

Mechanical Testing System Coupled with an Environmental Chamber for Interpenetrating Networks and Hydrogels

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ABSTRACT

Interpenetrating networks (IPNs) and hydrogels have many biomedical applications. Different applications require varying mechanical properties. The mechanical properties of IPNs and hydrogels are dependent on their chemical composition, as well as the temperature and pH of their surroundings. At present, there is limited published work to describe these properties and conditions. The goal of this project is to design a testing system(s), which will allow our client to identify the dynamic stress-strain relationship, creep, and ultimate stress and strain under varying temperatures (4–40°C) and pH levels (4–8). Before the mechanical properties of IPNs can be tested, individual IPN samples must be made. A procedure to fabricate stencils, which are used to make IPN samples, was developed during a previous semester. Also, a prototype of an environmental chamber to be used for tensile testing with the Instron 1000 Material Testing System was built. This semester, the stencil procedure was revised and refined, and the environmental chamber prototype was modified and tested. Lastly, a creep testing system has been designed and a prototype has been built. Testing of this system, as well as a comparison between the available Instron 1000 and 5548 material testing systems will be the primary focus of the forthcoming design course, BME 402.

1. INTRODUCTION

Interpenetrating networks (IPNs) are formed by combining two or more different macromolecules in an intertangled structure (i.e. a gelatin and a polymer chain). These molecular structures can be formed through photopolymerization allowing for the conversion of photoreactive solutions into gels or solids under varying physiological conditions. Hydrogels are produced by cross-linking gelatin backbones to different chemicals (i.e. gelatin and glutaraldehyde), and does not involve a photoreactive procedure. Hydrogels are excellent in helping to create or maintain a moist environment, and provide absorption, desloughing and

debriding capacities to necrotic and fibrotic tissue [1]. Despite these desirable properties, many hydrogels, like those used by our client, involve the use of toxic cross-linkers, such as glutaraldehyde-fixed agent. This makes IPNs more suitable than hydrogels for biomedical applications, such as drug delivery, tissue engineering scaffolds, and wound healing. In the past, our client's research has focused on using gelatin and poly(ethylene glycol) (PEG) diacrylate (PEG-dAc) (Appendix F) as the two main components in the IPN formulation. For the purpose of clarity, IPNs and hydrogels will both be referred to as "gels" for the duration of this paper.

Although gels have been extensively used in biomedical research, there is limited published work that presents findings on the physical changes of gels in response to specific environmental conditions, and how these changes affect the specific gels' biofunctions and applications [2]. Because knowledge of the mechanical properties of biomedical gels allows researchers to evaluate the material intended for a particular application [3], an understanding of the mechanical characteristics of gels is essential. Thus, the ultimate goal of this project is to design a testing system(s) that will be used to elucidate the tensile and creep properties of gels in response to changes in their environment, such as pH and temperature.

The desired tensile properties of gels that our client would like to determine are Young's modulus, ultimate stress & strain, and yield stress & strain. These five parameters describe the elastic and plastic properties of gels. For this project, a commercially available material testing system (Instron 1000) will be used to induce a uniaxial tensile force upon a sample gel specimen. Before a tensile test, gel specimens are synthesized (Appendix F) and pretreated with appropriate environmental conditions for a specified length of time (2, 168, or 336 hours). During a tensile test, a specimen is secured at each end, using a pair of Instron 2711 grips, and is subjected to a constant rate of displacement (range: 5-10 mm/min) induced by the Instron 1000. During each test, the resulting load vs. displacement data is plotted by a strip chart recorder that is connected

to the Instron 1000. Measurements of the initial cross-sectional area and gauge length of the specimen will allow the load vs. displacement curve to be converted to a stress-strain plot similar to the relationship shown below.

