27(2):226–233, 2006.

[23] Rommel G Bacabac, Theo H Smit, Stephen C Cowin, Jack JWA Van Loon, Frans TM Nieuwstadt, Rob Heethaar, and Jenneke Klein-Nulend. Dynamic shear stress in parallel-plate flow chambers. *Journal of biomechanics*, 38(1):159–167, 2005.

[24] Chong Wang, Brendon M Baker, Christopher S Chen, and Martin Alexander Schwartz. Endothelial cell sensing of flow direction. *Arteriosclerosis, thrombosis, and vascular biology*, 33(9), 2013.