Mechanical Testing System Coupled with an Environmental Chamber for Hydrogels

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ABSTRACT

Hydrogels have many biomedical applications. Different applications require varying mechanical properties. The mechanical properties of hydrogels are dependent on their composition and surrounding temperature and pH. There is limited published work to describe these properties and conditions. This goal of this project is to identify the dynamic stress-strain relationship, creep, and ultimate stress and strain under varying temperatures (4 to 40 ^{o}C) and pH levels (2 to 10). Before the mechanical properties of hydrogels can be tested, the individual hydrogel samples must be made. This requires developing a stencil procedure to make the hydrogel samples and building an environmental chamber for the mechanical testing system used. This semester, a stencil procedure was written and an environmental chamber was built. There was not enough time to test the chamber.

INTRODUCTION

Hydrogels are water-swollen, cross-linked polymeric structures. They can absorb water or biological fluids and have numerous biomedical applications: drug delivery, tissue engineering scaffolds, and wound healing. Biomedical products may include skin adhesive hydrogels for wound/burn care and electrodes, and anti-thrombogenic, biocompatible, lubricious medical device coatings [1]. Although hydrogels have been extensively used in biomedical research, there is limited published work that presents findings on the physical changes of hydrogels to a specific environmental condition and how these changes affect the specific hydrogel biofunctions and applications [2].

An understanding of hydrogels' mechanical characteristics is essential. The mechanical properties of biomedical gels allows researchers to evaluate the material intended for a particular application [3]. The ultimate goal of the design is to elucidate the dynamic properties of hydrogels in response to changes in their environment, such as pH and temperature.

DESIGN OBJECTIVES

The client, Prof. Weiyuan John Kao, is a professor in the School of Pharmacy and also has an appointment in the Biomedical Engineering Department at the University of Wisconsin-Madison. Prof. Kao would like the following mechanical properties of hydrogels to be identified: the dynamic stress-strain relationship, creep, and ultimate stress and strain. He would also like these properties to be observed under varying temperature (4 to 40 ^{o}C) and pH levels (2 to 10), which mimic biological conditions. The structure and environment (pH, temperature, and blood compounds) regulate hydrogels' degradation [3]. These environmental factors are critical in affecting the mechanical and chemical properties of biomedical materials. Research has shown hydrogels that undergo changes in pH levels and temperature often degrade, exhibit swelling changes, and lose their elasticity [4]. These changes are also dependent on the composition of the hydrogels. Therefore, the composition of the hydrogels will be varied during the determination of the mechanical properties to observe these effects.

Before the mechanical properties of hydrogels can be tested, the individual hydrogel samples must be made. In order to complete this task, a procedure must be developed to prepare a stencil from which to make the hydrogel samples. The testing equipment to be used must also be established, including an environmental chamber, which must be designed in order to vary the pH and temperature.

Stencil Procedure

The client has specified that the hydrogel samples must be a "dog-bone" shape as required for testing by the American Society of Testing and Materials (ASTM). The approximate dimensions for the samples are 280 μm thickness, 11 mm gauge length, and 2 mm neck width as shown in Figure 1. The stencil procedure developed produces a stencil that allows three hydrogel samples to be made at a time with the required dimensions. The procedure was designed to be relatively fast, ensure uniform thickness of the stencil, and maximize hydrogel production. Having a uniform stencil will prevent variance during the sample preparation, which could have a significant effect in the collection of mechanical properties data. The product design specification for the stencil procedure is attached in Appendix A.

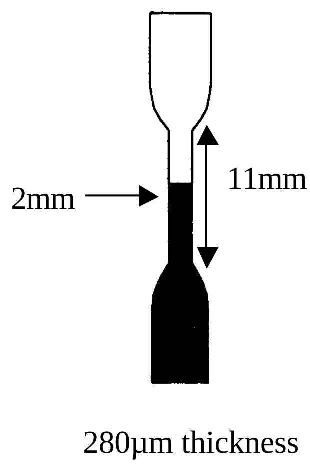

Figure 1. The dog bone shape, required by ASTM for mechanical testing, has the approximate dimensions shown above.

Environmental Chamber

The client has specified that the environmental chamber must maintain the temperature and pH of the buffer solution used during the mechanical tests (length of 0.5 to 5 minutes); not interfere with the stress, strain, and creep tests; and be compatible with the Instron 1000 and the Instron 5548 mechanical testing systems shown in Figure 2. The Instron 1000 is located in 1313 Engineering Hall and the Instron 5548 is in Prof. Wendy Crone's lab in the Engineering Research Building. The two different Instron machines each require a separate environmental chamber to be built for use with them because they have different dimensions, which are shown in Appendix B. The dimensions of the grips and grip attachments for the Instron 1000 are given in Appendix C. The chamber must maintain the temperature of a solution in the range of 4 to 40

^oC and the pH in the range of 2 to 10. The product design specification for the environmental chamber is attached in Appendix D.

(www.instron.com)

Figure 2. The environmental chamber is to be designed for use with the Instron 1000 (shown on the left) and the Instron 5548 (shown on the right) mechanical testing systems.

Therefore, the objective for this semester was to establish a stencil procedure, which produces ASTM approved hydrogel samples, and to design and build an environmental chamber to be used with the Instron 1000 mechanical testing system in order to test the mechanical properties of hydrogels.

CURRENT PROGRESS

Stencil Procedure

An ASTM approved stencil procedure has been developed. The stencils are made of Polydimethylsiloxane (PDMS). PDMS is used for several reasons. It is inexpensive, permeable to gases for biomedical applications, transparent, and bonds easily [5]. PDMS is prepared in the laboratory by mixing a PDMS prepolymer and curing agent (SLYGARD 184 Silicone Elastomer Kit, Dow Corning, Midland, MI) together and degassing for approximately one hour. To make the stencil, the PDMS mixture is poured over the EPON Master, a silicon wafer, and sandwiched in between aluminum disks by placing steel weights on top as demonstrated in Figure 3. The

EPON Master, shown in Figure 4, serves as a cookie-cutter to cut out the stencil from the PDMS layer. The transparency is placed on top of the PDMS layer to provide an easy, clean way to remove the top weights. The pyrex disk is placed on top of the transparency to provide a flat, level surface for the weights and to ensure uniform thickness of the stencil. The PDMS sandwich is cured for three hours at 80 ^{o}C. After curing, the PDMS stencil can be peeled off from inbetween the EPON Master and transparency. Figure 5 shows the final stencil product. The complete stencil procedure is attached in Appendix E.