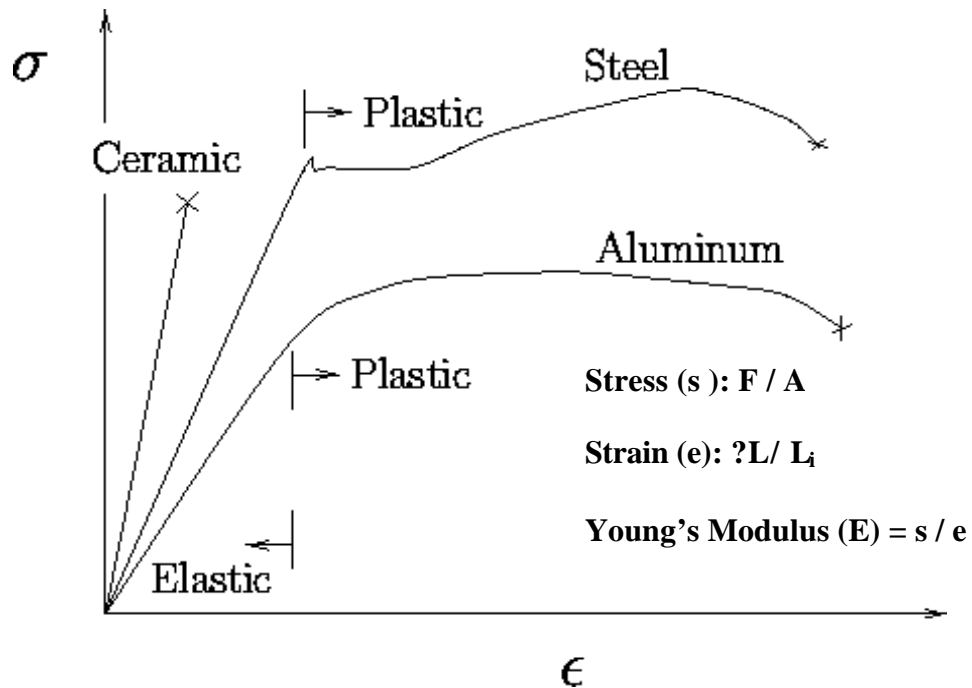


Figure 1. A representative graph showing stress-strain relationship of different materials.

Creep tests are performed in a distinct manner from tensile tests, and provide additional information about a gel's mechanical properties. Specifically, a creep test records the plastic deformation of a material over time, while under a constant load. As in a tensile test (Figure 1), a creep test shows the plastic and elastic deformation of a material. For both tests, the observed viscoelastic (creep) and tensile properties for a given material will depend greatly on the surrounding temperature and applied load. In particular to creep testing, certain materials may not exhibit creep at absolute temperatures below one-half of their melting point [4].

To perform a creep test of a gel, a constant tensile load is applied to a specimen, and the elongation of the specimen in the direction of the applied load is measured over time. The test is

usually performed to the point of failure of the specimen using a relatively light load (10-200 kg), so the duration of the test can often be notably long (i.e. 1 mo – 1 yr).

Creep testing machines have traditionally been composed of five main components: a chamber, a pair of grips, a loading apparatus, and an extensometer. In general, there are two fundamental design concepts for loading the material: the sample can be loaded from the bottom, by a suspended weight; or the sample can be loaded from the top using a lever or pulley system with an attached weight.

In contrast to the tensile testing component of this design project, there are no commercially available material testing systems that perform creep tests on soft biomaterials, like gels. Our client, Dr. Kao, is interested in researching the creep properties of gels and consequently must obtain a device with which these properties can be investigated.

2. DESIGN OBJECTIVES

The client, Prof. Weiyuan John Kao, is a professor in the School of Pharmacy and also has an appointment in the College of Engineering's Department of Biomedical Engineering, both at the University of Wisconsin-Madison. Prof. Kao is interested in learning the following mechanical properties of gels: the dynamic stress-strain relationship, creep, and the yield and ultimate stresses and strains. He would also like to observe these properties under varying temperature ($37 \pm 3^\circ\text{C}$) and pH levels (4.5–8), which mimic physiological conditions. The structure and environment (pH, temperature, and blood compounds) regulate the degradation of gels [3]. These environmental factors are critical in affecting the mechanical and chemical properties of biomedical materials. Research has shown that gels whose surroundings undergo changes in pH and temperature, often degrade, exhibit changes in swelling, and change their mechanical strength [4]. These physical modifications of the gels are also dependent on their

own chemical composition. Therefore, gel specimens of different compositions will be tested by our client, to determine the effects on their mechanical properties.

