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Fabrication of PDMS and SU-8 Micro-Cantilevers for Studying the Biomechanics of
Cardiomyocytes

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Andrea Marquez Navarro

Thesis Committee:
Professor William Tang, Chair
Associate Professor Elliot Botvinick
Assistant Professor Anna Grosberg

2015

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ABSTRACT OF THE THESIS

Fabrication of PDMS and SU-8 Micro-Cantilevers for Studying the Biomechanics of

Cardiomyocytes

By

Andrea Marquez Navarro

Master of Science in Biomedical Engineering

University of California, Irvine, 2015

Professor William Tang, Chair

It is essential to study the biomechanics of cardiomyocytes *in vitro* in order to develop drugs and diagnostic tools to treat cardiovascular diseases. Here a grooved and pegged micro-cantilever to observe and quantify the contractile behavior of cardiomyocytes was developed. The platform was based on muscular thin films technologies; however, this platform presents some unique features.

The aim of the project was to develop a simple platform to measure the amount of cantilever deformation as the shape and degree of the cantilever bending under stress can then be used to quantify the contractility of the cells. The end result is a micro-cantilever platform that can be used to study cardiomyocytes and their responses to various drugs.

The micro-cantilever contains pegs and grooves that cause the cardiomyoctes grown on the platform such that it mimics the anisotropic structure of native cardiac tissue. Two sets of micro-platforms were fabricated out of two different materials:

poly(dimethylsiloxane) (PDMS) and SU-8 photoresist. SU-8 and PDMS are both biocompatible and have their own advantages and disadvantages. The SU-8 micro-platforms were fabricated using multilayer photolithography while the PDMS micro-cantilevers were fabricated using multilayer soft lithography. HL-1 cardiomyocytes were then cultured on to SU-8 substrates and primary neonatal rat cardiomyocytes were cultured onto PDMS cantilevers. Concurrently, finite element analysis was performed in order to determine the dimensions that caused the most cantilever deflection.

CHAPTER 1

INTRODUCTION

1.1 Importance of Studying Cardiomyocytes

Cardiovascular diseases are the leading cause of morbidity and mortality in the United States [1]. The pharmaceutical industry also has a great need for drug testing platforms for cardiotoxicity as safety liabilities related to the cardiovascular system account for 45% of drug withdrawal [2]. Therefore, there is a high demand for a relevant and cost-effective cardiovascular model to perform early stage drug discovery studies and observe their effects on the biomechanics of cardiomyocytes.

One of the difficulties in studying the biomechanics of cardiomyocytes is the microenvironment of cardiac tissue must be replicated. In order to better study drugs' effects on cardiomyocytes, it is necessary to emulate the heart's native structure. In the human heart, extracellular matrix (ECM) fibrils naturally align cardiac cells [3]. The cells anisotropy promotes mechanical contraction and electrical propagation resulting in higher contractile force [4].

There have been many reports on methods in which to more closely replicate the anisotropic characteristics of cardiac tissue *in vitro* and its use in studying the biomechanics of cardiomyocytes. In this work, two sets of micro-platforms were designed and tested: SU-8 substrates and PDMS micro-cantilevers. The designs of these works were based on improvements on previous methods of studying cardiomyocytes.

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1.2   Existing 2D Methods of Studying Cardiomyocytes *In Vitro*

There are many existing approaches used to study cardiomyocytes *in vitro*. Here, we describe three that influenced the design of the micro-platforms.

Muscular Thin Films

Alford et al fabricated and tested muscular thin films (MTFs) with neonatal rat ventricular myocytes. The rectangular MTFs were fabricated out of PDMS and then microcontact printed with lines of extracellular matrix so that the cardiomyocytes align in an anisotropic manner. One end was fixed creating a cantilever like structure. They then were able to estimate the stress the cells exerted based on the radius of curvature the films bent to when the cells contracted [5]. Grosberg et al designed a "heart on a chip" based on this muscular thin film (MTF) technology [4]. They were able to measure up to eight samples in real time with this type of device.

Biomimetic Heart Sheets

Chen et al cultured human pluripotent stem-cell-derived cardiomyocytes (hPSC-CMs) on polystyrene and polyethylene shrink-film [6]. The flat sheets were made from polystyrene, while the lined and wrinkled sheets were made from polyethylene shrink-film. The films were then hot embossed using PDMS to transfer the necessary features. The authors found that the wrinkled films showed the most cardiomyocyte alignment and sarcomere orientation. They were then able to test various drugs using the platforms and measure cardiotoxicity.

Micropatterned Cantilevers

You et al fabricated flat, peg patterned, grooved, and peg and grooved cantilevers [7]. These were fabricated using compressive PDMS molding. They cultured neonatal

ventricular myocytes on them and observed that peg and grooved patterned beams experienced the most beam bending. You et al were able to measure the beam bending by measuring the displacement of the cantilever with a horizontal microscope and comparing the displacements to a finite element model to determine the stress generated.

Two of three platforms described here use PDMS as the material for the thin films. PDMS is prone to absorbing molecules in cell media which can affect cell experiments while SU-8 does not [8]. While the platforms made from the polyethylene and polystyrene film are more tedious to fabricate than PDMS platforms or SU-8 platforms.

Although this is not a comprehensive review, several requirements for cardiomyocyte testing are apparent. In general, the devices need to mimic the anisotropic nature of the heart, be simple to fabricate, have a fixed end, and be able to measure stress through measurements of beam deflection or radius of curvature. If possible, multiple cantilevers should be measured at a time.

1.3   Advantages of Micro-Fabrication

The two methods of fabrication used to create these platforms were photolithography and soft lithography. The majority of the fabrication was done in a clean room environment where the process conditions were able to be precisely controlled and small feature sizes (≥20 μm) and even smaller layer thicknesses (≥5 μm) could be achieved. Fabrication was batch processed, allowing for multiple platforms to be fabricated at one time and required minimal use of expensive capital equipment. This allowed us to create many iterations of the micro-platforms with minimal expense.

Multilayer SU-8 Fabrication

SU-8 is an epoxy based negative photoresist used for many types fabrication. With the use of SU-8, feature thicknesses down to 0.5 microns can be achieved with SU-8 2000.5 [9]. SU-8 is most often used to make the master molds for standard soft lithography. However, it can also be used to make the devices themselves. When SU-8 is exposed to UV light it is cross-linked making it insoluble to SU-8 developer. SU-8 is desirable as a material for cantilever fabrication as it is chemically inert, transparent allowing for cell observation, and biocompatible [10].

The challenge with SU-8 is in fabricating multilayer free-floating platform. Several methods were considered and discussed in detail later.

Multilayer Soft Lithography Fabrication

Soft lithography is a micro-fabrication technique that uses a patterned elastomer as a mold to make micropatterns or microstructures. A master mold is first made using lithographic techniques. Then an elastomeric structure, e.g. polydimethylsiloxane (PDMS), is cast from the master. Soft lithography is capable of generating structures as small as 30 nm [10]. PDMS has many advantages over SU-8. It is more flexible, and relatively inexpensive.

PDMS is normally cast and then used in a single layer. In order to get free floating structures, two layers must be cast and then bonded together. Several methods were researched and discussed later in the thesis.

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1.4   Cell Lines

HL-1 Cell Line

For biological experiments with SU-8 substrates, the HL-1 cardiomyocytes were cultured on the devices. Claycomb et al. developed the cell line, deriving it from the AT-1 mouse atrial cardiomyocyte tumor lineage [11]. The HL-1 cells have the structural characteristics that are typical of embryonic atrial cardiomyocytes and express genes that match cardiac muscle cells. The most important quality of these cells for the purposes of this thesis is that they maintain spontaneous contractile activity. Although these are derived from tumor cells and their biological properties may differ significantly from normal cells, their contractile behavior allows for the initial testing and development of the SU-8 and PDMS micro-platforms. HL-1 cells can also be serially passaged and stored long term in liquid nitrogen, making these cells easier to work with and obtain than primary cells.

Rat Neonatal Ventricular Myocytes

Primary rat cells were also used with PDMS micro-cantilever. They are capable of exerting a much higher contractile force than HL-1 cells. These primary rat cells were harvested from Sprague Dawley rats when the pups were 2 days old. Although these cells are more costly and difficult to obtain, their ability to contract at higher forces make them ideal for testing beam bending on the PDMS micro-cantilevers.

CHAPTER 2

MICRO-PLATFORM DESIGN

2.1 Introduction

The current micro-cantilever design is the latest enhancement of previous generations demonstrated in the Microbiomechanics Laboratory and muscular thin films from other groups. The cantilevers previously made in our lab are similar in design and method of fabrication. However, the current approach has several improvements with its method of imaging and its addition of pegs and grooves to promote cell elongation and cell alignment. The addition of a proximity chrome mirror to facilitate imaging allows for imaging of beam bending using a simple inverted microscope.

In order to test a wide variety of unique beam dimensions, the platform was designed with 180 individual beams with 90 unique geometries. It was designed to test several different beam geometries and determine which geometry demonstrates the most cantilever bending.

2.2 Beam Bending

There are many existing methods to calculate surface stress due to cell contraction through beam bending. Several groups have reported using a modified version of Stoney's equation.

$$\sigma = \frac{Et^3}{6R(1-\upsilon)h^2\left(1+\frac{t}{h}\right)}$$

Where σ is cardiomyocyte contractile stress, E is elastic modulus of the PDMS, t is PDMS thickness, R is MTF radius of curvature, h is 2D myocardium thickness and υ is Poisson's

ratio of the PDMS [12], [13]. This method of stress calculation assumes the cell layer is thin compared to the substrate and undergoes isotropic contraction.

Alford et al. described a method of correlating the two by considering the MTF as a bilayer beam consisting of a passive material layer and an active cell layer. The cell layer undergoes contraction described as finite volumetric negative growth. When the cells contract along their long axes while they are attached to the beam, this contraction results in cell stress and substrate bending. With this method, the stress the cells generated can be calculated from the measured radius of curvature. The moduli and thicknesses of both the beam layer and cell layer are also taken into account and modeled as Neo-Hookean solids [5]. This method made fewer assumption than Stoney's equation. It does not assume that the cells undergo isotropic contraction but considers orthotropic contraction and makes no assumptions about the relative substrate and tissue thickness [5].

These two methods of calculation were kept in mind in designing the micro-cantilevers beams used to study the cardiomyocytes.

2.3   Micro-Platform Design

Micro-Cantilever Design and Dimensions

The design of the cantilevers was heavily influenced by previous work done by Allison Baker and by J. You et al. Baker had previously worked to create a similar design that consisted of grooved micro-cantilevers [14]. The current design continues the work by Baker and adding the pegs from J. You et al [7].

In order maximize the number of unique beam widths and lengths options, the beams were organized in groups of 30. Each group had a different beam width and consequently different numbers of grooves (see Table 1). The lengths of the beams started

at 100 µm and increased by 100 µm until ending at the longest beam which is 1600 µm. The

diameters of the pegs were kept constant at 20 µm in diameter and repeated every 100 µm

(see Figure 2.1). These dimensions were based on the results of J. You et al. [7]. They found

that PDMS cantilevers with grooves and pegs spaced every 100 µm exhibited the most

beam bending. The smallest achievable dimension for the pegs using our method of

fabrication was 20 µm in diameter. Unfortunately, due to limitations with using a

transparency and photolithography, we could not achieve as fine dimensions as their

cantilevers. They had created their mold using a high-precision quartz mask and etching

[7].
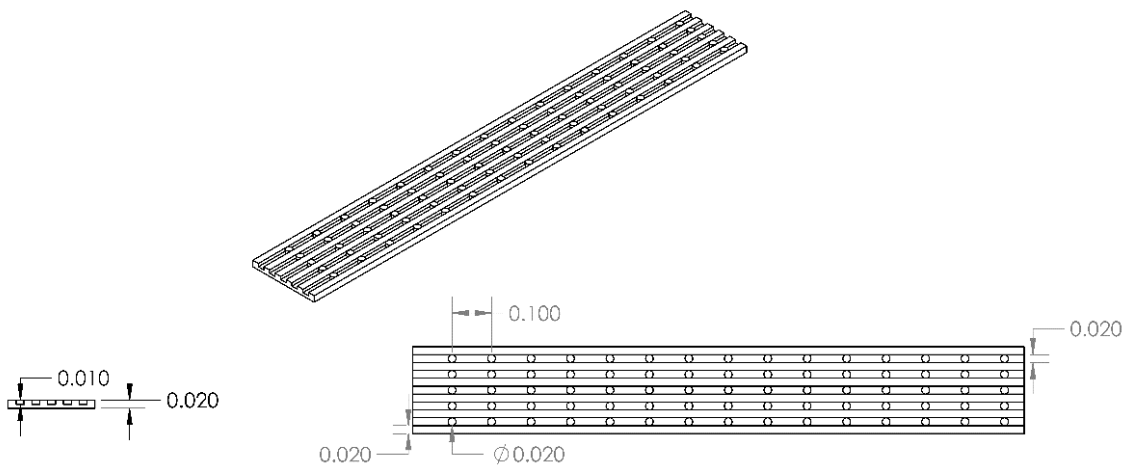
Figure 2.1 Cantilever Drawing with Dimensions
Units are in millimeters
Drawing created with SolidWorks (Dassault Systèmes)

The dimensions for the thicknesses and widths of the grooves were also based on

other groups' work on muscular thin films. Several groups have used thickness ranging

from 14 µm to 60 µm [4], [12], [15]. 20 µm was chosen as the thickness of the beams with

grooves because it was feasible to reach this thickness using photolithography and soft

lithography. The dimensions of the grooves were designed to be 20 µm wide and 10 µm in

depth. The widths of the grooves were chosen based on lengths of HL-1 cell and the dimensions used for microcontact printing: 14 μm to 15 μm in length and 20 μm width stamps respectively [5], [16]. The depths of the grooves were designed to be as thin as possible while still being feasible to fabricate.