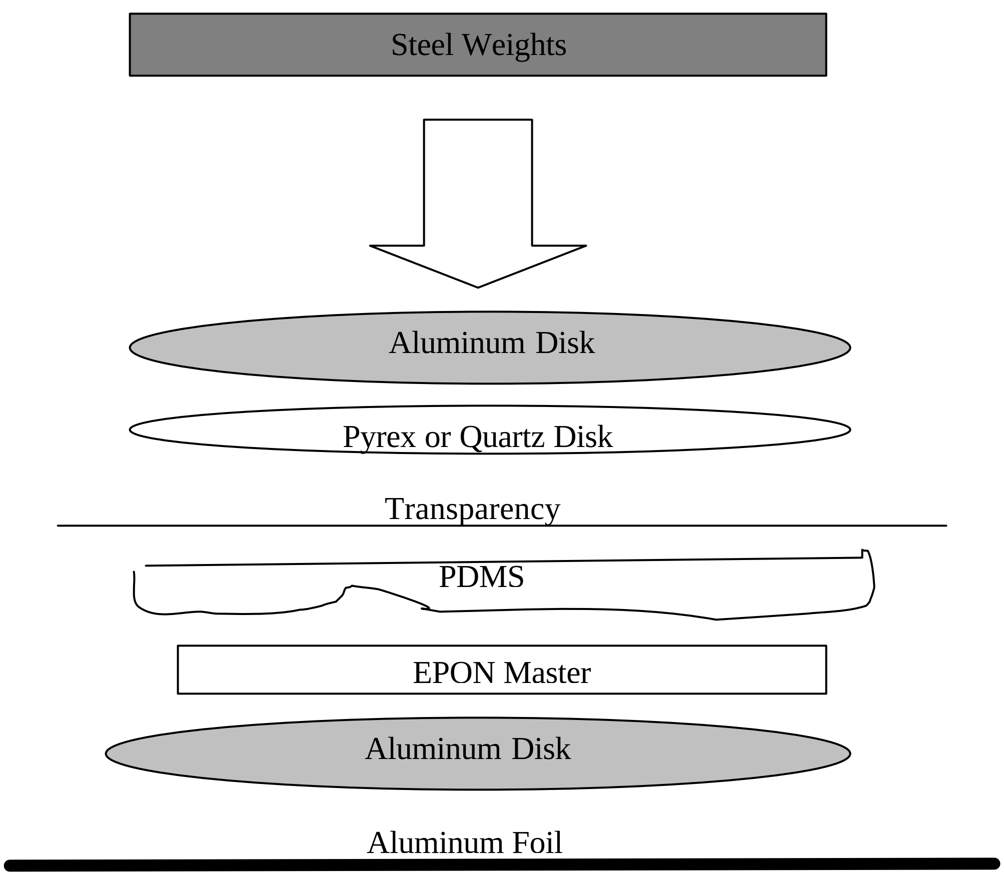

Figure 3. A graphical representation of the PDMS tension stencil procedure assembly.

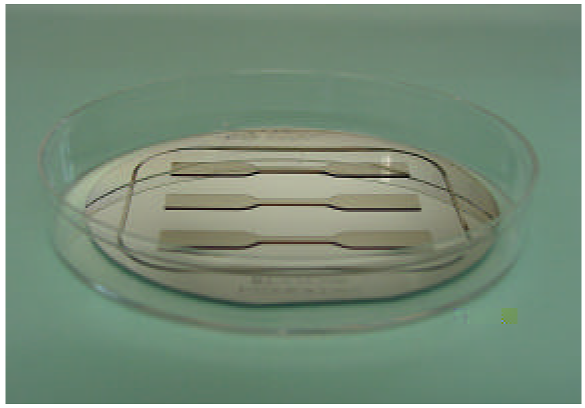

Figure 4. EPON Master, which serves as the cookie-cutter for the stencil.

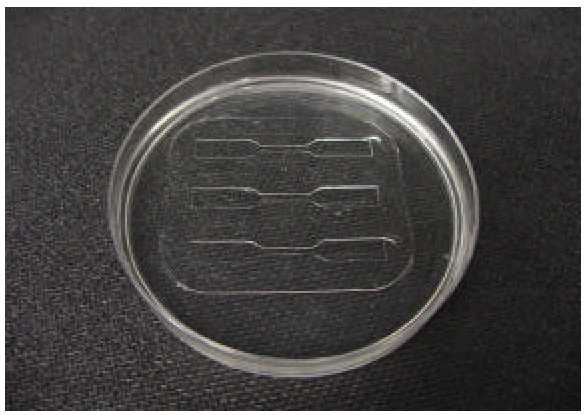

Figure 5. The PDMS stencil.

Once the stencils are made, they are stored in a petri dish filled with PDMS, which preserves them for up to one week. The procedure for making this base is attached in Appendix F. The entire stencil and base protocol has been tested twice with satisfying results. A similar ASTM, PDMS stencil procedure has been used by Prof. Crone, which this procedure is based. Prof. Crone's procedure is attached in Appendix G.

The stencil procedure has both advantages and disadvantages. It is simple and repeatable, but allows only a limited number of hydrogel samples and is relatively slow. One stencil makes three hydrogel samples and takes four to five hours to make. However, three stencils may be made at once with the starting materials available at our client's lab. This is enough for the purpose of the project at this stage.

Performance Testing of PDMS Stencil procedure

Upon testing of the PDMS stencil preparation procedure by researchers in Dr. Kao's lab, researchers provided suggestions for improvement. First, it was noticed that one should be careful in pouring an approximate amount of PDMS into the EPON master. For a close approximation, it was determined that only a small circular area (2 inches in diameter) in the EPON master should be covered with PDMS solution. The reason being is that if excess PDMS is poured it will be wasted and will lengthen the curing process because more PDMS requires more time in the oven. Also, it will make the weight allocation more difficult because the weights will move as the viscous solution is uniformly spread out. During testing, it was noticed that bubbles formed in the finished stencils. It was inferred that the continuous opening and closing of the oven to check the curing progress causes this problem. Therefore, it was decided to add a vacuum system to the oven, which will prevent air bubbles from forming in the PDMS stencils while the curing process takes place. Lastly, before testing, the cooling process of the stencils had not been quantified. It was determined that overnight cooling works better than a few hours.