Before the mechanical properties of gels can be tested, the individual gel specimens must be made. In order to complete this task, a procedure to fabricate a stencil was developed during the preceding design course, BME 301. Gel specimens are then made using one of these stencils. For this semester, the design team was divided into two groups: tensile testing and creep testing. The tensile testing team's goals included: update the existing dog-bone stencil procedure; test the compatibility of the environmental chamber prototype, built during BME 301, with the Instron 1000 Material Testing System; make the necessary modifications to the prototype; and conduct overall validation of the tensile testing system, including the Instron 1000, the environmental chamber, and any other additional components. The creep testing team's goals were to design and build a prototype of a creep testing system, to be used for studying the viscoelastic properties of gels. To avoid confusion, all persons from Dr. Kao's lab involved in using the tensile and creep testing systems will be referred to as "researchers".

3. PAST WORK

3.1 Stencil Procedure

The client specified that the gel specimens must be of 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 illustrated in Figure 2. The stencil procedure produces a stencil that allows three gel samples to be made at a time with these required dimensions. The procedure was designed to be relatively fast (3 hr), ensure uniform thickness of the stencil ($\pm 5 \mu\text{m}$), and maximize gel production (at least 2 good samples per stencil). Having a uniform stencil will minimize variance between sample specimens and therefore reduce the likelihood of anisotropy of the gel, which would have an

effect on the mechanical property data. The updated product design specification for the stencil procedure is attached in Appendix A.

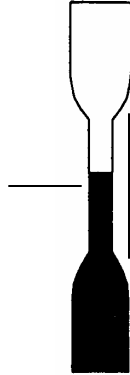


Figure 2. The dog bone shape, required by ASTM for mechanical testing, has the approximate dimensions shown above.

3.2 Tensile Testing

The client specified that the environmental chamber must: maintain a relatively constant temperature ($\pm 3^{\circ}\text{C}$ of desired temperature) and pH (± 0.5 pH units of desired pH level) of the buffer solution used during the mechanical tests (duration of 0.5 to 5 minutes); not interfere with the testing procedure, or the stress or strain properties of the material; and be compatible with the Instron 1000 Material Testing System (located in 1313 Engineering Hall) shown in Figure 3. The chamber must withstand the temperature of a solution in the range of 4 to 40°C and the pH in the range of 4.5 to 8. The selection of grips to secure the gel specimens in place on the Instron 1000 is critical to the function of the tensile testing system. The grips must be strong enough to secure the gels in place, but sensitive enough to avoid causing wear or premature fracture of the gel specimen. The design team discovered the Instron 2711 Lever Action Grips and selected them as the best potential grips, as they were designed for a smaller, more sensitive material testing system. The Instron 2711 grips will be referred to simply as “grips”. Construction of an

environmental chamber prototype was completed at the end of the preceding semester (Figure 4), but due to time constraints, no testing was conducted. The product design specification for the environmental chamber is attached in Appendix B.



Figure 3. The environmental chamber was designed for use with the Instron 1000 Material Testing System, available at 1313 Engineering Hall Materials Testing Laboratory.