Within each beam cluster, the distance between the centers of the tips of the beams was a constant 500 μm. Each group of 30 beams had 15 unique beams. Each beam had an identical pair in the group with the same dimensions. These pairs of beams were included in case one of the beams became damaged and nonfunctional during the fabrication process. It was also more likely to see beam deflection since it would increase the chances of seeing cardiomyocytes contracting on a beam. These dimensions were kept constant for all versions of the cantilevers.
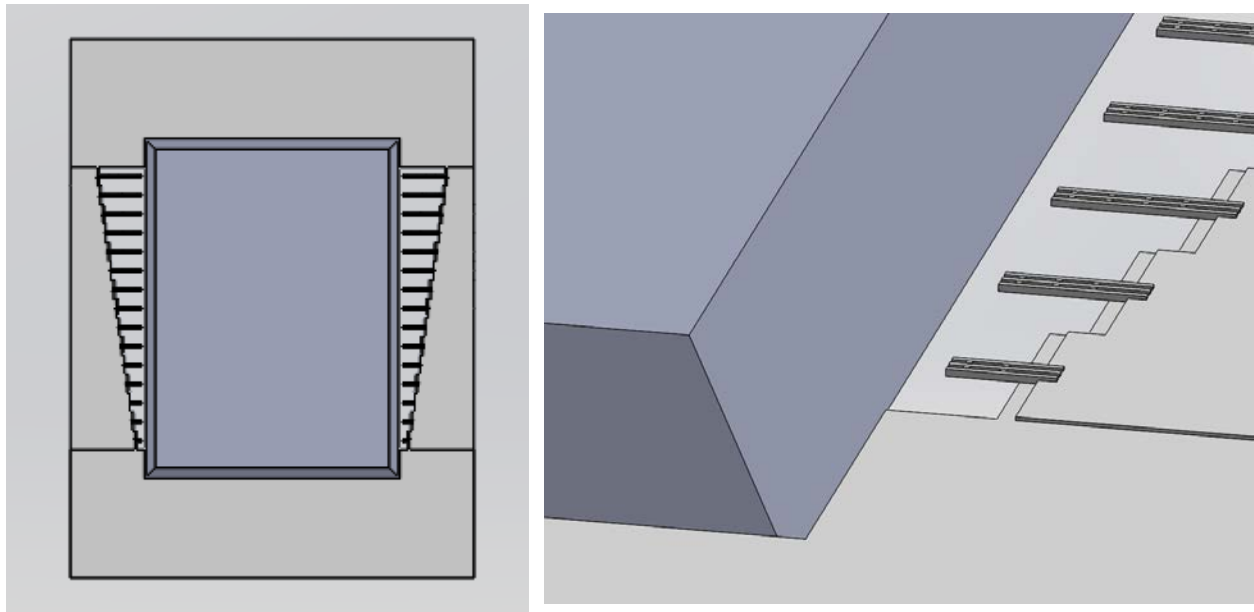
Figure 2.2 Model of Micro-Platform Design
In the center is the chrome mirror. This was placed into the PDMS support section. Six groups of these micro-platforms with varying widths were fabricated at a time.
Model created with SolidWorks (Dassault Systèmes)

Beam Cluster	Width [μm]	Number of Grooves per Beam
1	100	2
2	140	3
3	180	4
4	220	5
5	260	6
6	240	7

Table 1 Beam Dimensions

For all three iterations, groups of beams were clustered into 6 groups based on their widths. Within these groups, the lengths of the beams varied from 200 μm to 1600 μm.

Cantilever Support Design
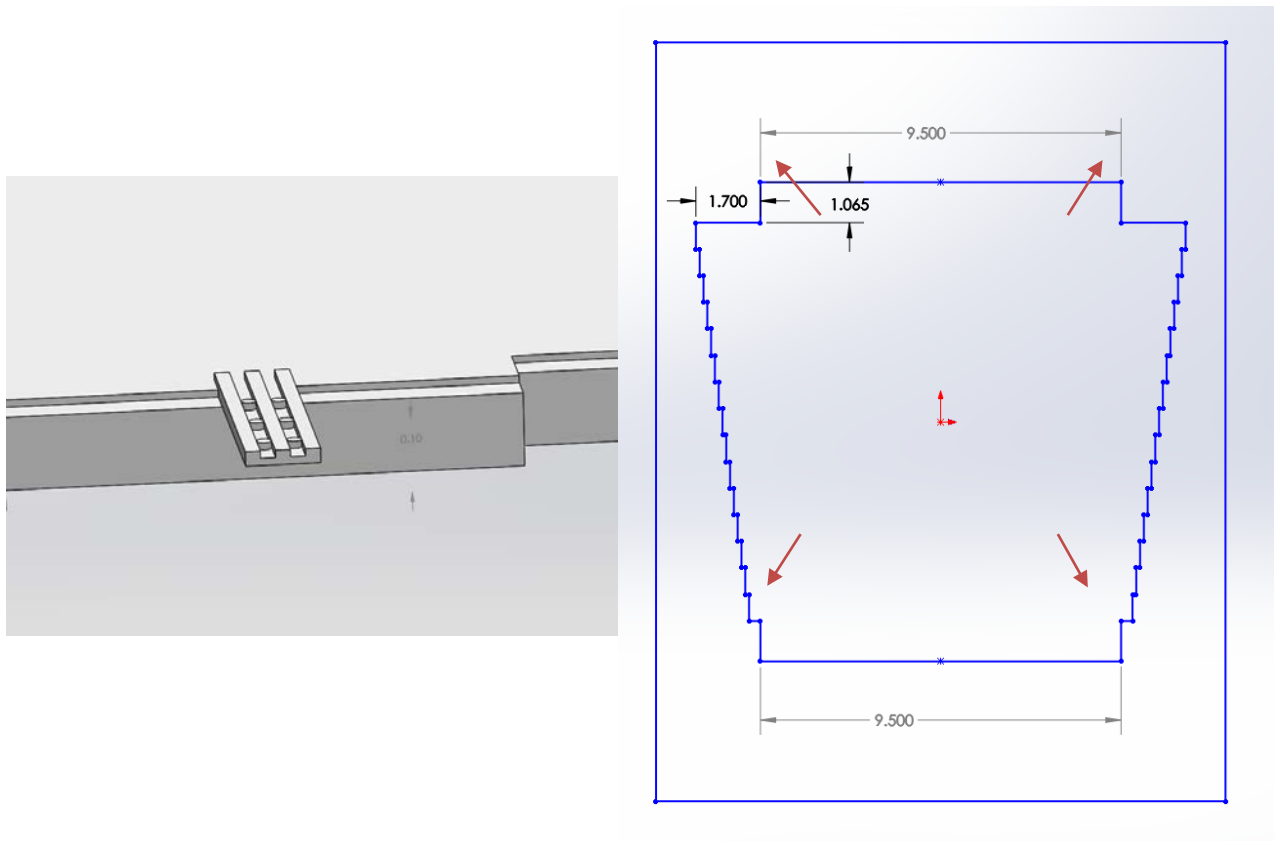
Figure 2.3 Cantilever Attached to Support Section and Sketch of Support Section
The cantilever sits 100 μm above the bottom of the support section.
Units are in mm
Model created with SolidWorks (Dassault Systèmes)

The support section was designed to allow the cantilevers to be free floating. The wells in the supports were 100 μm so that if cells were to fall beneath the cantilevers, they would not attach to the underside of the cantilevers and interfere with the beam bending. The support section would also keep the cantilevers within the working distance of the mirror. It also contained a well that was slightly larger than the dimension of the designed mirrors that would fit inside the well: 9.5 mm x 12.6 mm. The well was designed to be slightly larger to accommodate tolerance of the mirror dimensions. The arrows in the figure above indicate the areas of PDMS that would help align and keep the mirror in place.

Mirror Design
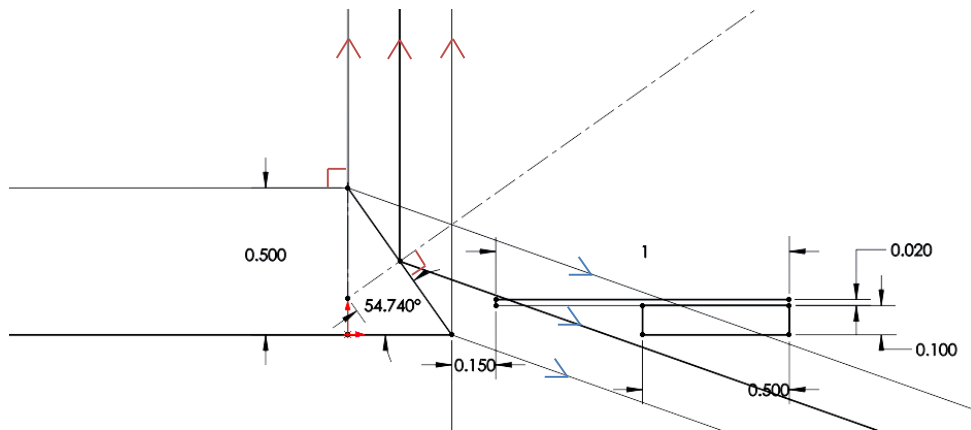
Figure 2.4 Diagram of Mirror with Cantilever
Units are in mm
Diagram created with SolidWorks (Dassault Systèmes)
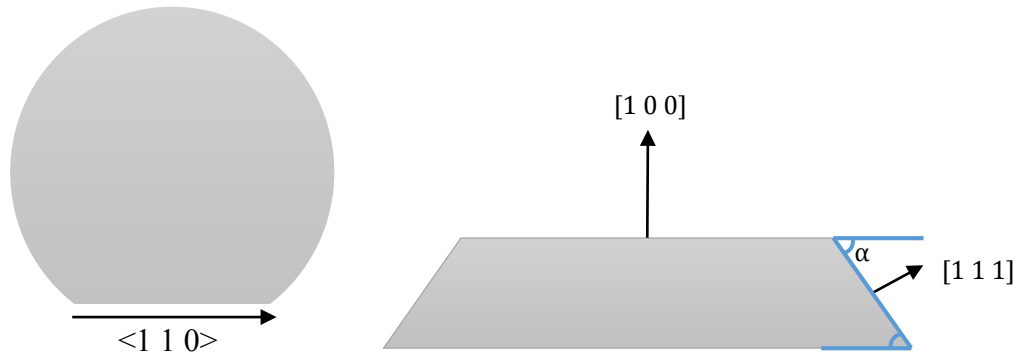
Figure 2.5 Top View of Silicon Wafer (Left) Side View of Etched Silicon Wafer (Right)
Wafers used for this project were all [1 0 0] oriented

The mirror was the next portion designed. The mirrors were fabricated out of

[1 0 0] oriented silicon wafers. These silicon mirrors were created through anisotropic wet

etching with KOH. If a rectangular mask is accurately aligned along the <1 1 0> direction

(the flat portion) of a [1 0 0] oriented silicon wafer and etched, the wafer will be etched

along the [1 1 1] plane. This results in the angles seen in Figure 2.4.

The manner in which these wafers will etch is very predictable. [1 0 0] wafers will

always etch on the [1 1 1] plane which result in a sidewall with an angle of 54.74° [10]. This

can be calculated with the formula below where h, k, and l are the miller indices of the two

planes.

$$\cos(\alpha) = \frac{h_1 h_2 + k_1 k_2 + l_1 l_2}{\sqrt{h_1^2 + k_1^2 + l_1^2}\sqrt{h_2^2 + k_2^2 + l_2^2}} \text{ [17]}$$

With this angle and the thickness of the wafer as the height of a right triangle, the

other lengths of the triangle can be calculated. This allows the calculation of the length and

width of the mirror. The mirrors and support section were designed such that the

cantilevers would be 150 μm away from the mirror and would stay within the imaging

range of the mirror throughout its bending.

2.4   Mask Designs

After the platform was designed, the next step was to design the photomasks that

would be used in the clean room during the micro-fabrication process. The first mask for all

iterations of the micro-platform was for patterning chrome alignment marks since it was

essential that the masks be aligned properly. The alignment marks on the last three masks

consist of plus symbols. The alignment marks on the first mask are four boxes in which the

plus symbol can be centered. Each layer was created in SolidWorks (Dassault Systèmes) as

a sketch in each of their own part file. These were then exported to AutoCAD (Autodesk)

where they were arranged and combined into one file to be printed. Finally, the masks

were printed at 24,500 dots-per-inch on a 0.007 inch thick transparency film (Fuji) by

CAD/Art Services Inc. (Bandon, OR).

Three different designs for the micro-platforms were created. The first set of platforms fabricated were SU-8 substrates, then SU-8 cantilevers with mirrors, and finally PDMS cantilevers with mirrors.

SU-8 Cantilevers
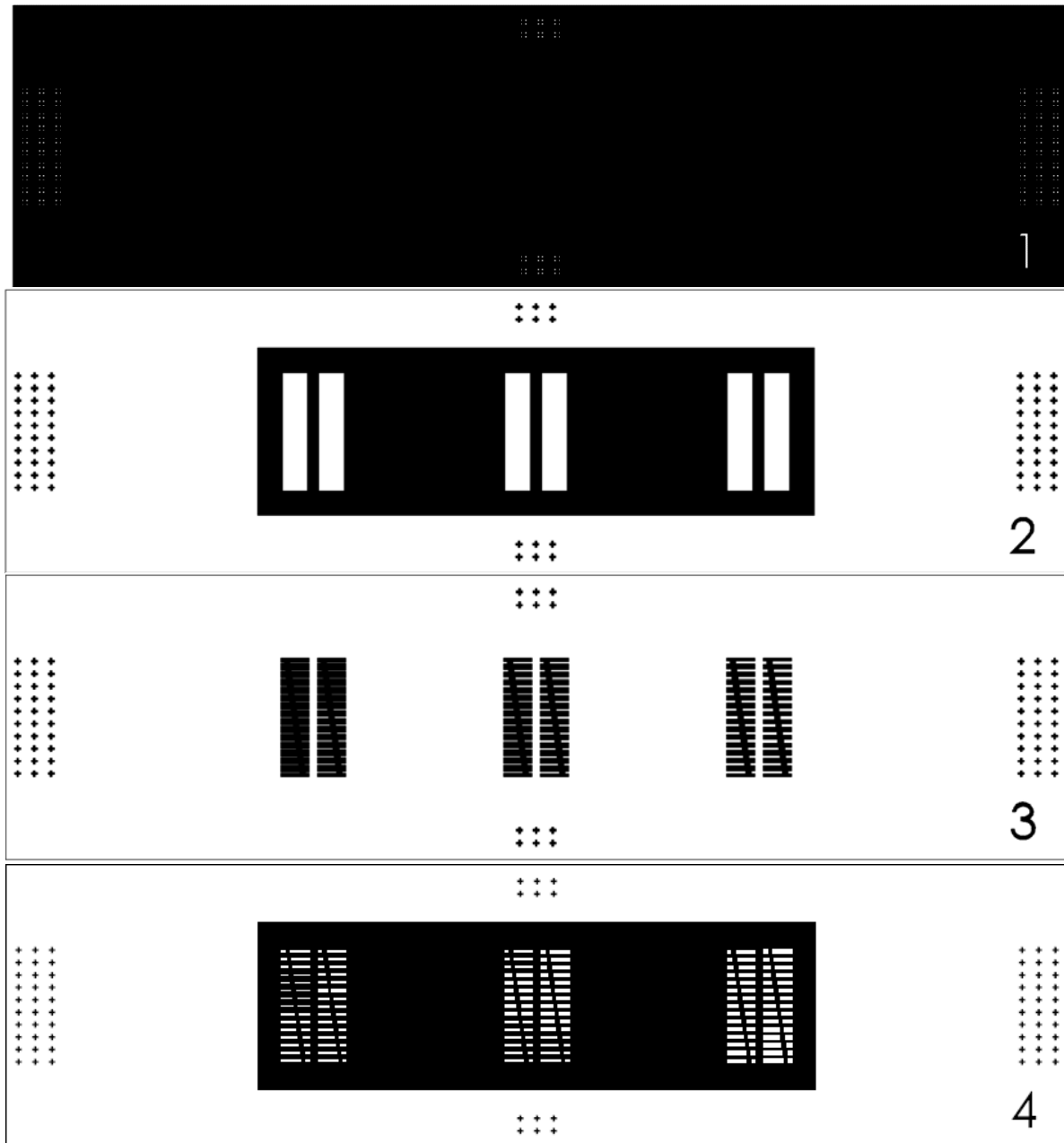
Figure 2.6 SU-8 Cantilevers Photomask
Mask 1 is used to pattern chrome alignment marks. It is necessary to use chrome alignment marks since this design consists of 3 layers. The alignment marks facilitate mask alignment. The alignment marks on this mask are of the opposite polarity but align exactly with the marks on the following three masks.
Mask 2 is used to create the support section.
Mask 3 is used to create the cantilevers.
Mask 4 is used to create the pegs and grooves.