Environmental Chamber

The purpose of the environmental chamber is to maintain pH and temperature during stress, strain, and creep testing of hydrogels. The testing procedure had to be kept in mind when designing the chamber, so it would not interfere with the tests. The description of a tensile test is outlined below, as well as restrictions of the chamber design. In selecting materials for the construction of the environmental chamber, initial temperature tests were performed on materials to determine their thermal properties. Three alternative designs and one final design for the environmental chamber were developed.

Tensile Testing

Prior to this semester, researchers in Prof. Kao's lab subjected hydrogel samples to tensile testing using an Instron Model 5548 testing machine. Load-displacement curves were recorded and converted to stress-strain data. These tests lasted from 0.5 to 5 minutes, which follow ASTM standards. The steps for the tensile testing are outlined below.

Prior to testing, the first step is to place the environmental chamber onto the Instron machine. The chamber has to enclose the hydrogel, which is attached to the Instron machine by two grips. The hydrogel is gripped at one end by the top part of the machine, and then lowered so it can be gripped at the other end to the bottom grip. It is crucial that the grips are aligned, so the hydrogel is held perpendicular to the lab bench so that is does not experience off-axis forces and break outside of the gauge length area. It is important that the user has access to the grips and the hydrogel while setting up the test, so the chamber must provide room for this.

The second step is to fill the environmental chamber with a solution of desired temperature and pH level. The chamber should prevent leakage to protect the surrounding machinery and instrumentation. The third step is to follow the testing instructions specific to the Instron machine. The hydrogel sample starts elongating vertically, perpendicular to the lab bench. The testing is monitored by observing the sample through the chamber. Therefore, the chamber should be transparent. The fourth step of the test is to stop the machine as soon as the sample fractures. The time to fracture, the location of the fracture, and other relevant information is recorded. The final step is to remove the hydrogel and drain the solution from the chamber using a vacuum system. Then the chamber is removed from the machine and stored for later use.

Initial Temperature Testing

Simple temperature tests were performed during the brainstorming phase of designing the environmental chamber to determine the decrease in temperature of water in different materials over a three minute period, the approximate time required for one mechanical test of a hydrogel sample. The client hypothesized that it will be unnecessary to monitor the temperature in the environmental chamber, assuming the temperature drop over three minutes will be negligible. These initial temperature tests were performed to confirm this assumption. The temperature drop of water was tested in a 250 mL pyrex beaker and in a polyethylene container of the same size. The temperature drop over three minutes was two degrees Celcius in the pyrex beaker and zero degrees Celcius in the polyethylene container. These preliminary results indicated that it was reasonable to assume the temperature drop over three minutes is negligible as long as an insulating material is chosen. However, these results must still be confirmed for larger volumes closer to the actual volume of the environmental chamber. There was not enough time this semester to perform temperature tests on a larger scale or for the final design made of acrylic and PVC.

Environmental Chamber Design

The nature of the testing requires certain characteristics of materials from which the environmental chamber will be made. The materials used should be durable, thermally insulating, transparent, easy to manufacture, resistant to degradation with changes in pH and temperature, and inexpensive. Low density polyethylene (LDPE), polypropylene (PP), Lexan, acrylic, and transparent polyvinylchloride (PVC) were researched. LDPE is thermally insulating, widely available, and inexpensive. Also, LDPE may be ideal for a collapsible design because it is soft and flexible. A disadvantage is that PE is translucent, and the user would not have a clear view of the sample. PP is transparent and allows the user to observe the samples during tests. It has greater toughness and rigidity than LDPE, and may be optimal for a chamber with sturdy walls. Lexan is transparent like PP but harder. Acrylic, a durable, transparent, thermally insulating material, is easy to manufacture and commercially available in many forms. Transparent PVC, which is also thermally insulating, is available in a flexible form that could be used for a collapsible design.

A variety of designs for the environmental chamber were discussed. Design 1, shown in Figure 6, is a cylindrical chamber with a flat bottom and a hole for the grip apparatus to enter through. An o-ring is placed around the circumference of the grip apparatus to prevent leakage of the testing solution. The problem with this design is that the permanent chamber walls will prevent easy access to manipulate the hydrogel and the grips.

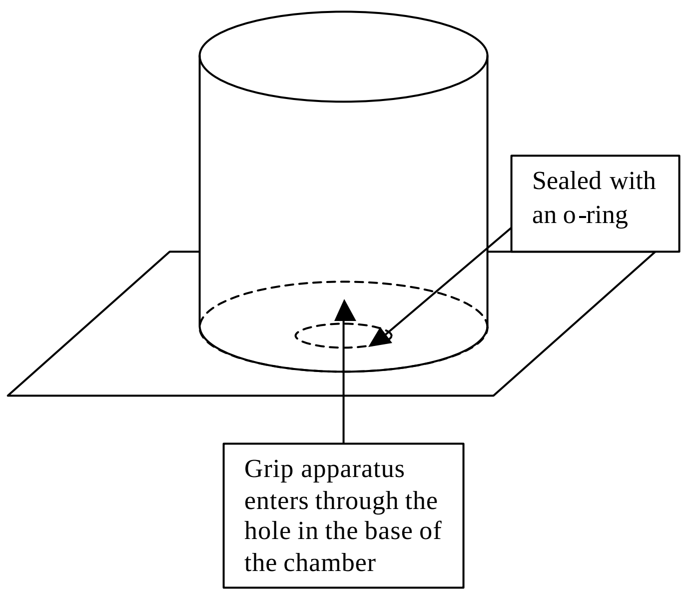

Figure 6: Design 1.

Design 2 is also a cylinder with a stationary base. As shown in Figure 7, removable sides can be taken off during the alignment of the hydrogel, and then replaced before filling the chamber with the testing solution. The walls require seams along the sides of the chamber and around the base. Seams can be sealed with silicone elastomer. Leakage can be reduced by securing the chamber with two belts around the circumference of the chamber near the top and bottom. A disadvantage to this design is the large number of seams that may cause leakage of the testing solution. In addition, if all the solution is not removed with the vacuum system, taking off walls when the solution is still present may result in leakage.