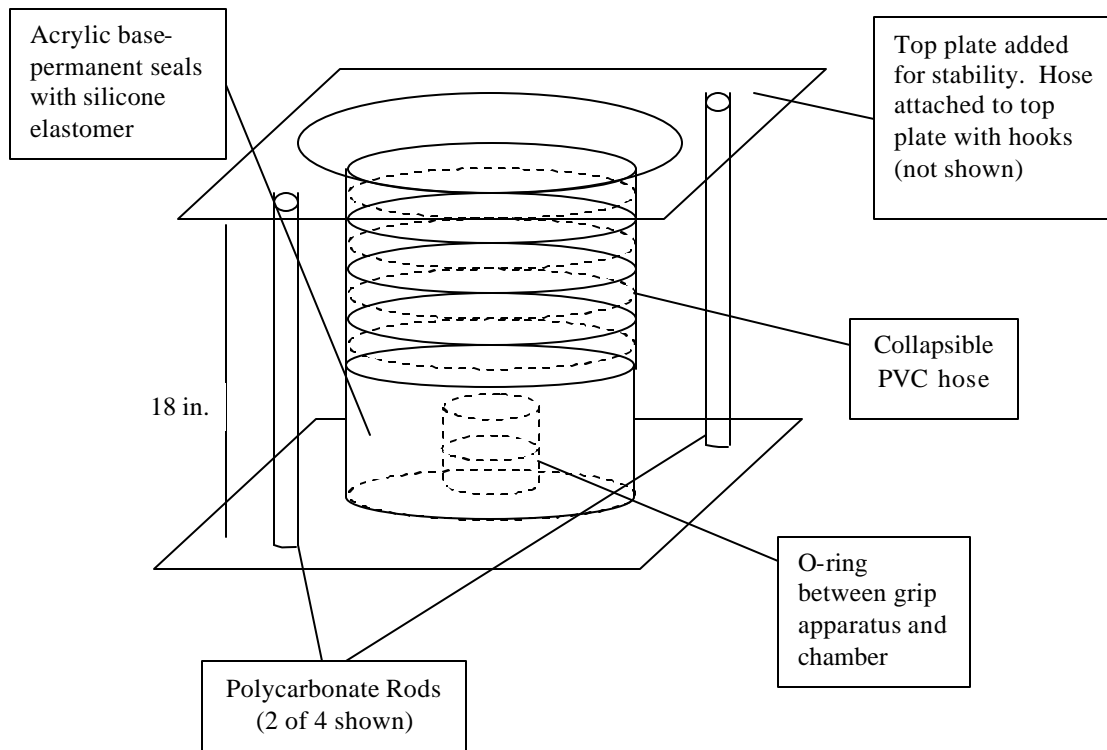


Figure 4. A sketch of the final environmental chamber design.

3.3 Creep Testing

This component of the design project was added on at the beginning of the current semester. Thus, no prior work was completed. The creep testing team is responsible for the design and construction of a prototype system that will be used to study creep properties of gels.

4. CURRENT PROGRESS

4.1 Stencil Procedure

The stencil procedure that follows ASTM guidelines and was originally developed during BME 301 has been reviewed and revised, based on the feedback given by researchers. The stencils are made of Polydimethylsiloxane (PDMS), which is used for several reasons. It is inexpensive (less than \$40.00 per kit), permeable to gases, transparent, and bonds easily [5]. PDMS is prepared in the laboratory by mixing a PDMS pre-polymer and curing agent (SYLGARD 184 Silicone Elastomer Kit, Dow Corning, Midland, MI), and degassing for approximately one hour. To make the stencil, the PDMS mixture is poured over the EPON Master (a silicon wafer) and sandwiched between two aluminum disks by placing steel weights on top (Appendix C). The EPON Master, shown in Figure 5, serves as a cookie-cutter to remove 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 placement of the weights and thus ensure uniform thickness of the stencil. The PDMS sandwich is cured for three hours at 80°C. After curing, the PDMS stencil can be peeled away from the EPON Master and transparency. Figure 6 shows the final stencil product. The complete stencil procedure is attached in Appendix C.

Once the stencils are made, they are stored in a petri dish filled with a PDMS structural base, which preserves them for up to one month. The procedure for making this base is attached

in Appendix D. A similar PDMS stencil procedure, upon which this procedure is based, has been used by Professor Wendy Crone (Appendix H).

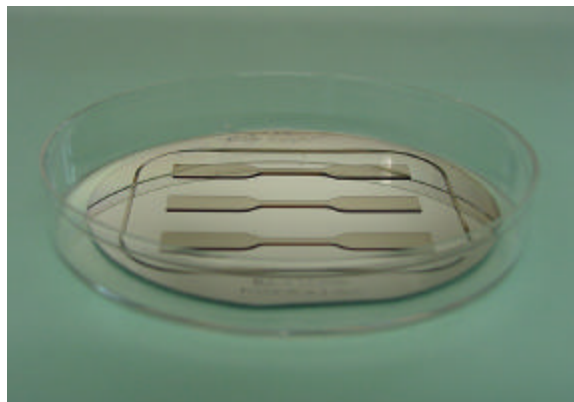


Figure 5. EPON Master, which serves as the cookie-cutter for the stencil.