SU-8 Cantilevers with Mirrors
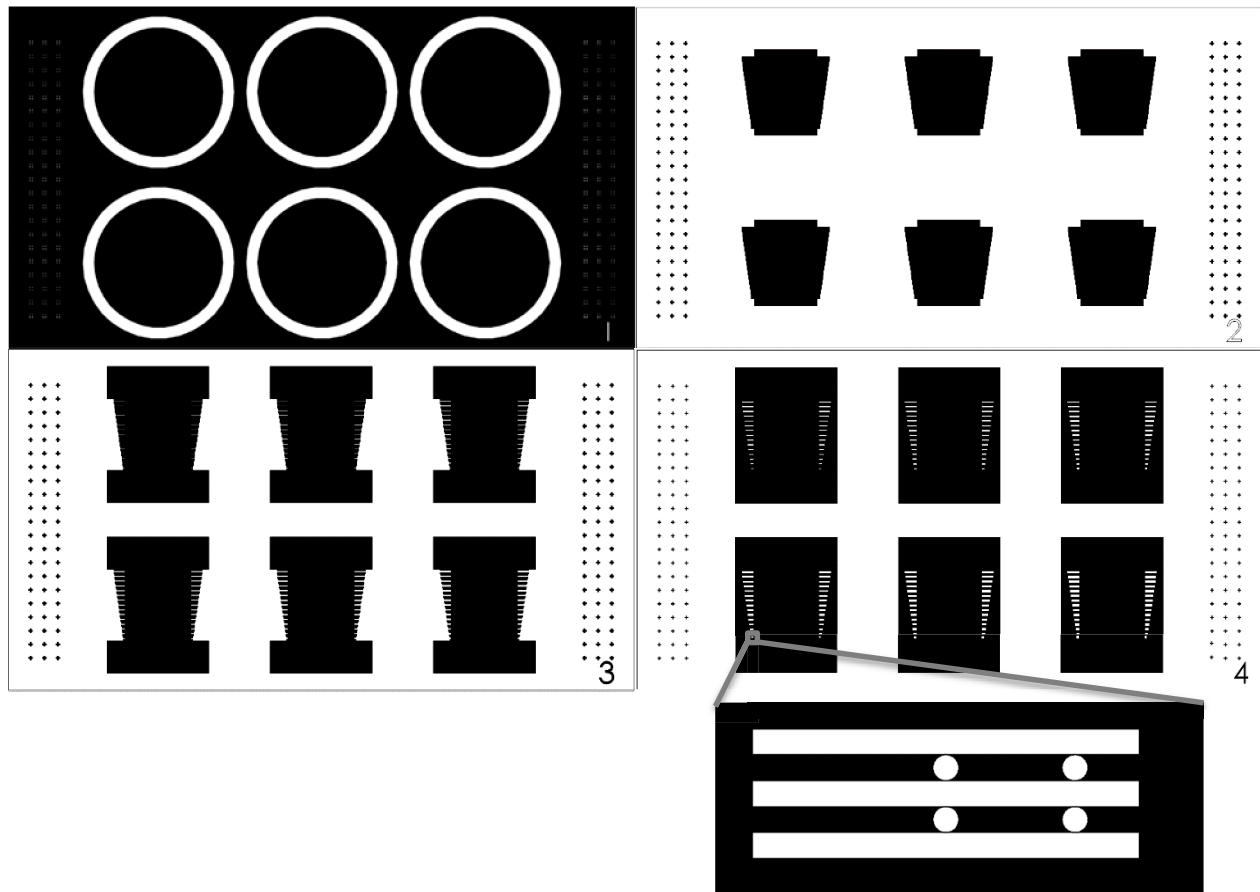
Figure 2.7 Photomask for SU-8 Cantilevers with Mirrors
Mask 1 is used to pattern chrome alignment marks. The circles are to facilitate alignment of PVC tubing for wells.
Mask 2 is used to create the support section.
Mask 3 is used to create the cantilevers.
Mask 4 is used to create the pegs and grooves.
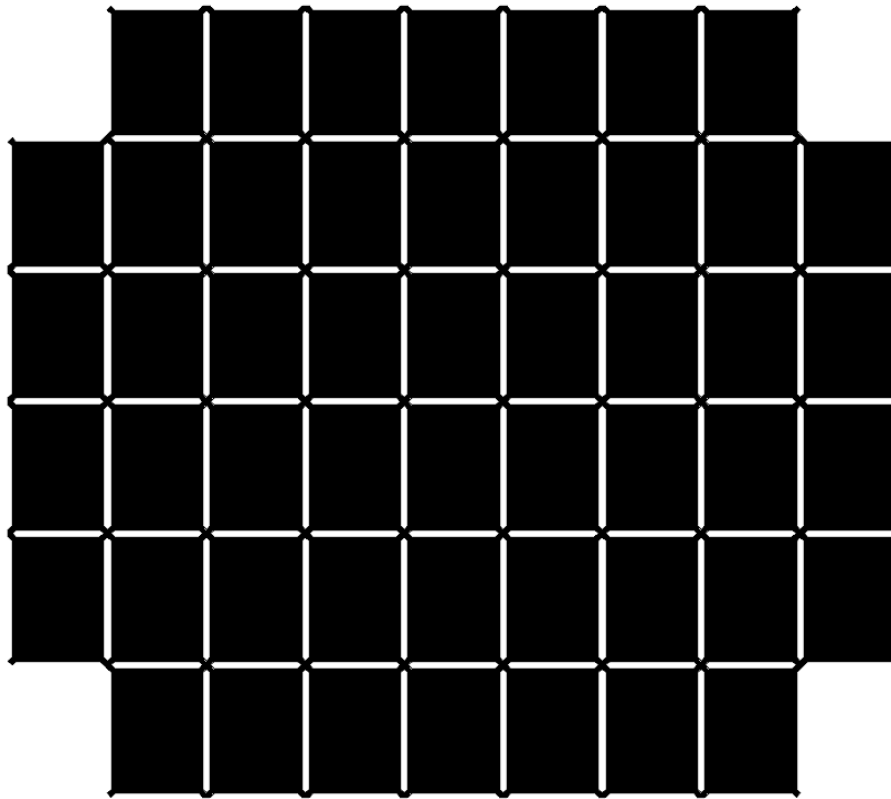
Figure 2.8 Photomask for Mirrors
This photomask is used to fabricate the mirrors using a positive photoresist. The x's in between the corners
of the rectangles protect the mirrors from over-etching where two planes meet. This design for mirrors were
used for both the SU-8 cantilevers with mirrors and PDMS cantilevers with mirrors.

PDMS Cantilevers with Mirrors
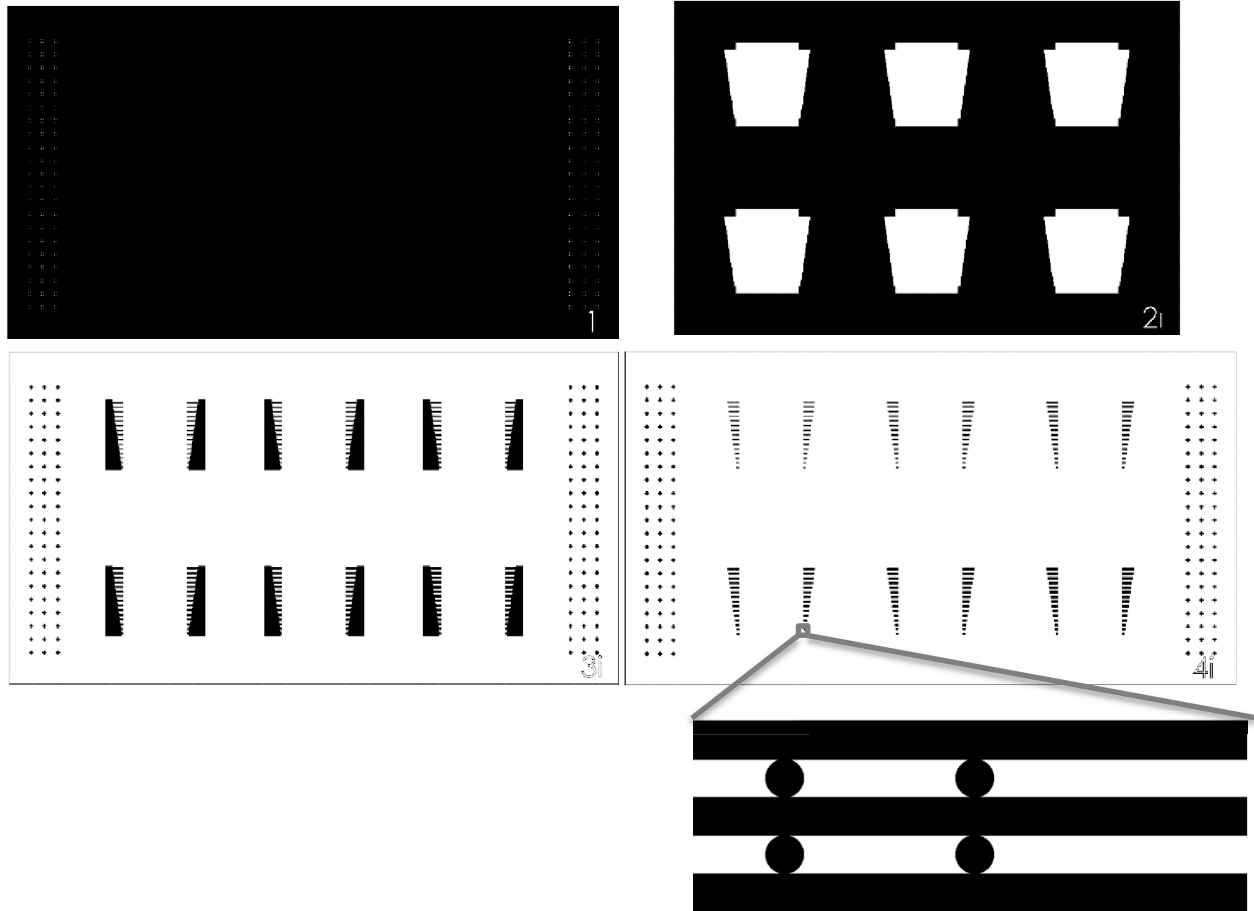
Figure 2.9 Photomasks for PDMS Cantilevers with Mirrors
These are an inverted version of the SU-8 cantilevers

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CHAPTER 3

SIMULATION

3.1 Introduction

Finite element analysis (FEA) is a method of finding approximate solutions for partial differential equations that would be too difficult or time consuming to solve analytically. The model is discretized, or divided into, smaller bodies or finite elements connected at the nodes or boundary lines. A node is location in space where the degrees of freedom (DOF) are defined. DOFs specify the state of an element and are variables. The partial differential equations are then solved at the nodes or boundaries [18]. This method was used to model cantilever bending as a result of cell contraction.

When the cells contract it induces stress on the cantilever, making it bend. Cell contraction force can then be detected according to the micro-cantilever bending vertical displacement. With the use of the finite element analysis, micro-cantilever beams were modeled with different lengths and widths in order to compare their deflection when the appropriate cardiomyocyte stress was applied.

3.2 Models

The models of all 90 geometries of cantilevers were created first in SolidWorks using design tables. This was then imported into COMSOL 5.0 using SolidWorks LiveLink and synchronized.

The models used in the simulations were different than the original SolidWorks models described earlier. The resulting PDMS beams and SU-8 beams were different due to resolution issues using a transparency instead of a glass mask. Rather than having circular pegs, the pegs in the

Figure 3.1 PDMS Cantilevers at 10x Magnification
The SU-8 substrates had similar resulting pegs.

PDMS cantilever mold and in the SU-8 cantilevers merged with the grooves resulting in the beams seen in Figure 3.1.
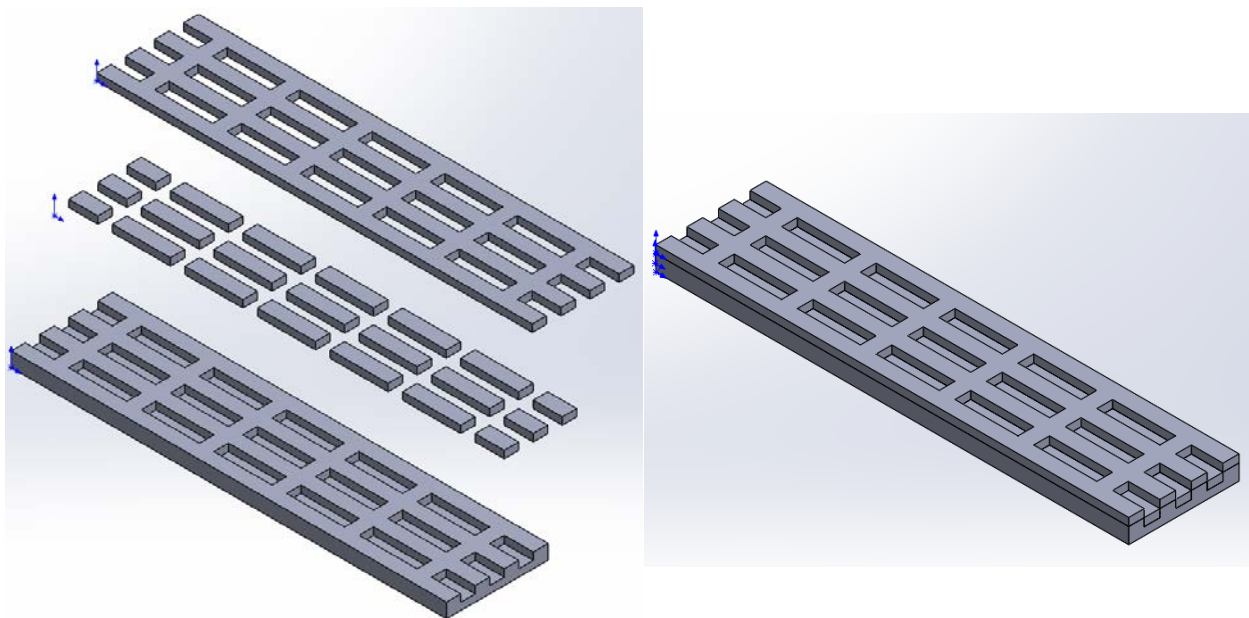
Figure 3.2 SolidWorks Model for Simulation
Exploded view of SolidWorks model on the left.
The two top layers represent cardiomyocytes. The topmost layer goes on top of the grooves and the second layer goes in between the grooves.