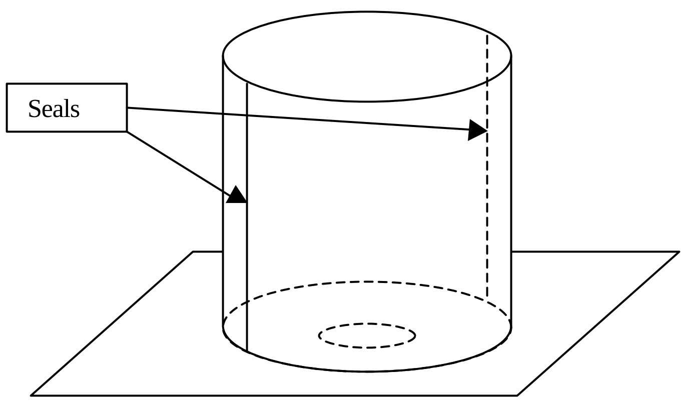

Figure 7. Design 2.

Design 3 consists of two cylindrical pieces, one on top of the other as shown in Figure 8. The top piece has a temporary seal on the sides, so that is can be attached after the hydrogel sample has been aligned. The seam around the base has been eliminated by moving it approximately 2 to 3 inches above the base. The bottom piece has no seals, so that if all of the testing solution is not removed with the vacuum apparatus, the remaining solution can collect in the base and remain there while the top part of the cylinder is removed. The grip apparatus enters through a hole in the base and through the small, inner cylinder. This design has two disadvantages: the seams on the sides can cause solution leakage and the base is difficult to manufacture.

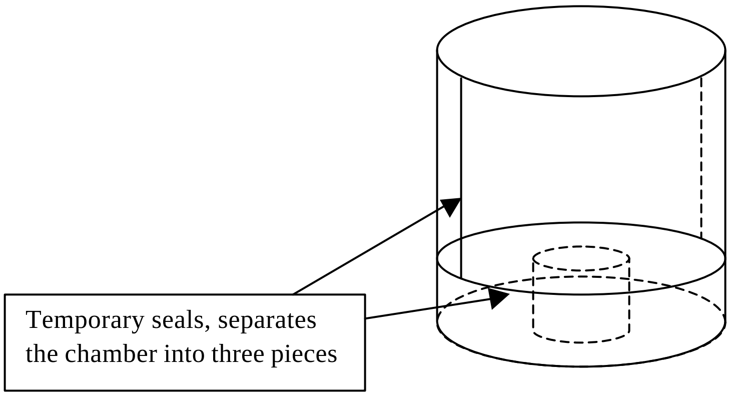

Figure 8: Design 3

The final design, shown in Figure 9, solves these problems, as well as, having the advantages of the designs 1, 2, and 3.

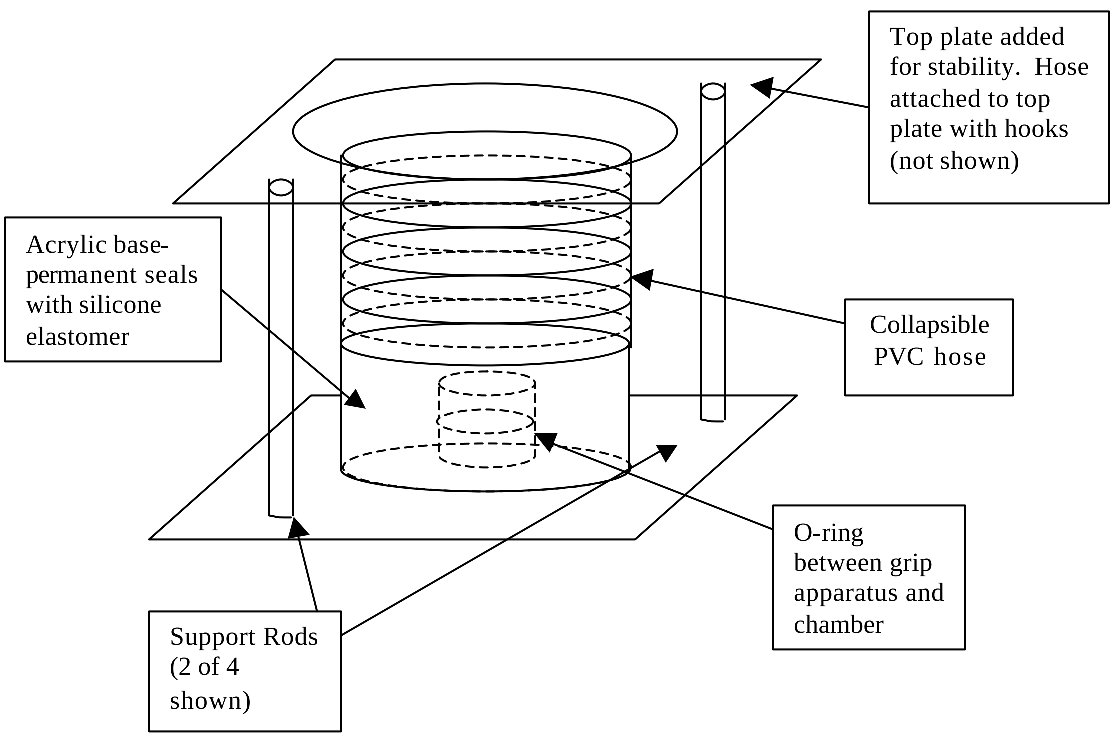

Figure 4: Final Design

The final design consists of a similar base as discussed in design 3, but the base consists of three separate pieces: a flat plate, large cylinder, and a smaller cylinder. These three pieces are permanently sealed together and allow collection of any solution not removed by the vacuum system. This design is easier to manufacture than design 3 and minimizes leakage. The top of the chamber is made of soft plastic, which is permanently sealed to the bottom piece. The plastic can be pushed down while the grips are adjusted, eliminating the seals on the side of the chamber. This design is advantageous because the walls can be elongated for hydrogels that have extreme elastic properties. The soft plastic is held up by hooks, which attach to a plate fixed on top of four rods attached to each corner of the base for support.

The bottom three pieces are made from acrylic. Acrylic was chosen because of its durability, high refractive index, and availability. These pieces were ordered from Acry Fab, a company in Sun Prairie, Wisconsin, and were glued together and then sealed with silicon elastomer. The top plate is also made of acrylic. An o-ring was placed inside the inner cylinder, enabling a tight seal to be formed with the grip apparatus and chamber during testing. Clear PVC hose, reinforced with a steel wire helix, was chosen for the soft, plastic region (ordered from McMaster-Carr). The support rods are made of polycarbonate because it is a durable, inexpensive, and readily available material. (Dimensional drawings of the final design and each individual part would have been included in the Appendix along with an itemized list of costs but some final dimensions and prices could not be obtained in time from the ME shop.)