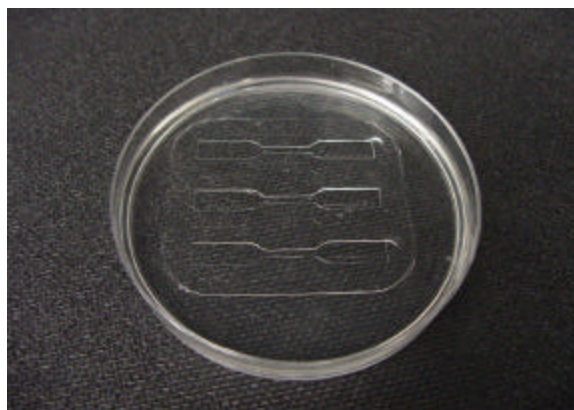


Figure 6. A finished PDMS stencil.

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

Since its original development, researchers in Dr. Kao's lab have used the PDMS stencil procedure about 50 times, and have provided the design team with feedback and 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) of the EPON master should be covered with PDMS solution. Pouring an excess amount of PDMS onto the EPON master will result in wasted PDMS, and will lengthen the duration of the curing process, as an increase in PDMS requires an increased time in the oven. Also, it will make the weight placement more difficult because the weights will move as the viscous solution is uniformly spread out. During testing, it was observed that bubbles formed in the finished stencils. It was inferred that the continuous opening and closing of the oven to check the curing progress is the cause of 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 during the curing process. Lastly, the original procedure did not quantify the cooling process of the stencils. It was determined by the researchers that overnight cooling (12 hr) yields better stencils than cooling for only a few hours (2-4 hr) does.

4.2 Tensile Testing

Over the course of this semester, the tensile testing system (including the environmental chamber, Instron 1000 Material Testing System, Instron 2711 grips, and additional components) was tested, modified, and validated.

4.2.1 Compatibility and Modification

The environmental chamber was tested for compatibility with the Instron 1000 material testing system (Figure 7) The base of the chamber (6.5" x 6.5") was narrow enough to fit in the Instron 1000, but the chamber's height (18.5 inches) interfered with the testing system set-up.

The top plate of the chamber obstructed the downward travel of the crosshead on the Instron 1000, ultimately preventing the top grip from lowering to the initial testing position. To solve this problem, the team decided to remove the top plate of the chamber, so that the crosshead could be lowered to any desired position. Although the top plate was originally designed to stabilize the four polycarbonate rods, it was determined by observation that removing the top plate would not considerably decrease the stability of the chamber.



Figure 7. Illustration of environmental chamber on Instron 1000. The chamber's top plate has been removed to allow free travel of the load cell and top grip, and the PVC hose is lowered for orientation of bottom grip within the chamber.

While these compatibility observations were being conducted, a very substantial compatibility issue was brought to the attention of the design team. John Dreger (Manager, 1313 Engineering Hall – Instructional Structures and Materials Testing Laboratory) informed the design team that no liquid solutions could be brought into contact with any part of the Instron 1000. Any leak from the environmental chamber or spill during testing could potentially cause the machine to rust and wear prematurely. To eliminate the possibility of any such accidents, some design modifications were made to the whole tensile testing system. Since the Instron

2711 grips are constructed from aluminum and plastic, John Dreger informed us that they are submersible and will not rust and wear.

Upon closer inspection of the environmental chamber and the Instron 1000, it was determined that modifications must be made to the overall design of the testing system in order to prevent leakage of buffer solution from the chamber. The only interface of concern between the chamber and the Instron 1000 was where the inner cylinder of the chamber was designed to create a seal (o-ring) with the steel grip adaptor that attaches the bottom grip to the base of the Instron 1000 (Appendix K). There were three potential problems with this interfacing of components: the grip adaptor is made was steel, and would likely rust if contacted with buffer solution; the o-ring on the inside of the inner cylinder of the chamber did not fit tightly with the steel grip adaptor; and there was no seal between the inside of the grip adaptor and the grip apparatus, to prevent leakage through center and out the bottom of the grip adaptor.