3.3   Studies

After the models were created and imported into COMSOL, stationary studies were performed using multibody dynamics. The materials were then assigned the properties in Table 2 and one end of the cantilever was fixed (see Figure 3.3). The stress cardiomyocytes was assumed to be anisotropic and the initial stress exerted only in the x-x direction on the cardiomyocyte layer, and was taken to be 10 kPa, since primary neonatal rat cardiomyocytes on muscular thin films have been previously reported to exhibit an average peak systolic stress of 9.2 kPa [5]. Finally, a physics controlled mesh with an extremely fine element size was created (Figure 3.4) and the study run. This was done initially with SU-8 as the cantilever material and then with PDMS as the cantilever material.

	SU-8 [9]	PDMS [7]	Cardiomyocytes [7]
Young's Modulus	2000000000 Pa	75000 Pa	40000 Pa
Poisson's Ratio	.22	.49	.49

Table 2 Material Properties Used for Simulations
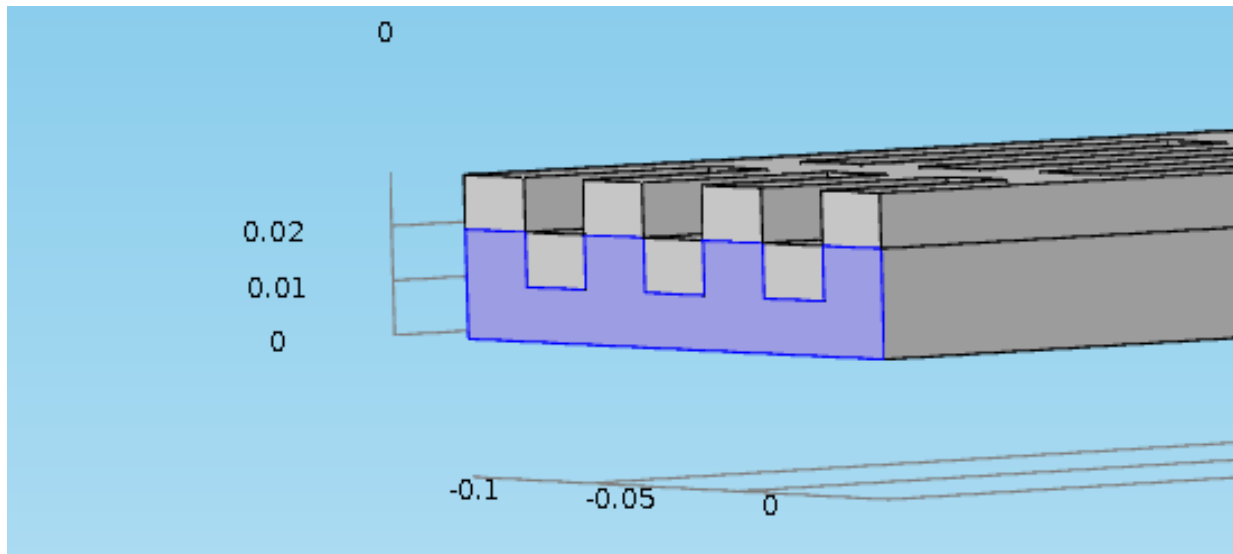
Figure 3.3 Fixed End of COMSOL Model
One end of the cantilever was fixed. All other parts of the cantilevers and cardiomyocytes remained free.
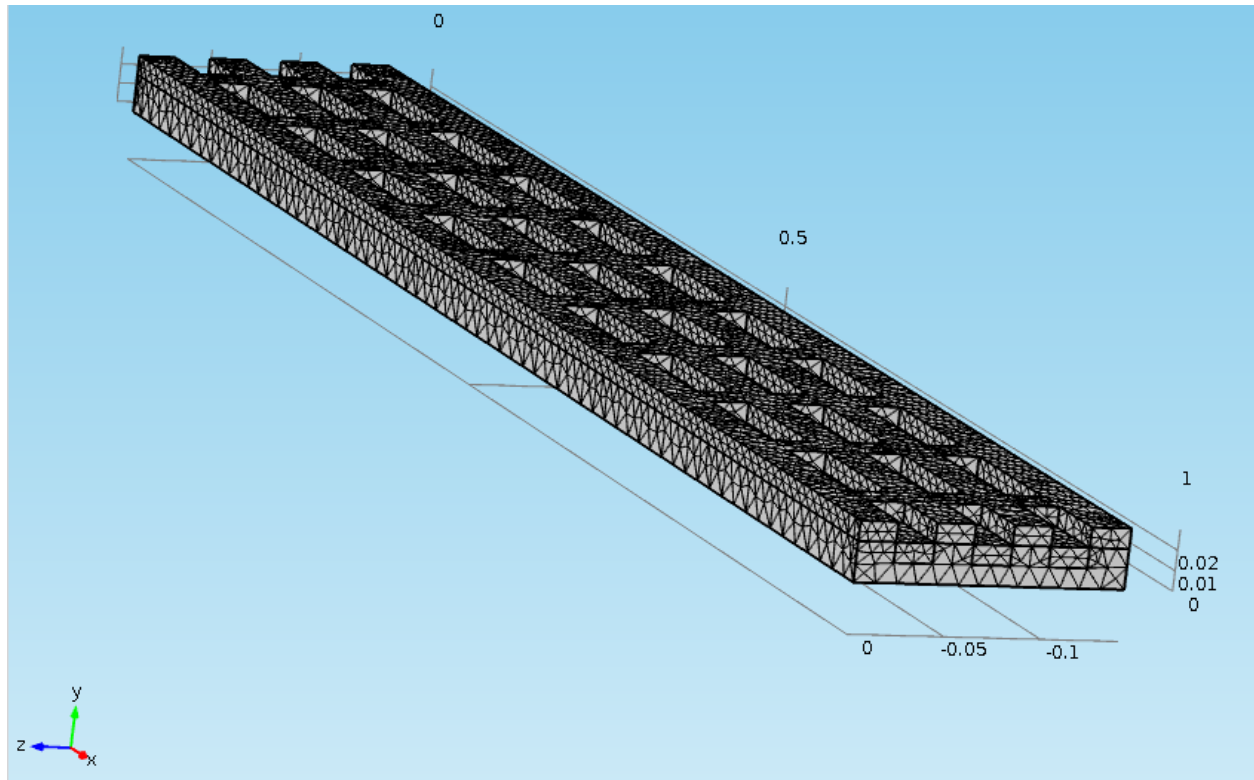
Figure 3.4 Mesh of the Cantilever Model
A similar mesh was created for all 90 geometries.

3.4 Results

The simulations were initially run with SU-8 micro-cantilevers with 100 μm widths. It was found that very little beam bending was observed (see Figure 3.7) for all lengths. This was likely due to SU-8's high elastic modulus. This led us to switch to using PDMS as the material for the micro-cantilevers for biological experiments and continue simulations with PDMS as the material. The maximum beam displacement for each of the geometries was recorded and summarized in the graphs shown later.

Based on the simulations, the change in width did not cause much change in bending displacement. However, the longer the cantilever was, the more it deflected. This shows that when more cardiomyocytes are aligned they exert greater stress and contract more efficiently.

Knowing this information, future micro-cantilever beams can be fabricated in lab and

experiments run to compare the results.
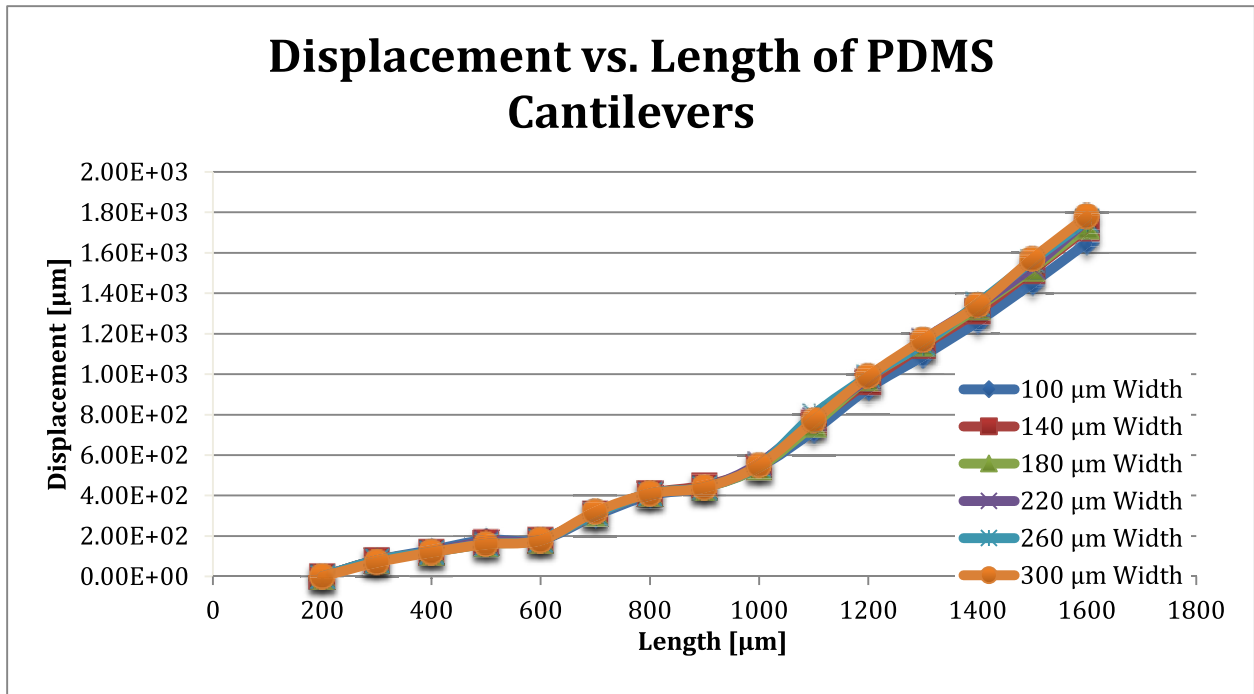
Figure 3.5 Displacement vs. Length Graph
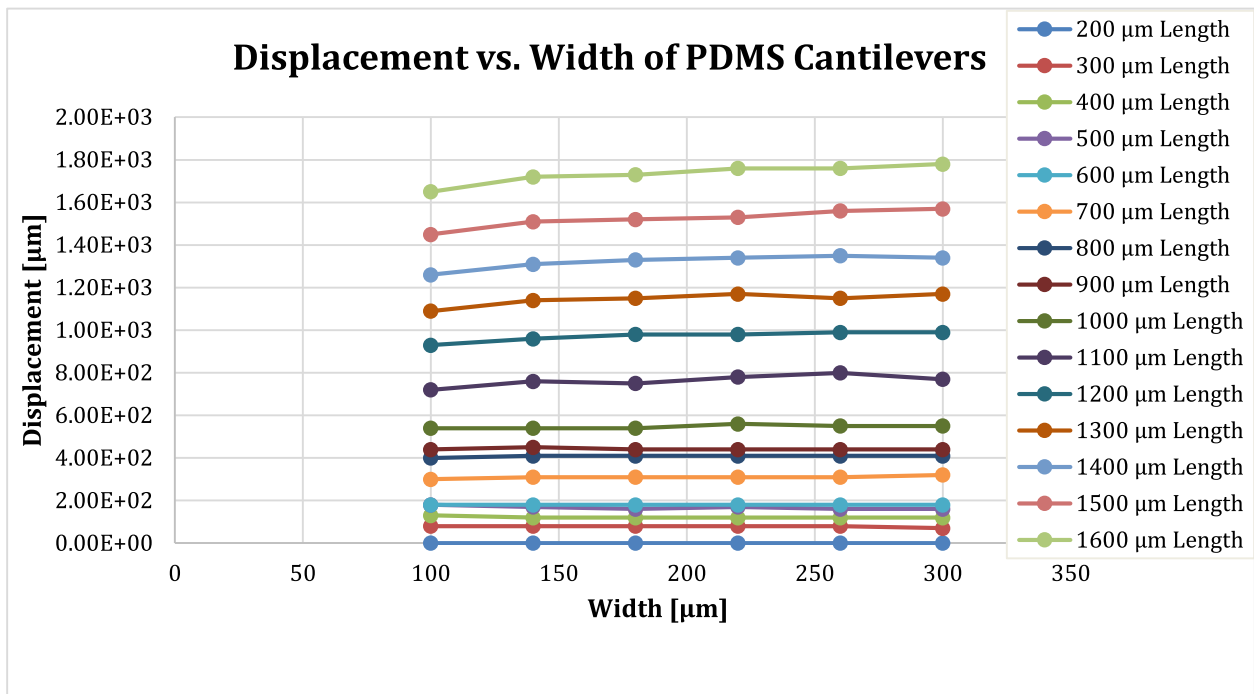
Figure 3.6 Displacement vs. Width Graph
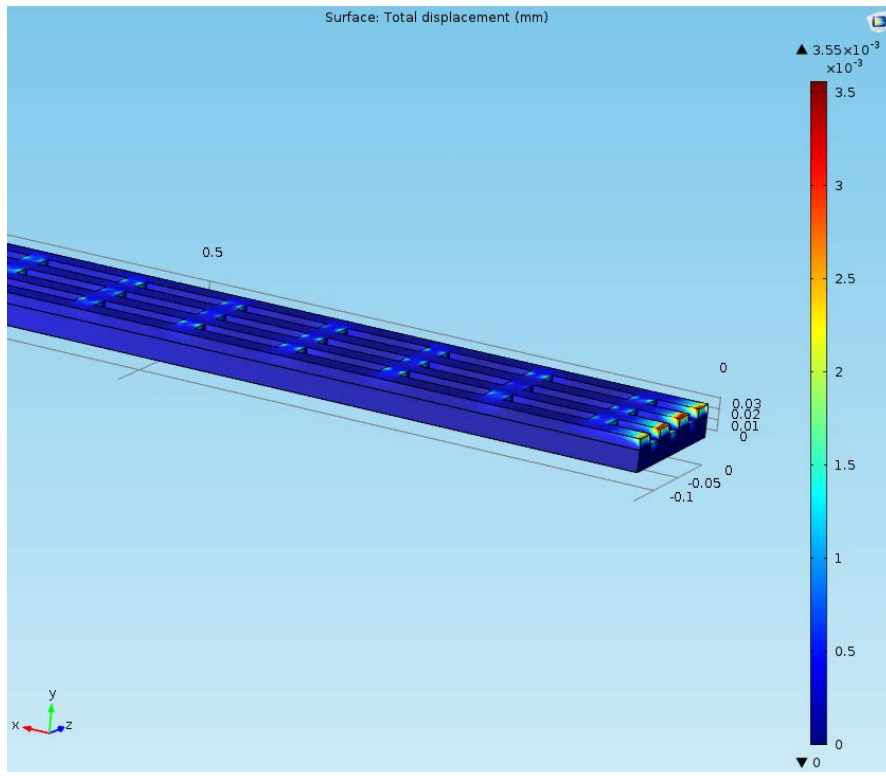
Figure 3.7 SU-8 Cantilever Simulation
Almost no beam bending was observed with the SU-8 cantilever simulations
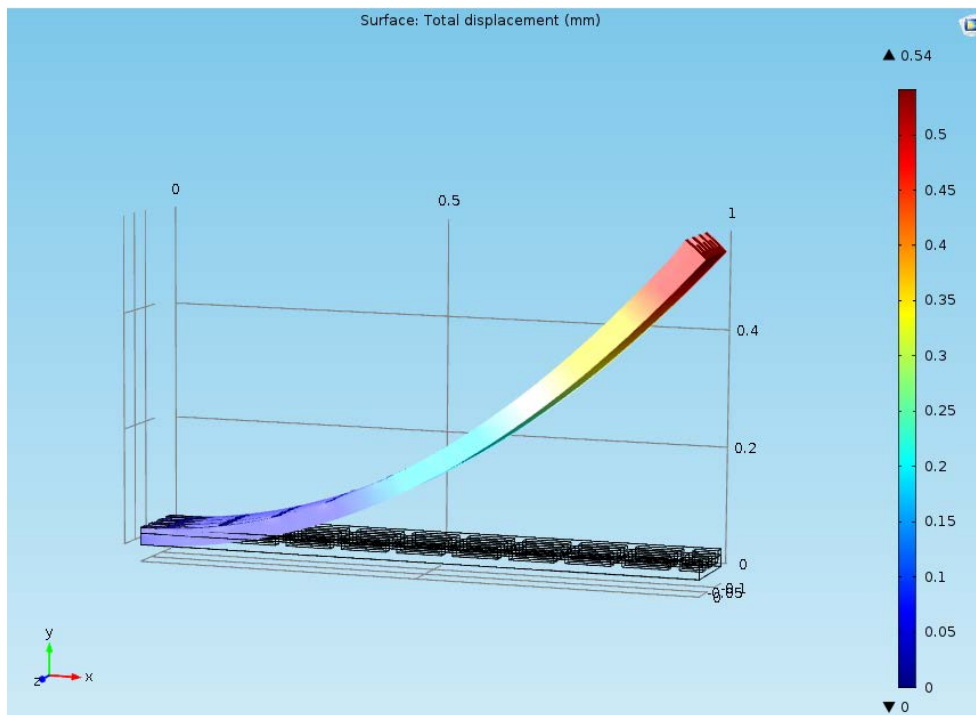
Figure 3.8 PDMS Cantilever Simulation