FUTURE WORK

The stencil procedure will be used in ongoing research, and more feedback will be provided on its efficacy. Also, the environmental chamber will be tested for use with the Instron 1000. Necessary changes will be made to the stencil procedure and environmental chamber. Temperature testing of the environmental chamber will be performed as well. An environmental chamber for the Instron 5548 machine will be developed, and adjustable rods may be added to both chamber designs.

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The Product Design Specification of the Mechanical Testing System Coupled with an Environmental Chamber for Hydrogels: Procedure for Making Polydimethyl Siloxane (PDMS) Stencils

Gabriel Martinez-Diaz, Darcee Nelson, Christy Palmer
4/4/02

Function: To establish a procedure to make tension stencils, approved by the American Society for Testing Materials (ASTM).

Client Requirements:

- Fast procedure to make ASTM approved PDMS stencils
- Method to ensure uniform thickness of approximately 280 µm stencils.

Design Requirements:

1. **Physical and Operational Characteristics**

   a. *Performance Requirements:* The stencil made should be disposable. The procedure should allow three stencils to be made at one time.

   b. *Safety:* Be careful when using the oven because it is very hot, 80 to 90°C. Although, the materials used are nontoxic, gloves should be worn.

   c. *Accuracy and Reliability:* The mixture of silicone elastomer and curing agent must be homogenous and properly degassed.

   d. *Life in Service:* The stencil is to be used only once and then thrown out. It should also be used within one week after it is made.

   e. *Shelf Life:* The stencil should be stored in the petri dish in which it is made.

   f. *Operating Environment:* The procedure should be carried out at room temperature and normal humidity.

   g. *Ergonomics:* N/A

   h. *Size:* The stencil should be 280 µm thick, have a gauge length of 11 mm, and a neck width of 2 mm.

   i. *Weight:* 10 parts by weight of silicone elastomer should be used to 1 part by weight of curing agent. To make one stencil, use 50 g of silicone elastomer and 5 g of curing agent.

j. *Materials:* The following materials should be used: Sylgard Brand 184 silicone elastomer, Sylgard Brand 184 curing agent, pyrex plates (3 mm thick, 8.5 cm diameter), EPON Master (~280 μm thick, 11 mm gauge length, 2 mm neck width), polystyrene petri dishes (100 mm diameter, 15 mm height), weights – 1 Al plates (3.5 in. diameter, ~102 g) and 3 Al plates (3 in. diameter, ~80 g), tweezers, presentation transparency.

k. *Aesthetics, Appearance, and Finish:* N/A

2. Production Characteristics

a. *Quantity:* One procedure is needed. At least 10 stencils are needed for client.

b. *Target Product Cost:* ?

3. Miscellaneous

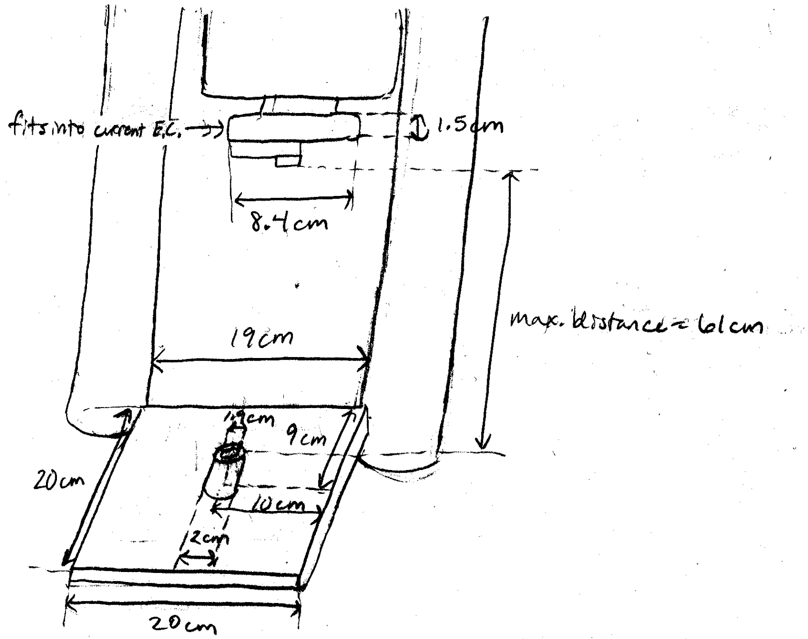
a. *Standards and Specifications:* ASTM approval is required.

b. *Customer:* N/A

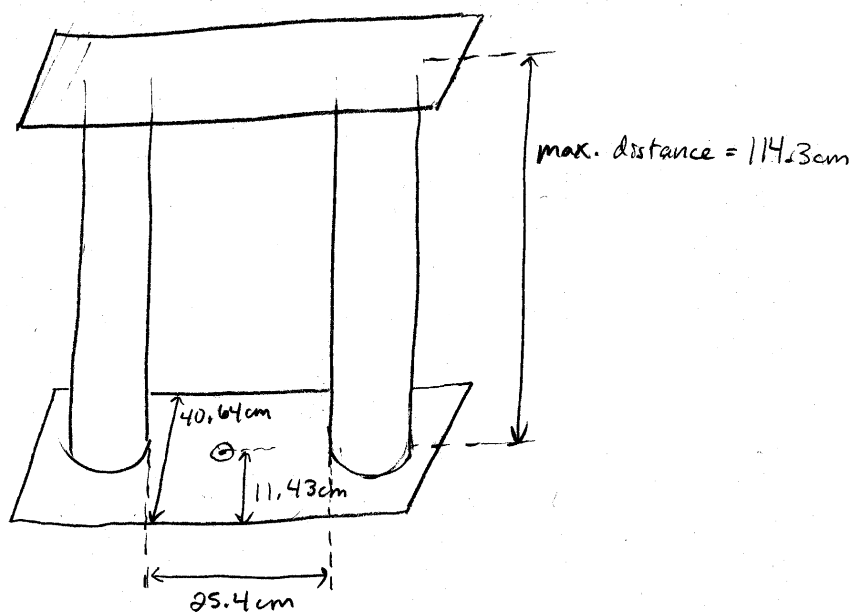
c. *Patient-Related Concerns:* N/A

d. *Competition:* Prof. Beebe and Prof. Crone have similar procedures.