A final solution to this problem was generated through a meeting with Dr. Kao, and subsequent meetings with Bill Hagquist (Director, College of Engineering Student Shops) and John Dreger. The final solution was to machine a new aluminum grip adaptor that would fit tightly against the o-ring of the inner cylinder and thus form a sufficient seal with the chamber (Figure 8). Bill Hagquist took measurements of the chamber and steel grip adaptor, in order to machine a custom fit aluminum grip adaptor (Appendix J). The new adaptor was designed to be longer than the original (4.373 in vs. 2.375 in), to increase the vertical distance between the bottom of the chamber and the bottom of the specimen at the initial testing position. This allows more of the environmental chamber to be filled with solution during testing (50.2 in^3), as the PVC hose can be adjusted to a greater height without interfering with the load cell at the initial testing position. A larger volume of solution in the chamber decrease the surface area to volume ratio, and thus the temperature of the solution will decrease at a slower rate.



Figure 8 Clockwise from top: aluminum grip adaptor, M10 Cap-head screw with gasket and washer, spring, and steel dowel pin. Ruler is pictured to provide scale.

To prevent leakage through the center of the cylindrical adaptor, a rubber gasket and washer were put on the M10 cap-head screw (8mm Allen wrench head), so that when the adaptor is tightened onto the table surface of the Instron 1000 a tight seal is formed. A spring, to provide an upward stabilizing force on the bottom grip, and a 2" steel dowel pin to secure the grip apparatus within the adaptor were also purchased. The new components are intended for use by researchers for future tensile testing using the Instron 1000 and the environmental chamber.

The function of the polycarbonate rods is to provide structure to the PVC hose, and provide it from buckling when filled with solution. However a method of attaching the PVC hose to the polycarbonate rods was not previously addressed. Therefore, with the help of Bill Hagquist, two steel shaft collars and adjusting knobs were purchased for this purpose. The collars were installed on diagonally opposing rods, and a tie wrap was used to attach the hose to them. The adjusting knobs will allow for quick adjustment of the shaft collars' position, which will allow the user to appropriately lower and raise the PVC in between tensile tests.

4.2.2 System Validation

Fifteen gel specimens made of Poly(ethylene glycol)-diacrylate (PEG-dAc) were tested to determine the sensitivity of the Instron 2711 grips. The samples were made using the IPNs and PDMS stencil procedures (Appendix C). The Instron 1000 was calibrated, and a 5 kg load cell was installed in the machine. The full-scale load was set at 0.5 kg, and the crosshead speed was set to 10 mm/min. Tests were completed at room temperature (23°C), without the use of the environmental chamber. All gel samples were positioned in the machine's grips according to the following steps:

1. Place one end of the gel in the bottom grip, so that the grip clamps the gel halfway between the bottom and top of the dog-bone end.
2. Lower the crosshead, so that the top grip can secure the free end of the gel without stretching it from its natural length.
3. Clamp the top grip to the free end of the gel halfway between the bottom and top of the dog-bone end.
4. Adjust the grips, so that the gel is flat and in one plane.
5. Adjust crosshead, so that the gel is at its natural length and no tensile load is being applied.

The fifteen specimens were positioned in the grips, one at a time, and tension tests were performed. Whether or not the gel fractured at the gauge length and approximately where it fractured was observed (Table 1).

Sample Trial	Point of Fracture
1	BG
2	GL
3	GL
4	GL
5	GL
6	GL
7	GL
8	BG
9	GL
10	GL
11	GL
12	GL
13	BG
14	GL
15	GL

Table 1. Point of fracture of gel specimens (BG- bottom grip, GL-gauge length).

The results of this test showed that 20% of the gels fractured at the point of contact with the bottom grip, while 80% of the samples broke at the gauge length. The Instron 1000 uses a strip chart recorder to record load vs. displacement plots during tensile testing. Full-scale deflection of the strip chart recorder was set to corresponded to a tensile load of 500g (most sensitive setting). Each whole vertical division (1 inch) on the strip chart recorder corresponded to a tensile load of 50 g and each small vertical division (0.1 inches) corresponded to a tensile load of 5 g. Using the plots from each of the tests, estimate calculations were made to determine failure loads of approximately 75-100g. Exact numbers were not recorded because the purpose of these tests was only to see if tensile testing of gels in atmospheric air would be feasible with the Instron 1000 and 2711 grips.