3.5 Comparison to In Vitro Results

More work is needed in order to draw any conclusion and to validate this model. Experiments need to be completed with primary rat cardiomyocytes and cardiomyocyte stress calculated using methods described in previous chapters, then compared to the result of these simulations.

CHAPTER 4

FABRICATION

4.1 Introduction

SU-8 and PDMS were chosen as the final materials to make for these platforms because of several qualities and advantages they have over other materials. Because this was a continuation of a previous project, the first micro-cantilevers were fabricated out of SU-8. SU-8 is advantageous over PDMS in that it is much less porous, making it a better material for drug discovery. PDMS's network polymer structure causes it to be porous and absorb molecules from the surrounding media. This characteristic of PDMS can affect the results of studies where a fixed volume of a drug is screened [8].

The micro-cantilevers were then fabricated with PDMS. The main advantage of PDMS over SU-8 is its lower elastic modulus. The resulting micro-cantilevers are more flexible making it easier to see cantilever bending as a result of cardiomyocyte contraction. This is needed since the contraction from the cardiomyocytes is measured by measuring the displacement of the cantilevers optically. As a result, the PDMS micro-cantilevers are more sensitive to cardiomyocyte contraction.

This chapter describes the fabrication protocols used to create three sets of micro-platforms: SU-8 substrates, SU-8 micro-cantilevers with mirrors, and PDMS micro-cantilevers with mirrors. The SU-8 substrates and SU-8 micro-cantilevers with mirrors were fabricated using multi-layer photolithography while the PDMS micro-cantilevers were fabricated using multi-layer soft lithography.

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4.2   Fabrication Technique for SU-8 Substrates

The SU-8 substrates were the first set of SU-8 devices fabricated and tested. They were fabricated using a similar method as SU-8 cantilevers. The only difference was that they were created without the use of a UV filter. This resulted in SU-8 substrates that were not free floating and were adhered to the glass slides.

These substrates were fabricated as initial test of the fabrication method, and for testing with HL-1 cardiomyocytes. Previous work in the lab has shown that cardiomyocytes can be cultured on SU-8. This was to see if the cardiomyocytes cultured on pegged and grooved substrates would align and contract on the substrates.

Clean Glass Slides

These substrates were fabricated on 2" x 3" glass slides to allow for easier imaging as its transparent properties would allow for the use of an inverted microscope while a silicon wafer would not. Before the SU-8 substrates were fabricated, the glass slides were cleaned with an RCA-1 solution which removed organic residue from the surface of the glass. RCA-1 cleanser is 5 parts water, 1 part ammonium hydroxide (NH_4OH), and 1 part 30% hydrogen peroxide (H_2O_2). 325 mL DI water was poured into a Pyrex beaker and then 65 mL NH_4OH added. The mixture was heated on a hotplate to 70 °C. The beaker was then removed from the hotplate and 65 mL of H_2O_2 added. The glass slides were soaked in this solution for 15 minutes and then placed in a container with DI water [19]. More DI water was run continuously from a tap into this container for several minutes.

After, rinsing with DI water, the slides went through a mild solvent rinse. The glass slides are rinsed on both sides with acetone. Next, they were rinsed with methanol and then rinsed with isopropanol. The wafer was then placed on a lint-free wipe and dried

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using nitrogen gas. Finally, the slides were dehydration baked in a 120 °C oven for at least two hours to remove any residual water or organic solvents. The slides were then ready for chrome alignment marks.

Pattern Chrome Alignment Marks

Microposit S1827 (Shipley) is positive photoresist that was used to cover the areas of the glass that needed to be protected from the deposited chrome. The glass slide was placed in the center of a spin coater (Laurell) and a small puddle of the photoresist was poured onto the center of the slide. The spin coater then spun the slide at an acceleration of 500 rpm/s until it reached 3300 rpm where it held steady for 30 seconds resulting in a 3 μm layer of photoresist. The slides were then placed in a 90 °C oven to soft bake for 10 minutes [20].

For a positive photoresist, areas exposed to UV light are developed away. Mask 1 (see Figure 2.6) was used to make the alignment marks. This was placed on top of the slide and exposed to 10 mJ/cm^2. The slides were submerged in MF-319 developer solution for approximately 50 seconds to remove the exposed areas.

The next step was metal deposition. An electron beam evaporator (CHA industries) was used to deposit a 1000 Å layer of chrome on the glass slides. The chrome was deposited evenly over the glass slides on areas with and without photoresist . The glass slides were next placed in a beaker of acetone which is then placed in an ultrasonic bath for 10 minutes to lift off the unwanted metal that was deposited on top of photoresist. They were then rinsed in first acetone, then methanol, and finally DI water [21]. After rinsing and drying, the slides were dehydration baked in a 200 °C oven for at least two hours. After this process, the slides were ready to pattern SU-8 layers.

Pattern SU-8 Layers

Fabricating the SU-8 substrates consisted of spinning two layers with three exposures. Since these substrates were used as practice for fabrication and cell culturing. These were made without using a UV filter, resulting in substrates that were not free floating.

The first layer was made with SU-8 2050. 60 µm were spun with a 10 second hold at 500 rpm at an acceleration of 100 rpm/s, then at a 30 second hold at 2500 rpm at an acceleration of 300 rpm/s [22]. The glass slide was next soft baked on a hot plate for 3 minutes at 65 °C and then 6 minutes at 95 °C. After soft baking, the glass slide was taped to silicon wafer to make it easier to handle. Mask 2 was aligned to the chrome alignment marks with an MA6 mask aligner (Karl Suss) and exposed to 160mJ/cm^2 of energy. Then the beams were exposed with mask 3 to 125 mJ/cm^2 with another post exposure bake of 6 minutes at 95 °C afterwards. After baking, 10 µm of SU-8 2010 were spun: 10 seconds at 300 rpm with an acceleration of 100 rpm/sec and then 30 seconds at 3500 rpm with an acceleration of 300 rpm/sec. This was exposed to 140 mJ/cm^2 with mask 4 and post exposure baked for 3 minutes at 95 °C.

After these layers were spun and exposed, the glass slides were placed in SU-8 developer in a beaker and the beaker placed in an ultrasonic water bath for approximately 10 minutes. They were rinsed with acetone, methanol, and isopropanol and then dried with nitrogen gas.

4.3 Fabrication Technique for SU-8 Micro-Cantilevers with Mirrors
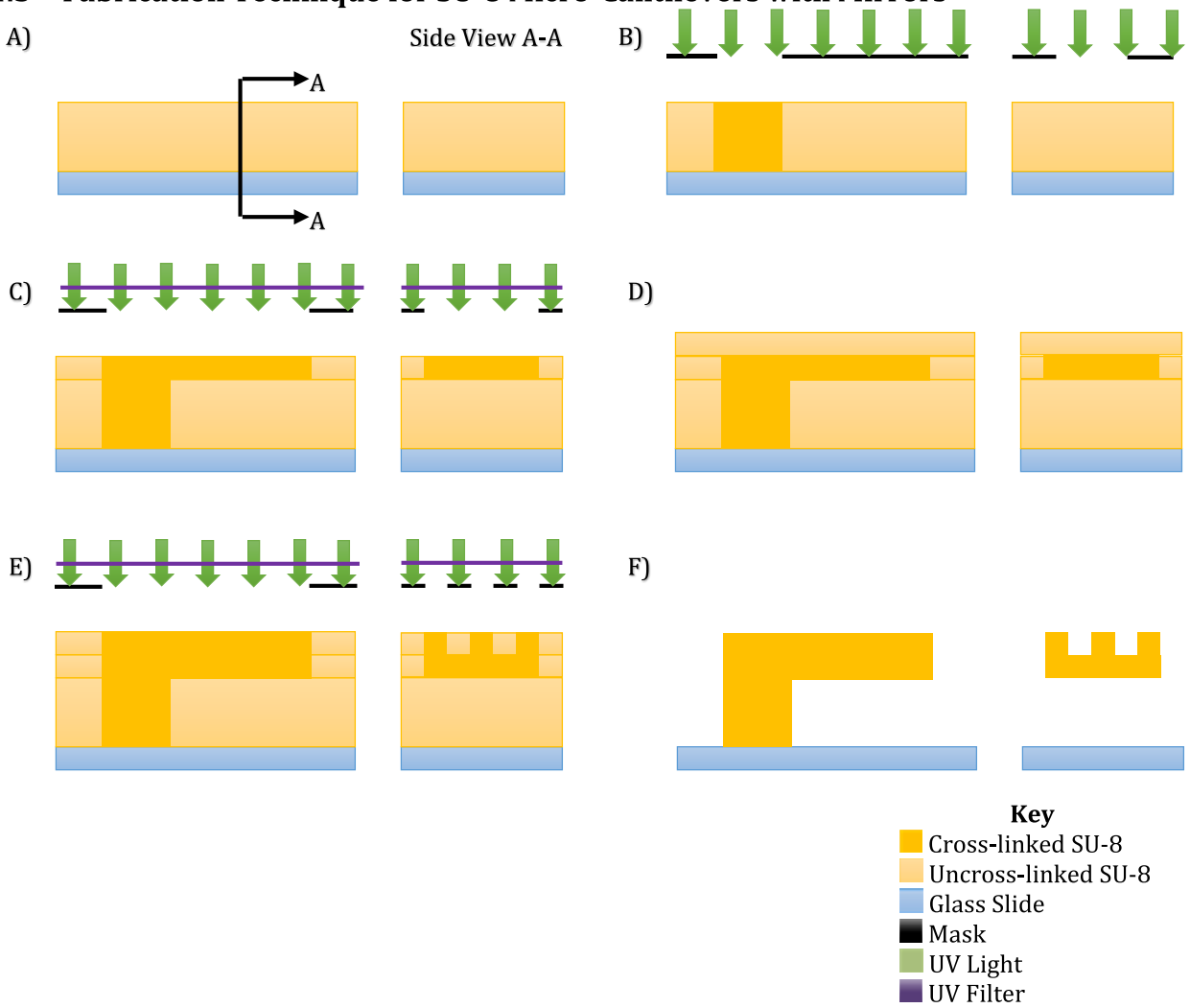
Figure 4.1 Fabrication Diagram of SU-8 Micro-cantilevers
These steps were done after chrome alignment marks were patterned.
A) Spin 60 μm of SU-8 2050
B) Expose with UV Light with Mask 2
C) Expose with UV Light with Filter with Mask 3
D) Spin 10 μm of SU-8 2010
E) Expose with UV Light with Filter with Mask 4
F) Develop with SU-8 Developer

Preparation	Pattern Chrome Alignment Marks	Pattern SU-8 Layers	Develop SU-8
Clean Wafers: HF Dip	Shipley •Spin photoresist •Expose with Mask 1 •Soft Bake •Develop	60 µm SU-8 2050 •Spin 60 um •Soft bake •Use mask 2 to expose •Post exposure bake •Expose with mask 3 with UV filter •Post exposure bake	Place in developer solution
	Rinse with mixture of acetone, methanol, and IPA	10 µm SU-8 2010 •Spin 10 um •Soft bake •Expose with mask 4 with UV filter •Post exposure bake	Rinse with DI water
	Dry with N$_2$ gas		Air dry and place in wafer carrier
	Dehydration Bake in 200°C oven		

Figure 4.2 SU-8 Micro-cantilevers with Mirrors Fabrication Process

The fabrication for the SU-8 micro-cantilevers has a similar process to the fabrication process for the SU-8 substrates described in the previous sections. The same amount of SU-8 was spun and exposed with the equivalent masks (see Figure 2.7). However, these were fabricated on silicon wafers because of the micro-platform's size. The wafers were prepared accordingly. These devices were fabricated without the third layer to see if this multi-layer method would work first with two layers.

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HF Dip

Before the SU-8 can be spun, the silicon wafers must be prepared. Diluted

hydrofluoric acid (HF) was used to remove the native silicon dioxide from the wafers. 2%

HF was created by pouring 480 mL of DI water into 20 mL of 49% HF in a propylene

beaker. The wafers were soaked for 1-2 minutes in the 2% HF solution. After soaking, the

wafer was rinsed with DI water and a wetting test performed to test for hydrophobicity.

Since oxide is hydrophilic and silicon is hydrophobic, if the water beads up and rolls off the

surface is clean of oxides. The wafer was dried with nitrogen gas and ready to pattern

photoresist.