Instron 5548



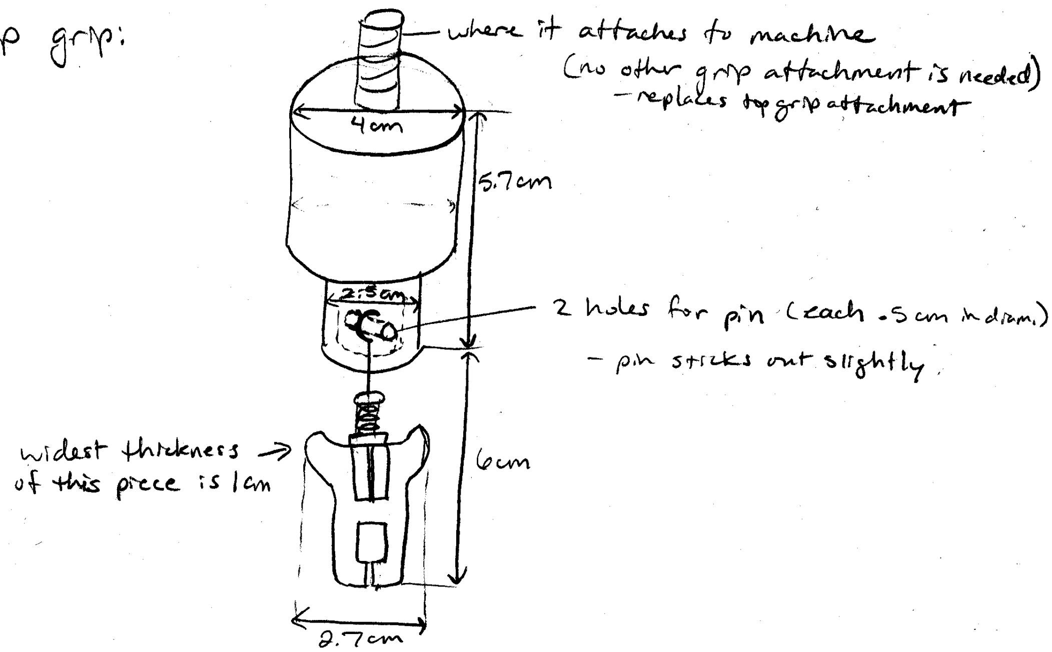
Instron 1000



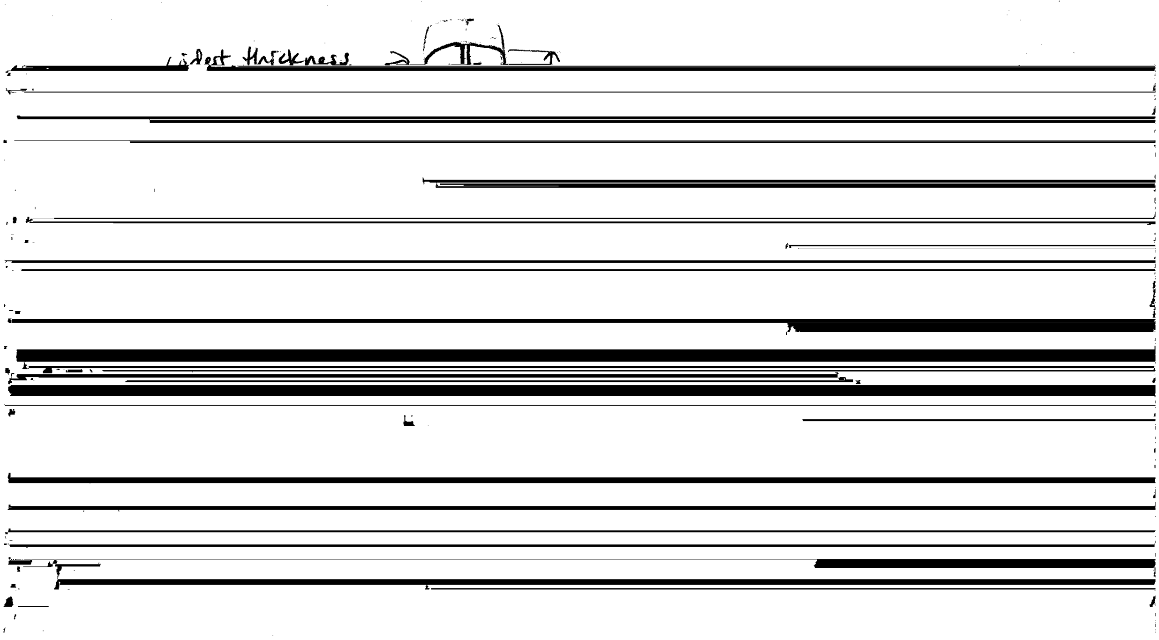
Appendix C

Wedge Grips to be Used with Instron 1000:

top grip:

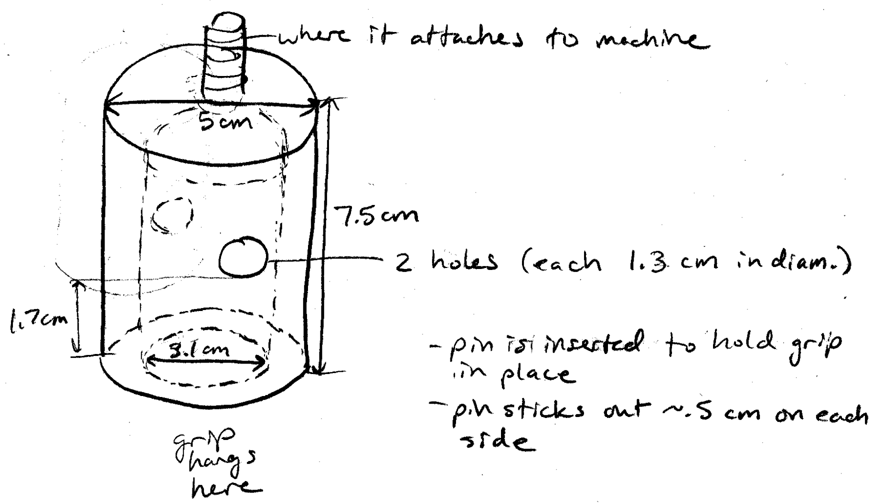


bottom grip:

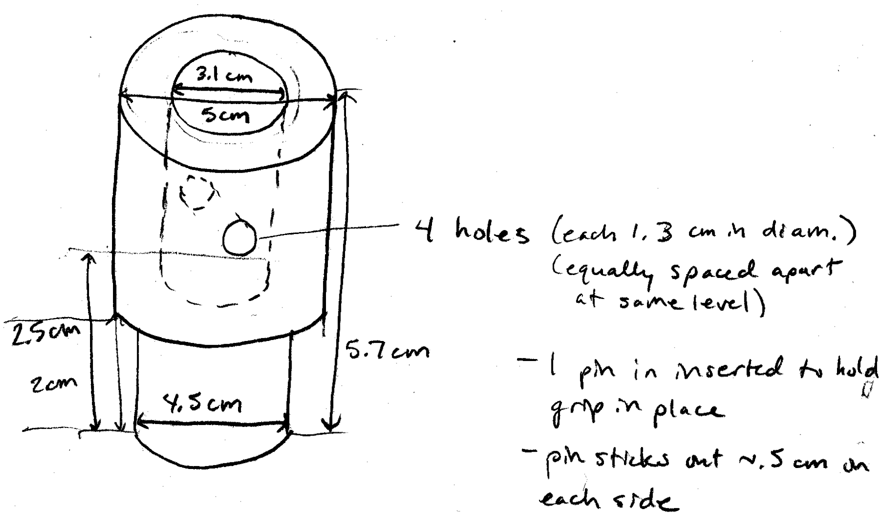


Standard Grip Attachments for Instron 1000:

top:



bottom:



The Product Design Specification of the Mechanical Testing System Coupled with an Environmental Chamber for Hydrogels: Environmental Chamber

Gabriel Martinez-Diaz, Darcee Nelson, Christy Palmer
4/4/02

Function: To provide a pH and temperature controlled environment for the mechanical testing of hydrogels.

Client Requirements:

- Maintain the pH of a solution in the range of 2 – 10
- Maintain the temperature of a solution in the range of 4 – 44 degrees Celsius
- No interference with the dynamic stress-strain relationship, creep, and ultimate stress and strain tests.
- Compatible with the Instron 5548 and the Instron 1000

Design Requirements:

1. Physical and Operational Characteristics

l. *Performance Requirements:* The environmental chamber should be able to be used as much as necessary (i.e. consecutive tests).

m. *Safety:* The chamber should be securely sealed to prevent potentially acidic (pH levels of 2-6), basic (pH levels of 8-10), very hot (4 to 40 degrees Celsius), and/or very cold solutions from escaping and causing injury to both the user and any surrounding lab equipment. Gloves should be worn when handling the solutions to be used in the chamber. Oven mitts should be worn if the solution to be used is very hot.

n. *Accuracy and Reliability:* The chamber should not interfere with the mechanical testing of the hydrogels.

o. *Life in Service:* The chamber should maintain the temperature and pH of a solution over a time period between 0.5 to 5 minutes, the length of each individual mechanical test approved by ASTM.

p. *Shelf Life:* The chamber should be stored clean and dry in a cool, dry place.

q. *Operating Environment:* The chamber is to be used at room temperature, atmospheric pressure, and normal humidity. It will also be exposed to solutions with temperature ranging from 4 – 40 degrees Celsius and pH ranging from 2 – 10.

r. *Ergonomics:* The chamber should be easy to transport.

s. *Size:* See the Instron 1000 and the Instron 5548 dimensions (to be attached later). The chamber should fit with both of these machines.

t. *Weight:* The chamber should be light enough to be lifted by one person.

u. *Materials:* The materials of the chamber should be durable, transparent, easy to manufacture, affordable, insulating, and able to withstand changes in temperature from 4 – 44 degrees Celsius and changes in pH from 2 – 10.

v. *Aesthetics, Appearance, and Finish:* The chamber should have a transparent shell so that the user can see the hydrogel sample inside. It should also have no sharp edges or extrusions.

2. Production Characteristics

c. *Quantity:* One environmental chamber is needed.

d. *Target Product Cost:* ? at this time.

3. Miscellaneous

e. *Standards and Specifications:* None.

f. *Customer:* See client requirements.

g. *Patient-Related Concerns:* N/A

h. *Competition:* Prof. Crone has an environmental chamber setup in her lab for similar purposes.

Appendix E

Procedure for Making Polydimethyl siloxane (PDMS) Stencils of Tension Samples

1. Put on gloves because the elastomer is very sticky.

2. Measure out (by weight) 10 parts of elastomer (large container) to 1 part hardener (small bottle) using separate clean weighing dishes.
    a. To make 1 PDMS Stencil, it is recommended to use 10 grams of elastomer and 1 gram of hardener.
    b. To make 1 PDMS base, in which the stencil will be placed, it's recommended to use 50 grams of elastomer and 5 grams of hardener.

3. Mix the elastomer and the hardener thoroughly with the tines of a plastic fork for approximately 5 minutes.
    a. If it is not mixed well, the PDMS will not cure properly.

4. Pour the mixture into a jar and degas it under vacuum for approximately 1hour.
    a. Make sure all bubbles are gone.
    b. For larger volumes to make more than 1 stencil and 1 base, it may longer than 1 hour.

5. Place EPON Master on top of aluminum disk, which is placed on aluminum foil.

6. Pour just enough degassed mixture over EPON Master to cover about 2/3 of it.
    a. Make sure you pour the mixture slowly starting at the center of the EPON Master; this will prevent forming bubbles.

7. Hold weight and EPON master in hand and gently tip it so that the PDMS mixture moves to cover the entire EPON Master.

8. Place a piece of copy machine transparency over the PDMS.
    a. Make sure transparency has a diameter slightly larger than the EPON master

9. Starting from the center of the master, use a small cylinder (pen or marker) to roll out any bubbles that may have formed under the transparency.
    a. Roll from the center in one direction, and then starting from the center again, roll in the opposite direction.

10. Place pyrex disk on top of transparency. Place aluminum disk on top of the pyrex disk. Place 2 steel weights on top of the aluminum disk to push out any excess PDMS.
    a. Hold weights in place as PDMS mixture is pushed out from underneath to prevent the transparency from sliding. Once the setup settles and no longer slides, then may let go.

11. Bake in an oven for 3 hours at 80 ^{o}C.

12. When done baking, let cool to room temperature. Then separate components.
    a. Throw away transparency and aluminum foil.
    b. Clean off any PDMS residues on EPON Master, pyrex disk, and weights with methanol.