It was concluded that the grips are sensitive enough to hold the gels, as no samples fractured due to the grips' force alone, but only fractured after a significant tensile load had been applied by the Instron 1000. Furthermore, fracture at the point of contact with the bottom grip

on three of the samples was thought to be a result of imperfections (cracks/holes) in the specimen prior to testing.

The modified environmental chamber prototype was tested to determine its ability to maintain a relatively constant solution temperature over time ($\pm 3^{\circ}\text{C}$ of desired temperature). This experiment also served to identify and eliminate any possible leaks that the environmental chamber could have. The most critical location to observe possible leakage was where the aluminum grip adaptor was designed to form a tight seal with the o-ring on the inside of the chamber.

The temperature change of the solution in the environmental chamber was measured using an alcohol thermometer, with the chamber in its testing position on the table surface of the Instron 1000. The following procedure was used to perform the temperature test:

1. Approximately 4 L of “tap water” in a beaker was heated to $37 \pm 1^{\circ}\text{C}$, using a hot plate, for each of the three trials.
2. Once each solution had reached the desired temperature ($37 \pm 1^{\circ}\text{C}$), it was poured into the environmental chamber.
3. In accord with ASTM standards, the temperature changes were measured with a thermometer every 30 seconds for the first 5 minutes, and then every 5 minutes, for a total of 30 minutes. The results of all trials are shown in Figure 9. The American Society for Testing Materials (ASTM) establishes that a tensile test should last between 0.5 minutes to 5 minutes [6].

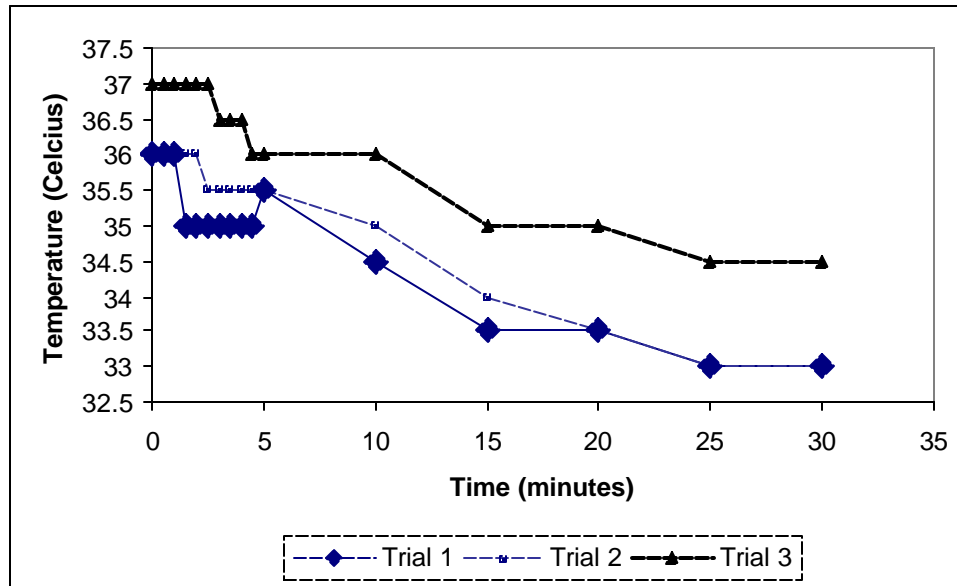


Figure 9. At all times, the thermometer was held in the middle of the chamber, as temperature was 1 degree warmer adjacent to the clear PVC hose. During trial 1, the solution was swirled with the thermometer before taking temperature at time 5 min.; this explains the increase in temperature, at 5 min. The room temperature was constant, at 23 °C, during the duration of all trials.