Pattern Photoresist

The SU-8 cantilevers are then fabricated using a similar process described on page

28. First, chrome alignment marks are patterned on the wafers using Mask 1 in Figure 2.7.

Then 60 μm of SU-8 was spun and exposed with mask 2 at 125 mJ/cm^2 to create the

support section and then post exposure baked. Then it was exposed with mask 3 through a

filter to create the beams. Mask 4 and filter would be used to create the pegs and grooves in

future work.

Two different sets of SU-8 micro-cantilevers were created: one made with an

exposure energy of 240 mJ/cm^2 with a 312 nm centered band pass filter (Omega Optical)

and one partially exposed with 60 mJ/cm^2 with no filter. Previous work in our lab was done

to characterize the 312 nm filter to correlate exposure energies with SU-8 thicknesses [23].

The wafers were then developed using SU-8 developer and post exposure baked.

The results of the fabrication for these micro-cantilevers are described later in the chapter.
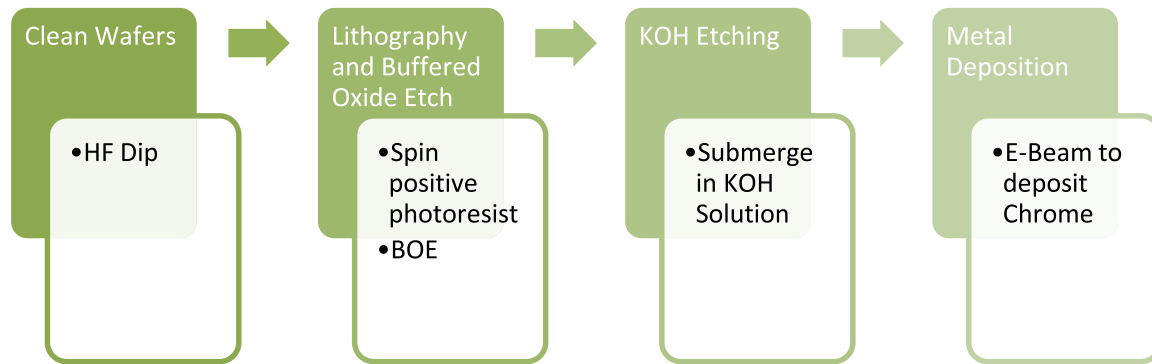
4.4   Fabrication Technique for Chrome Mirror

Clean Wafers	→	Lithography and Buffered Oxide Etch	→	KOH Etching	→	Metal Deposition
•HF Dip		•Spin positive photoresist •BOE		•Submerge in KOH Solution		•E-Beam to deposit Chrome

Figure 4.3 Mirror Fabrication Process

Buffered Oxide Etch

The first step of the fabrication of the chrome mirror was to prepare the wafers for etching. The photoresist was spun to protect the oxide. Then buffered oxide etch was done to remove the unwanted oxide to expose the silicon underneath for KOH etching.

The wafers used for this project were 500 μm in thickness. A 3 μm layer of Microposit S1827 (Shipley), a positive photoresist, was spun onto the backside (the non-reflective side) of the wafer. This was to protect the backside oxide from etching. This was then soft baked for 5 minutes at 65 °C and then 20 minutes at 95 °C. Another 3 μm layer of Shipley was spun on the topside (the reflective side) of the wafer. Because Microposit S1827 is a positive photoresist only the portions of the wafer that should be removed were exposed. The mask for the mirrors (see Figure 2.8) was then placed on top of the wafer on the spun photoresist and exposed 10 mJ/cm^2 using a UV exposure system (AB&M) [20]. The exposed photoresist was developed away using MF-319 developer solution [20]. The result was wafer with photoresist covering it in the pattern seen in Figure 2.8.

The wafer was then placed into buffered oxide etch (BOE) for approximately 1 hour until the exposed oxide was completely gone. BOE consists of 80 g of ammonium fluoride

(NH₄F), 120 mL of DI water, and 20 mL of 49% hydrofluoric acid (HF)[24]. Finally, the wafer went through a mild solvent rinse: acetone, methanol, and finally isopropanol.

Anisotropic Silicon Etch (20% KOH)

The next step was an anisotropic silicon etch with potassium hydroxide (KOH). 50 g of KOH pellets was weighed out and then mixed into 200 mL of deionized water. The mixture was stirred until the pellets were dissolved, then heated up to 80°C and kept stable.

Next, the wafer was placed into the heated KOH solution for approximately 4 hours until the wafer broke apart into the rectangular pieces designed in the mask. The KOH attacked the unexposed portions of the wafer causing it to break apart. The rectangular pieces were then soaked in DI water for approximately two minutes then dried.

Metallization

In order to make the silicon pieces reflective, they must first be metallized. The rectangular pieces were taped into place with Kapton tape along the long side of the pieces, so that the shorter edges are taped over, since these sides would not be needed as mirror surfaces. They were next placed into the electron beam evaporator (Cha Industries) and 500 Å of chrome were evaporated onto it.

4.5 Final SU-8 Micro-Platforms
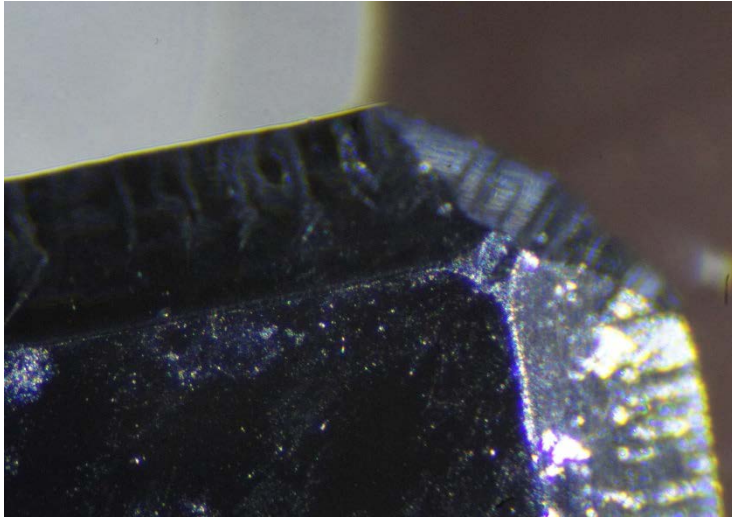
Chrome Mirrors

Figure 4.4 Completed Chrome Mirror

The resulting chrome mirrors were the correct dimensions to fit in the PDMS support section. However, they were not reflective nor smooth enough to be used. Therefore, they were not included in the biological experiments. Figure 4.4 shows the uneven surfaces around one of the corners of the mirror piece.

SU-8 Substrates

After the SU-8 substrates were fabricated, a small section (approximately 1 cm long) of a PVC tubing (McMaster-Carr) with 3/8 inch inner diameter was glued using uncured PDMS to the glass slides. This served as a well to hold the cell media. The PVC tubing with PDMS cured

Figure 4.5 SU-8 Substrates with PVC Wells

over several days at room temperature. The substrates were then sterilized through UV treatment for at least half an hour and were then ready to be coated with fibronectin.
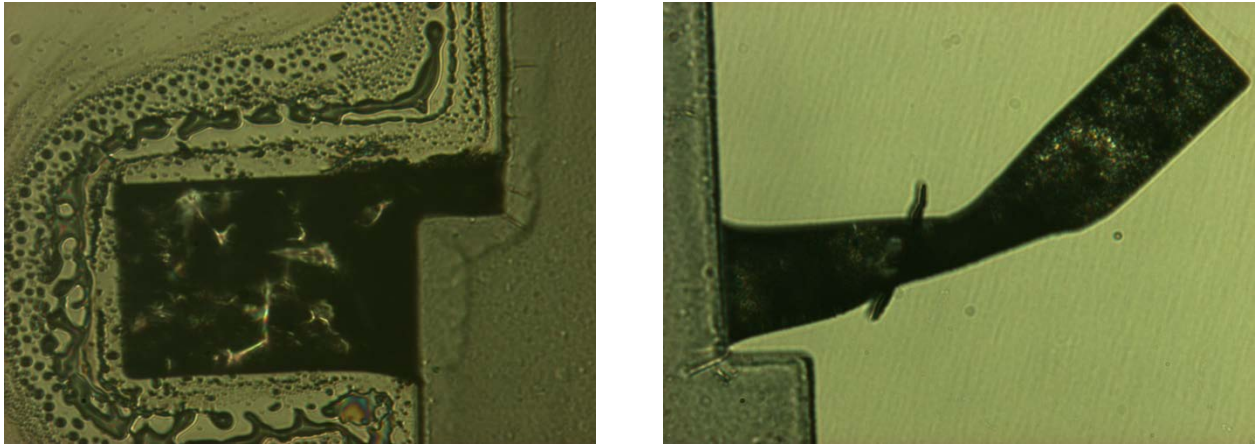
Figure 4.6 Partially Exposed Beam (Left) and Filtered Exposed Beam (Right)

As stated previously, two different sets of SU-8 micro-cantilevers were created: one made with an exposure energy of 240 mJ/cm² with a filter and one partially exposed with 60 mJ/cm² with no filter. Both sets of micro-cantilevers were probed with a DekTak 3 surface profilometer (Veeco Instruments). The thickness of the cantilevers exposed with the filter should theoretically be 20 μm, while the measured thickness was 7 μm. Both sets of beams were also found to be bent upwards instead of lying flat. The resulting beams were very fragile and prone to breaking when probed with the profilometer. However, they were exposed to much higher force than they would from cardiomyocytes.

These thin cantilevers are most likely a result of needing finer characterization of the UV filter. Finer increments need to be taken
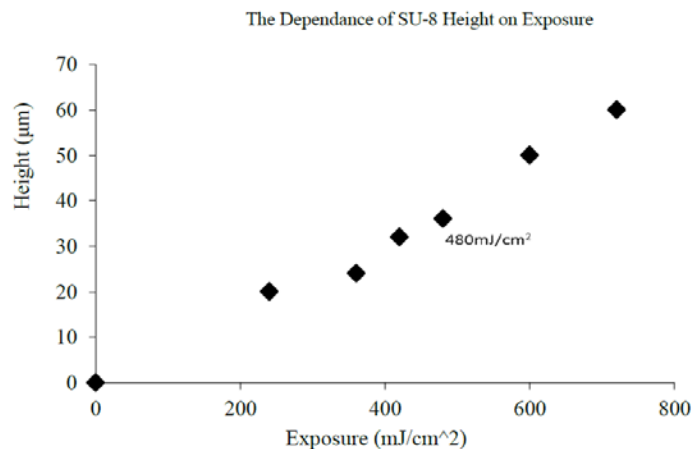

Figure 4.7 SU-8 Height Dependence on Exposure
This characterization was done using SU-8 2050 on a glass slide [23].

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with exposure energies when testing SU-8 thickness in response to exposure energy. The same experiment to characterize SU-8 2050 for use with the filter must also be repeated with SU-8 2010 and ideally on a wafer instead of a glass slide as done previously.

Although no biological experiments were conducted with these micro-cantilevers, the results of these micro-cantilevers are promising. Free standing SU-8 cantilevers were fabricated from the bottom-up. More work is needed in characterizing the UV filter which led us to switch over to PDMS micro-cantilevers in the meantime.

4.6 Fabrication Technique for PDMS Micro-Cantilever Molds

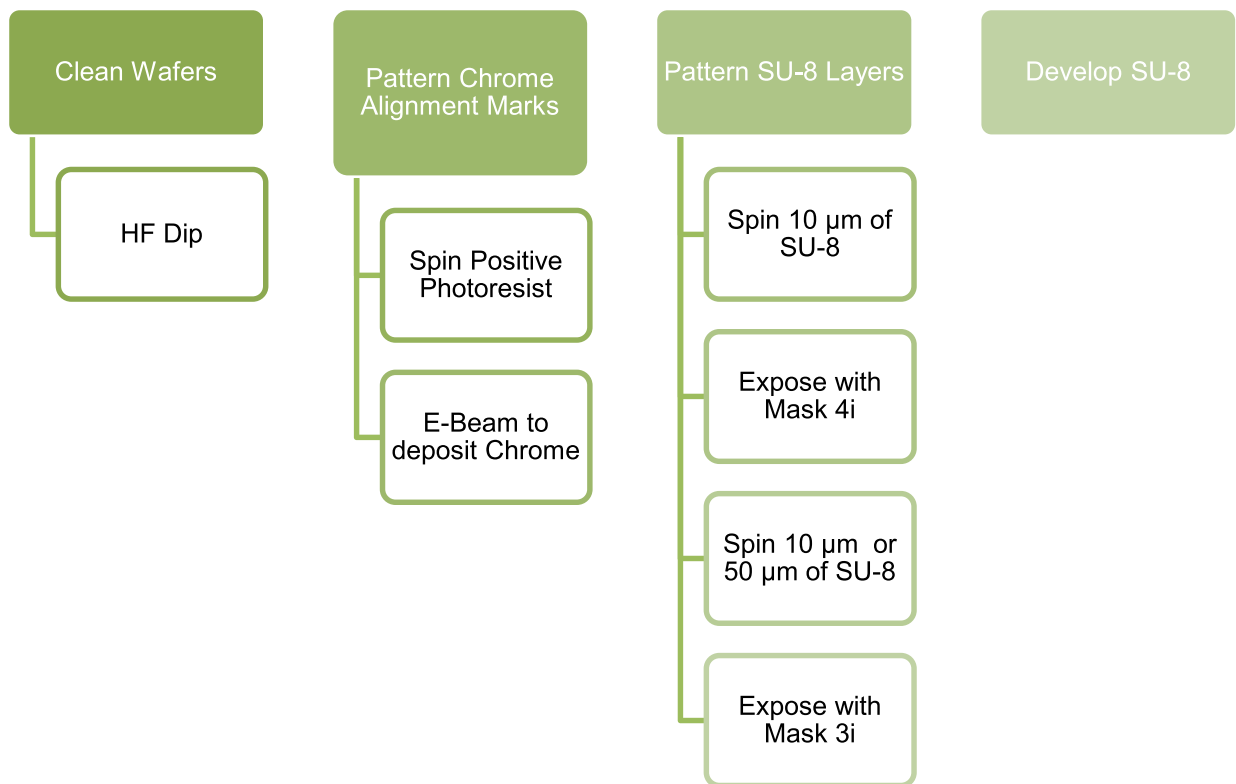
Figure 4.8 PDMS Micro-Cantilever Molds Fabrication Process

The fabrication process for making the PDMS micro-cantilever molds is similar to the process described previously. Two molds were necessary to fabricate the PDMS micro-platforms: one for the support sections and one for the micro-cantilevers. The wafers for both molds were first cleaned with an HF dip before SU-8 was spun on them.

The support section was fabricated using mask 2i, an inverted version of the mask 2 used to make the SU-8 micro-cantilevers. 100 μm of SU-8 2050 were spun on to the wafer at 500 rpm for 10 seconds at 100 rpm/s. Then at 1650 rpm for 30 seconds at 300 rpm/s [22]. The support mold was exposed to 160 mJ/cm^2 with mask 2i. This was then soft baked for 5 minutes at 65 °C and then 10 minutes at 95 °C. The mold was then developed as described previously with SU-8 developer.