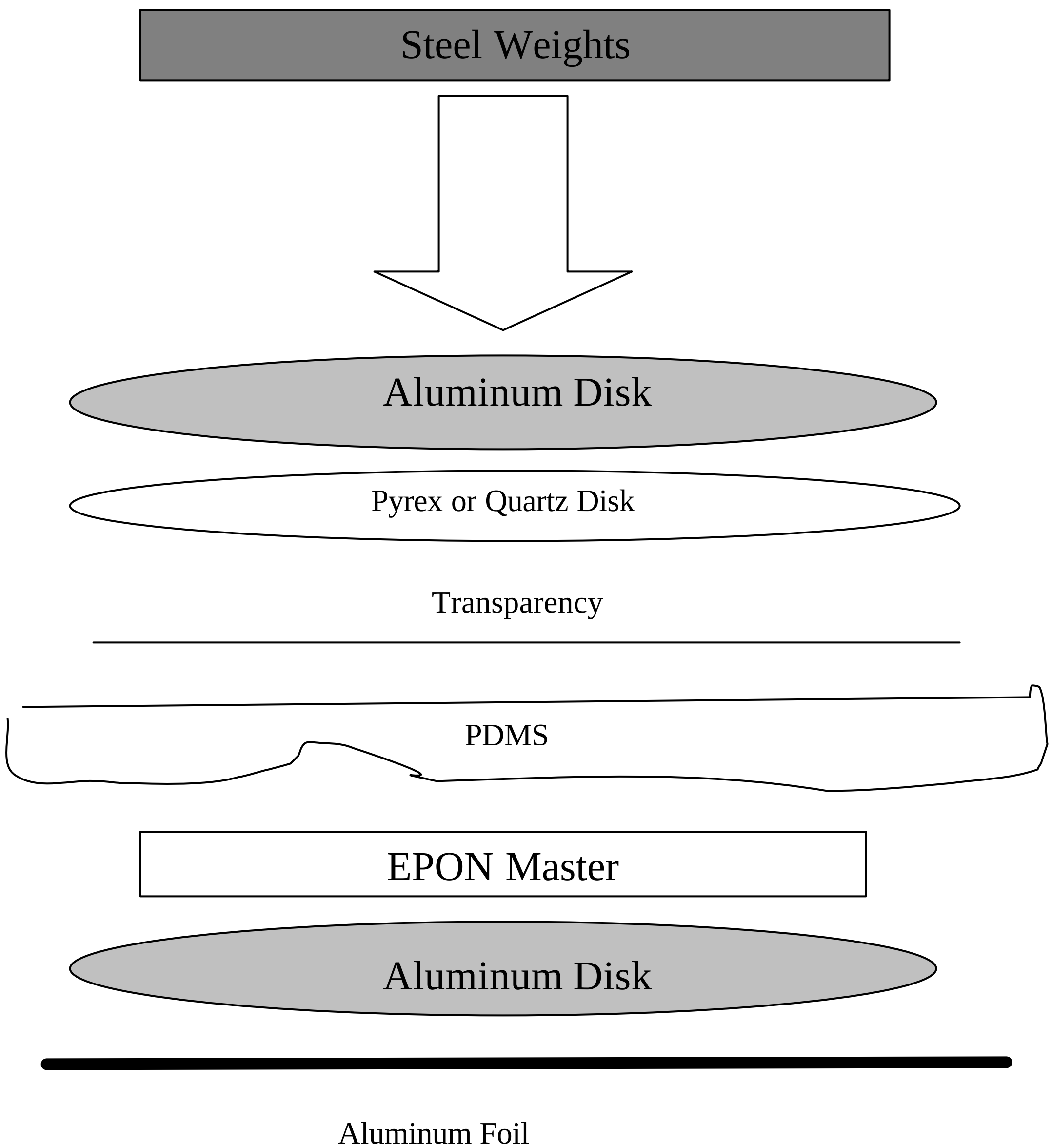


Figure 1. A graphical representation of the PDMS tension stencil procedure assembly.

Appendix F

Base for Tension Samples
And
Cover Slip for Tension Samples

1. Put on gloves because the elastomer is sticky.

2. Measure out (by weight) 10 parts of elastomer (large container) to 1 part hardener (small bottle) using separate clean weighing dishes.
    c. To make 1 PDMS Stencil, it's recommended to use 10 grams of elastomer and 1 gram of hardener.
    d. To make 1 PDMS base, in which the stencil will be placed, it's recommended to use 50 grams of elastomer and 5 grams of hardener.

3. Mix the elastomer and the hardener thoroughly with the tines of a plastic fork for approximately 5 minutes.
    a. If it is not mixed well, the PDMS will not cure properly.

4. Pour the mixture into a jar and degas it under vacuum for approximately 1 hour.
    a. Make sure all bubbles are gone.
    b. For larger volumes to make more than 1 stencil and 1 base, it may longer than 1 hour.

5. Pour degassed mixture into the bottom of the Petri Dish.
    a. Make sure you pour the mixture slowly starting at the center of the Petri Dish; this will prevent forming bubbles.
    b. Fill the Petri Dish up to half of its volume.

6. To make the Cover slip, use the top part of the Petri Dish.
    a. Pour a thin layer of the PDMS solution. It should not reach the border of the Petri Dish. Hold dish in hand and gently tip it so that the layer spreads out towards the edges of the dish.

7. Bake in an oven for 3 hours at 80 ^{o}C.

Procedure for making Polydimethylsiloxane (PDMS) Stencils of Tension Samples

1. Measure out (by weight) 10 parts of elastomer (large container) to 1 part hardener (small bottle) into a clean plastic weighing dish or cup.
2. Mix thoroughly with the tines of a plastic fork (if it is not mixed well, the PDMS will not cure properly).
3. Degas under vacuum for approximately 40 minutes or until all the bubbles are gone (larger volumes may take longer).
4. Pour degassed mixture over EPON master.
5. Place a piece of copy machine transparency (slightly larger than the EPON master) over the PDMS.
6. Starting from the center of the master, use a small cylinder (pen or marker) to roll out any bubbles that may have formed under the transparency. Roll from the center in one direction, and then starting from the center again, roll in the opposite direction.
7. Place weights over transparency and master to push out any excess PDMS.
8. Bake on hot plate for 3 hours at 80 °C.