Two versions of the cantilever mold were created: one that was 20 μm thick and one that was 60 μm thick. The cantilevers that were produced from the 20 μm thick mold were too thin and prone to tearing. No usable cantilevers from this mold were able to be produced so the 60 μm thick mold was fabricated.

The cantilevers were fabricated using masks 3i and 4i, inverted versions of the SU-8 cantilever masks. First, chrome alignment marks are patterned using mask 1 in Figure 2.9. Then, 10 μm of SU-8 2010 were spun on the wafer at 500 rpm for 10 seconds at 100 rpm/s and then for 30 seconds at 3500 rpm at 300 rpm/s. For the 20 μm thick mold, another 10 μm of SU-8 was spun, exposed, and post exposure baked in the same manner as the first layer. For the 60 μm mold, 50 μm of SU-8 2050 was spun at 500 rpm at 100 rpm/s for 10 seconds, then 3000 rpm at 300 rpm/s for 30 seconds. This was then exposed with mask 3i at 160 mJ/cm^2. The mold was post exposure baked for 2 minutes at 65 °C and then 6 minutes at 95 °C. Finally, the molds were developed using SU-8 developer and were ready to be hardbaked.
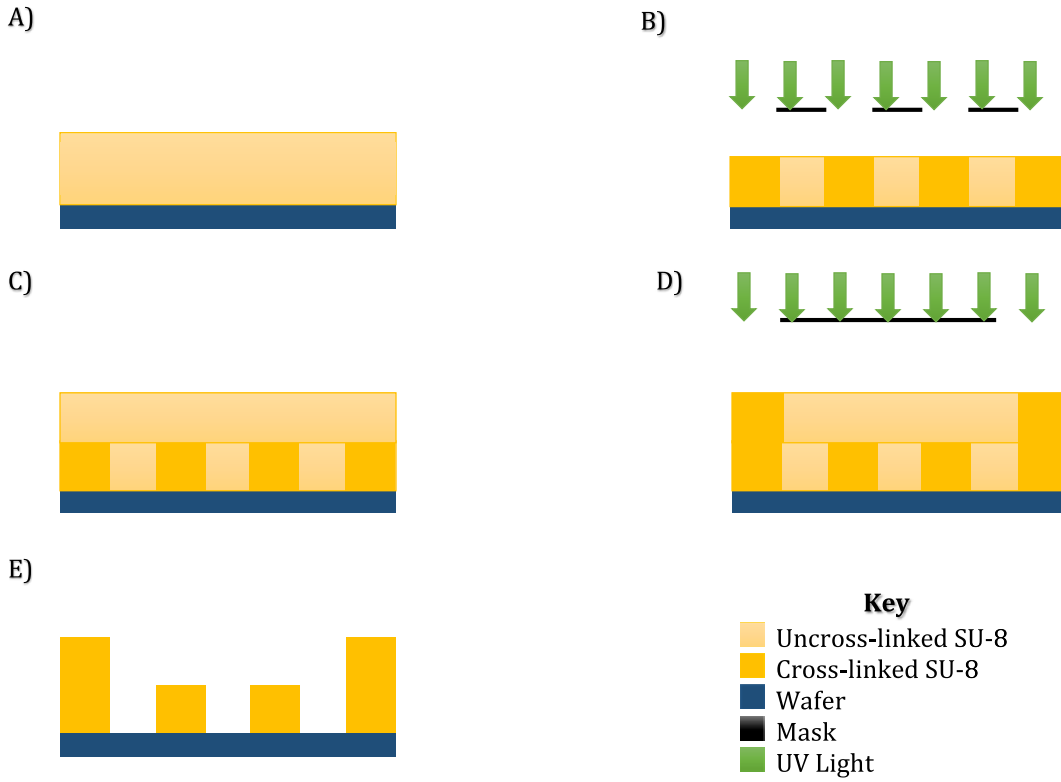
Figure 4.9 Fabrication Diagram of PDMS Cantilever Master Mold
Drawing not to scale
A) Spin 10 μm of SU-8 2010
B) Expose
C) Spin 10 μm of SU-8 2010 for 20 μm beams or 50 μm of SU-8 2050 for 60 μm beams
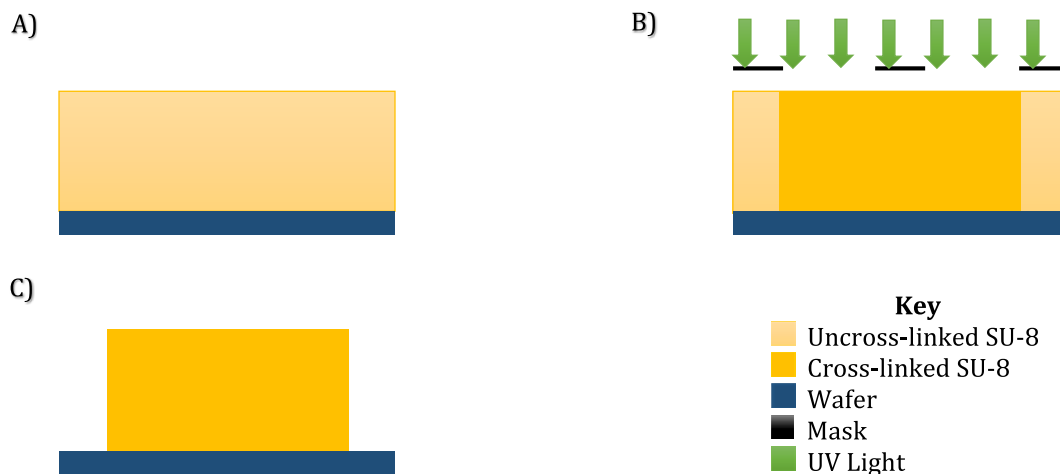D) Expose
E) Develop

Figure 4.10 Fabrication Diagram of Support Section Master Mold
Drawing not to scale
A)   Spin 100 μm of SU-8 2050
B)   Expose
C)   Develop

4.7   Fabrication of PDMS Micro-Cantilevers

Hardbake

Hardbaking was done after development to anneal and strengthen the SU-8 and increase SU-8 adhesion, increasing the lifespan of the master mold. This helps reduce cracking in the SU-8, especially since the wafer must be placed in a 70 °C oven.

After the SU-8 had been developed, they were placed on a hot plate and the temperature was slowly increased then decreased, waiting 10 minutes at each interval after the hotplate reached the specified temperature. The temperatures used were 65° C, 90° C, 120° C, 150° C, 120° C, 90° C, and finally 65° C. After 10 minutes at the final 65 °C interval, the hot plate was turned off and the wafer was cooled to room temperature to avoid cracking.

Silanize Mold

The master molds were treated with silane before the PDMS was cast. Normally, PDMS and SU-8 stick to each other as they are both organic polymers. In order to peel off the PDMS from the SU-8 master mold easily, the mold must be first silanized. The surface of the SU-8 coated wafer was treated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich) to prevent PDMS adhesion. The silane preferentially binds to the SU-8 and causes fluorocarbon residue to stick up from the surface of the SU-8. Consequently, this prevents the PDMS from sticking to the SU-8 on the wafer and makes it easier to peel off [25].

This work must be done in the fume hood. A pipette tip was dipped into the bottle of silane to and placed into a 15 mL centrifuge tube with the cap screwed on loosely to allow silane fumes to escape. The wafer and silane loaded centrifuge tube was then placed in a desiccator overnight. The first time set of molds were silanized, they were silanized for approximately two hours since previous groups and lab members have done so [14]. Unfortunately, after a couple uses with this method of silanization, PDMS began sticking to portions of the mold. This is possibly due to a combination of the small features on the mold and the length of exposure to the silane fumes. Silanizing the molds overnight and resilanizing them after every three uses remedied this problem.

Pouring and Curing

A)

B)

C)

D)

E)

F)

Key
- ■ Cross-linked SU-8
- ■ Wafer
- ■ PDMS
- ■ Uncured PDMS
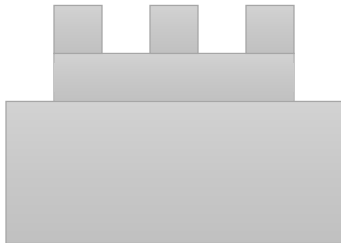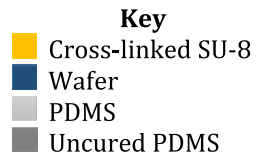
Figure 4.11 Fabrication Diagram of PDMS Cantilever and Support Assembly

Diagram not to scale

A) PDMS is poured onto the cantilever mold. Then it is scraped with a razor blade to make it flush with the SU-8 mold.
B) PDMS is also poured on the support mold.
C) The PDMS is peeled off of the support mold.
D) Uncured PDMS is then placed swabbed on the cantilevers and the support section is then aligned to the cantilevers and allowed to cure
E) The support is peeled off the cantilever mold.
F) Side view of the cantilever

The thin films and the bases were made out of PDMS. This was made using a silicon elastomer base (Sylgard 184, Dow Corning) mixed with a cross-linker (Sylgard 184, Dow Corning) at a ratio of 10:1 by weight. These two components were mixed vigorously until white and foamy, then placed in a vacuum chamber to remove all air bubbles. Next, this mixture was poured over the silanized SU-8 molds.

In order to make the cantilever layer, it was necessary to remove all excess PDMS that does not fall into the indentations of the mold. The beams must stay disconnected so the PDMS must be flush with the SU-8 structures so that the resulting beams were 10 μm in height with 10 μm high grooves. A straight razor blade was used to scrape across the mold many times in many directions to ensure that any excess PDMS was removed and prevented the beams from connecting.

In order to make the support section of the platform, PDMS was also poured on the mold but the excess PDMS was not removed. The thickness for this support section was not too important. It needed to be thick enough to be easy to handle, but thin enough so that when placed in a 6 well plate 3 mL of media would be enough to cover the surface of the cantilever. This resulted in support sections approximately 2 mm in thickness.

After the PDMS was poured, the two molds were placed back in the vacuum chamber to remove any bubbles introduced when pouring. These were then placed in a 70° C oven for a minimum of two hours to cure.

After curing, the cross-linked PDMS on the support mold was cut with a razor blade into rectangles and peeled off the mold. The PDMS on the beams mold was left in the mold until the two components were bonded together.

Bonding PDMS Layers

Several methods were considered for bonding the two PDMS layers together. Oxygen plasma bonding is a common method but other groups have also used sandwich molding, transferring a PDMS structure with a PDMS layer treated with silane, and UV curable glue [25]–[28].
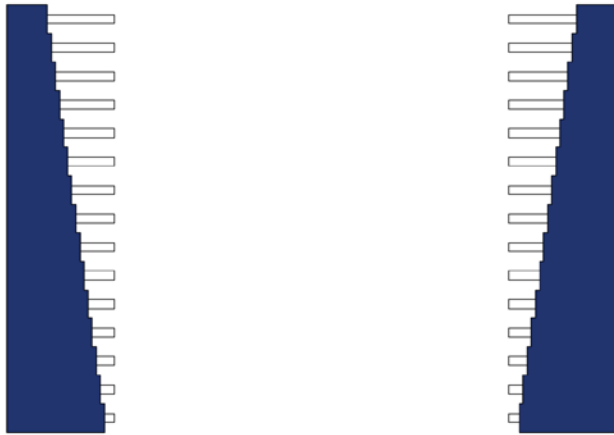

Figure 4.12 Areas to Apply PDMS Glue
Uncured PDMS is applied to the blue areas on the mold to bond the two layers of PDMS.

The simplest, cheapest, and easiest method to use for this project was to use uncured PDMS as an adhesive. This was done by preparing PDMS as described earlier in previous sections (see page 42). A foam tipped cotton swab was dipped into the degassed PDMS and a very thin layer of PDMS was spread over the sides but not over the beam area (see Figure 4.12). This was done carefully so as not to put any PDMS glue on the beams. If too much PDMS is placed in the blue area, this can spread to the beam area when support section is laid on.

Next, while using tweezers, the rectangular support section was placed over the PDMS beams, aligned, and placed on top of the uncured PDMS. The aligning process was very difficult as it must be done by eye. Future designs should take this into account to make it easier to align. Alignment marks should be added to the support section and the cantilever layer. The wafer was then placed again in the 70 °C oven for at least another two hours. This type of adhesive has a bond strength of over 600 kPa [28], a far higher pressure than this platform will ever be subjected to.

Removing PDMS Structures from Master Mold

After the two layers have cured together, the platform was peeled off from the master mold. During this process it was very easy to rip beams off from where they were anchored to the platform. To minimize this, it was found that one must peel the beams in the direction that they are facing. One must peel at the base of the beam and lift toward the end of the beam then repeat on the other side. It was also easier to peel when isopropyl alcohol is squirted using a squeeze bottle in between the PDMS and the mold after the initial peeling.

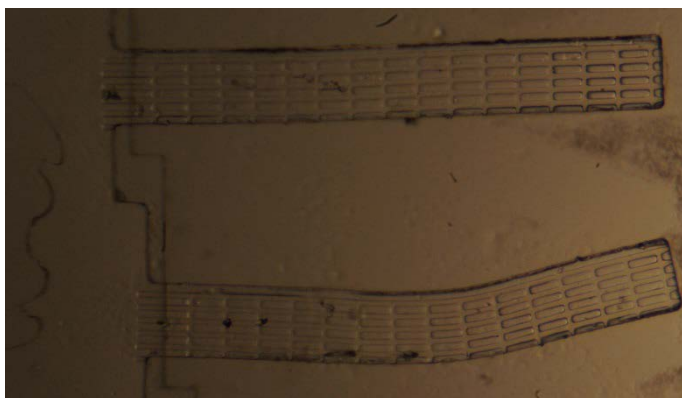## 4.8 Final PDMS Platforms

Figure 4.13 PDMS Beams at 10x Magnification

The final platforms were close to what the original design called out for. The beams were all free floating and none were stuck to the bottom of the platforms. Unfortunately some would rip, but the majority of the beams would be fine. Some problems during assembly occurred with alignment and PDMS glue spreading into the wrong areas. The alignment issues can be resolved in future iterations with the addition of alignment marks. In order to avoid PDMS spreading, less PDMS glue should be used. As stated previously, the chrome mirrors were not reflective enough, so they were not glued to the micro-platforms.

After assembly, the PDMS glue was placed on the bottom of the platforms. The micro-platforms were then glued to the bottom of a 6 well plate and again placed in a 70 °C
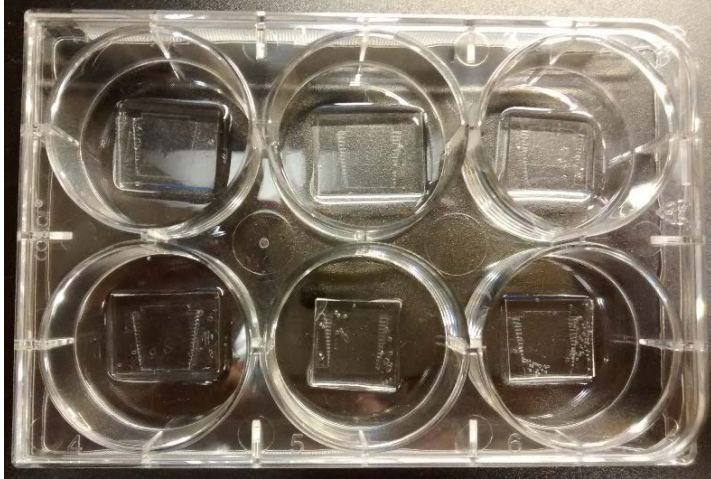
Figure 4.14 PDMS Micro-Platforms Glued to 6 Well Plate
PDMS glue is used to attach the micro-platforms to the 6 well plate then placed in a 70 °C oven

oven for at least 2 hours. The 6 well plate was then UV treated for at least half an hour to sterilize it. Finally, the micro-platforms were ready to be coated with fibronectin.

CHAPTER 5

BIOLOGICAL EXPERIMENTS

5.1   Introduction

Once the fabrication process of the SU-8 substrates and PDMS micro-platforms were developed and tested, several micro-platforms were fabricated for experimentation with cardiomyocyte cells. First, HL-1 cardiomyoctes were cultured on SU-8 substrates. After SU-8 substrates experiments were completed and PDMS micro-cantilevers fabricated, primary neonatal rat ventricular myocytes were cultured on those micro-cantilevers. In this chapter, cell culturing protocols for HL-1 cells and primary cells are discussed and experimental results from seeding cardiomyocytes on SU-8 substrates and PDMS micro-cantilevers are presented.

The HL-1 cardiomyocyte cell line was used for initial cell experiments on SU-8 substrates because of the cell line's ability to be indefinitely passaged and stored, its cost effectiveness, and its spontaneous contractile activity [11]. Primary rat cells were then used with PDMS micro-cantilevers because of the stress they are capable of generating. Alford et al reported primary neonatal rat cardiomyocytes having a peak systolic stress of 9.2 kPa much higher than the 1 – 2 kPa HL-1 cells are capable of generating [5], [14].

5.2 HL-1 Cardiomyocytes on SU-8 Substrates

Cell Culture

Prior to seeding HL-1 cardiomyocytes, the SU-8 substrates were coated with fibronectin from bovine plasma (Sigma-Aldrich) to promote cell adhesion. The substrates were coated with the fibronectin for at least 1 hour before it was aspirated and the cells were seeded.
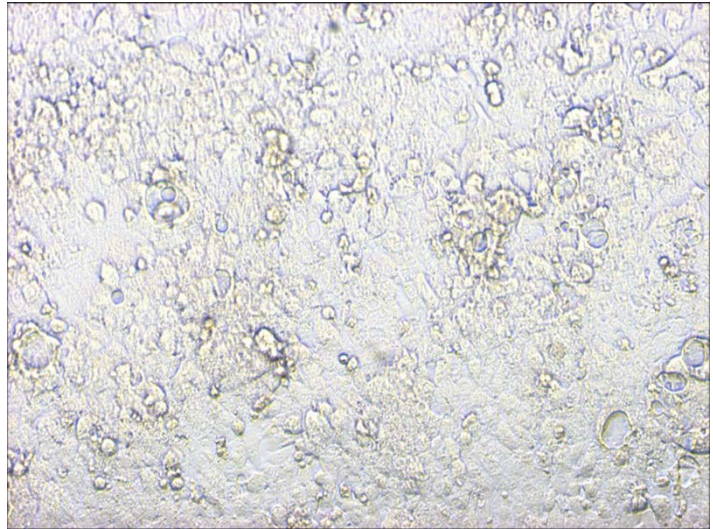
Figure 5.1 Confluent HL-1 Cardiomyocytes on a Tissue Culture Flask

The cardiomyocytes were passaged when confluent from a T25 and seeded into the 3 wells of the substrate at a seeding density of 1000 cells/mm^2. The cells were fed 1 mL of supplemented Claycomb medium (Sigma-Aldrich) daily and fed double the amount on Saturdays to avoid feeding on Sunday.

Claycomb medium is supplemented with fetal bovine serum (Sigma-Aldrich), Penicillin/Streptomycin (Invitrogen), Norepinephrine (Sigma-Aldrich), and L-glutamine (Sigma-Aldrich). These were then mixed to the concentrations in the table below.

Component Name	Volume Added (mL)	Final Concentration
Claycomb Medium	86	-
Fetal Bovine Serum	10	10%
Penicillin/Streptomycin	2	200 U/mL:200 µg/mL
Norepinephrine	1	0.1 mM
L-Glutamine	1	2 mM

Table 3 Supplemented Claycomb Medium Components and Concentrations

Results

Cardiomyocytes were grown on the SU-8 substrates for 3 days at which point they reached confluency. Cells appeared to align preferentially along the grooves and pegs of the substrate, especially compared to areas of the SU-8 substrate that were flat and without pegs and grooves.

Previously, in tissue culture flasks, cells have been seen beating in large groups of cells. However, this behavior was
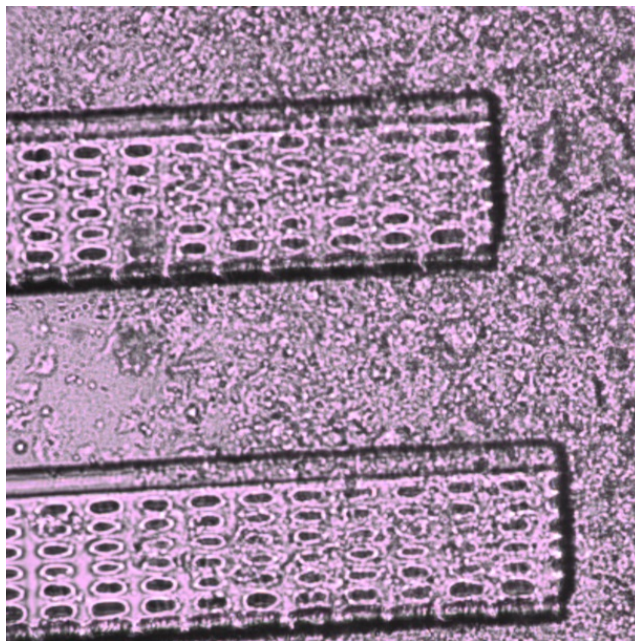
Figure 5.2 HL-1 Cells on SU-8 Substrates

not seen on the SU-8 substrates. Rather, cells in small isolated areas were seen beating on day 3 (see Figure 5.2). This is possibly due to the height of the pegs and grooves. The 10 μm thickness pegs and grooves, may be too high and preventing large groups of cardiomyocytes from interacting with each other, thus preventing cell contraction. More work needs to be done with thinner grooves to see if this aids in cell beating.

5.3   Neonatal Rat Ventricular Myocytes on PDMS

After experiments with SU-8 substrates were completed, the neonatal rat ventricular myocytes were cultured on PMDS micro-cantilevers

Cell Culture

Prior to cell seeding, the PDMS micro-platforms were coated with human fibronectin (Corning) and incubated at room temperature. The fibronectin was only on the PDMS micro-platforms and not on plastic portions of the 6 well plate. After an hour, the

fibronectin was aspirated and replaced with PBS. The devices were stored at 4°C for two days until cells were seeded on them.

Neonatal rat ventricular myocytes were harvested and cultured in accordance with Grosberg et al. [4]. Two day old neonatal Sprague-Dawley rats (Charles River Laboratories) were sacrificed and their ventricles were extracted and homogenized in Hanks balanced salt solution. This was then incubated at 4°C overnight in 1mg/ml trypsin and the tissue was digested with 1mg/ml collagenase at 37°C. The released myocytes were resuspended in M199 culture medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 10mM HEPES, 0.1 mM MEM non-essential amino acids, 3.5 g/L glucose, 2mM L-glutamine, 2 mg/L vitamin b-12, and 50 U/ml penicillin.

The cells were then seeded at 300,000 cells per micro-platform and fed 10% FBS medium on day 1. Starting on day 2, the cells were fed every other day with 2% FBS M199 medium.

Results

The cells were observed for 4 days. Cell beating was observed on some of the beams and on the micro-platform on day 3 and day 4. However, no beam bending was observed. This was possibly due to the low amount of the cells seeded directly onto the PDMS cantilevers. It may also be due to the material properties of the PDMS. Possibly too much curing agent was used and resulted in stiffer beams. A disproportionate amount of cells landed on the surrounding micro-platform rather than the cantilevers. Consequently, although cells were beating, not enough of the cells were beating on the beam to cause contraction or the beam was too stiff for the beams to bend. This led to the beams remaining stationary.
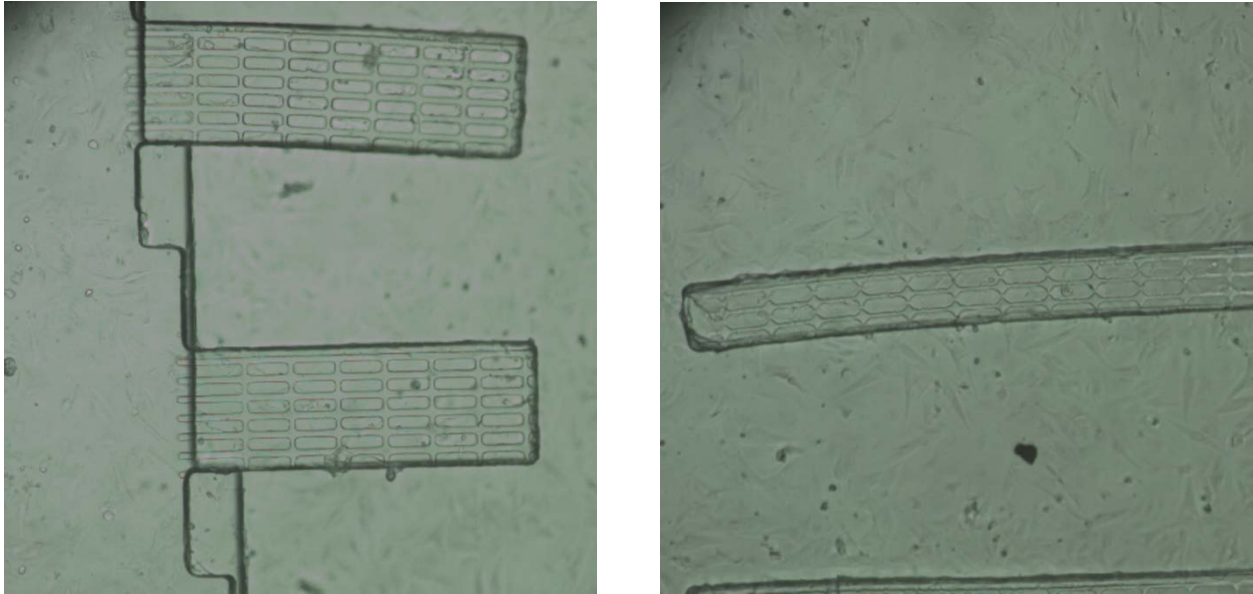
Figure 5.3 Neonatal Rat Ventricular Myocytes on PDMS Micro-Cantilevers Seen at 10x
Primary rat cells were seeded onto the PDMS micro-cantilevers and observed over 4 days.

5.4   Important Findings

Cell beating was observed with both types of cells on both SU-8 substrates and PDMS

micro-cantilevers. Through these experiments we were able to demonstrate that SU-8 was

a viable material for cardiomyocyte cell culture. Although no beam bending was observed

on the PDMS, the initial findings are optimistic. Future work should focus on having more

of the cells land on the cantilever and testing if the PDMS is too stiff. With more cells that

are contracting on the micro-cantilever or a more flexible beam, beam bending can be

observed and then measured.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1 Concluding Remarks

The micro-platforms researched in this thesis offer a potentially simple and cost effective tool to measure cardiomyocyte contraction. The designs of these micro-platforms were heavily based on micro-cantilevers and muscular thin film technology, in which the radius of curvature or beam deflection can be measured and used to calculate stress due to cardiomyocyte contraction. Two sets of micro-platforms, SU-8 substrates and PDMS micro-cantilevers, were designed, fabricated, and tested with HL-1 cardiomyocytes and primary cardiomyocytes respectively. The micro-platforms consisted of 180 beams of varying lengths and widths with pegs and grooves to aid in cell alignment.

Finite element analysis was conducted to determine the best geometries for the SU-8 and PDMS micro-cantilevers. It was found that the widths of the cantilevers did not affect the amount of bending as much the lengths of the cantilevers did. In general, the longer the beam, the more beam deflection can be observed when the same stress was applied.

The SU-8 substrates were the first micro-platforms tested to see if SU-8 is a viable material to culture cardiomyocytes on. SU-8 is superior to PDMS in that it does not absorb small molecules like PDMS does. These substrates with 10 μm thick pegs and grooves were fixed to the glass slide and did not allow for beam bending. Small groups of cells were seen beating on day 3. Flat SU-8 cantilevers without pegs and grooves were also fabricated using a UV filter. These cantilever were not usable since many were bent up and broke easily.

This is possibly due to their incorrect thickness. Their measured thicknesses were not what was specified and were much thinner than needed.

After work was completed with SU-8, PDMS micro-cantilevers were fabricated using multi-layer techniques. First, 20 μm micro-cantilever molds were fabricated. Unfortunately, the resulting cantilevers from these molds were prone to tearing. This led to the fabrication of 60 μm micro-cantilevers instead. These micro-cantilevers had the same geometries as those fabricated with SU-8. PDMS has a lower elastic modulus making them more sensitive to cardiomyocyte contraction. Primary neonatal rat ventricular myocytes were then cultured onto the 60 μm thick cantilevers and cell contraction was observed on day 3 and 4. Although no beam bending was observed, this is likely due to the low density of cells that landed and adhered to the cantilevers rather than the surrounding PDMS platform. Future work needs to address this issue and the issues described previously.

6.2   Future Work

Although significant improvement has been made from previous iterations of this design, further work needs to be completed in order to fully develop these micro-platforms for use in drug testing. First, modeling of the micro-cantilever bending needs to be further refined and developed. The updated model may include a modified Stoney's equation or other models used for muscular thin films.

Fabrication work needs to be improved as well. For the SU-8 cantilevers, the UV filter needs to be characterized for use with SU-8 2010 specifically. For the PDMS cantilevers to bend, the material properties need to be tested to see if it is flexible enough to observe beam bending. The mirror can also be made through traditional machining as well instead of using clean room techniques. Although this may be tedious for such small mirrors, it may

result in better mirrors. The use of 1002F SU-8 photoresist as the structural material should also be explored. It has a lower Young's modulus and may be easier to exhibit beam bending [29].

Future cell culture work should focus on using primary cells as they contract at higher stresses. The rat cardiomyoctes should be seeded at higher densities to ensure that more of the cells land on the cantilevers. The cells should also be stained after seeding to check for cell alignment. More experiments with cells are necessary to validate the micro-platforms.

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