The mean \pm standard deviation of the differences in temperature test was $2.83 \pm 0.290^{\circ}\text{C}$. Based on these results, it was concluded that the temperature change from the baseline temperature of 37°C is permissible. If the solution should ever remain in the chamber for more than 0.5 hours, an aquarium heater or other heat source will be considered for use to maintain a constant temperature. Based on this preliminary testing, the client accepted a decrease of 3°C for the duration of each experiment.

To observe whether or not the chamber and bottom grip adaptor would create a seal, 4L of water were poured into the chamber, while both were in their testing positions on the Instron 1000. A leak was immediately discovered around the o-ring, and by adding vacuum grease to the o-ring and the groove that it sits in, no additional leakage was observed throughout the remainder of the testing.

The final validation testing performed on the modified tensile testing system was to observe the data recorded by the Instron 1000 and strip chart recorder during an actual tensile test, while the environmental chamber is filled with a buffer solution (Appendix G). Five gel specimens of 40% PEG-dAc and 60% gelatin were fabricated (Appendix F), and a buffer solution was prepared and heated to 37°C using a hot plate. The aluminum grip adaptor was tightly secured to the Instron 1000, and the environmental chamber was situated, so that a seal was formed with the grip adaptor. Each of the five samples was then secured in the grips, prior to filling the chamber with solution (Section 4.2.3).

For the first two samples, it was observed that air bubbles entered the chamber from the valve inlet, traveled upwards and caused complete fracture of the gel specimens prior to the actual tensile test. It should also be noted that neither of these samples were pretreated with any solution. The root cause of these air bubbles was determined to be the excess length in the cannula tubing, where pockets of air had accumulated and were then released into the chamber. Prior to testing the remaining three specimens, the length of the tubing was shortened from about 6 ft. to 3 ft. Also, about twice the amount of volume of solution (i.e. 4L to 8L) was added to the carboy reservoir to induce a larger pressure gradient and continuous flow of solution into the chamber during filling.

The third gel sample, which was also not pretreated, was secured in the grips, and the chamber was filled to the highest submersible portion of the top grip. This is the location of the end of a steel screw, which secures the top grip to the load cell. Just as all other parts of the Instron 1000, this must not make contact with any moist solution. For this trial, no bubbles were observed to enter the chamber, and the gel specimen did not fracture during filling. Following a desired set-up, a tensile test was performed, with a crosshead speed of 10 mm/min and a full-scale load of 2kg. The resulting plot showed zero load over 2 -3 mm of displacement, before the

specimen fractured at its gauge length. The fourth and fifth gel specimens were each pretreated in the buffer solution for approximately two hours before tensile testing. The crosshead speed was not changed, but the full-scale load was reduced to 500g, for maximum sensitivity. The results from the fourth sample were identical to that of the third, with a fracture at gauge length over a measurable displacement from the gel's natural length. Lastly, the fifth sample was tested, and a small load (~2.5 g) was recorded over a displacement similar to the previous tests before the specimen fractured, again at its gauge length.

The results from this testing indicate that, while the device functions properly and further testing should be completed, the efficacy and sensitivity of the Instron 1000 for tensile testing of gels, may not be satisfactory for the researchers.

4.2.3 System Enhancements

Aside from the modifications made to the chamber and the grip adaptor of the Instron 1000, some modifying enhancements were considered, to increase the overall ease of use of the system, increase the time efficiency for researchers using the system, and decrease the likelihood for experimental error or laboratory accidents. Essentially, a well-defined method for draining and filling the chamber with solution and maintaining the solution at a desired temperature had not been addressed. Thus, three design alternatives were sought and subsequently compared with each other.

The original design idea was to attach a length of cannula tubing to a valve, connected to the outlet hole at the base of the chamber. The solution would be heated in a beaker to the desired temperature, and then poured into the chamber, while attempting to not pour directly on the gel specimen already secured by the grips, as this may cause premature fracture of the gel. Following a test, the solution would be drained into the beaker, and would then have to be heated up to the desired temperature again to repeat the testing cycle. This method would have been

simple and only required a valve and about 6 feet of cannula tubing. However, the constant transferring of solution between the beaker(s) and chamber, and the use of a hot plate presented potential hazards (i.e. spilling hot water on Instron 1000 or researcher's extremities), and would require multiple simultaneous tasks for a single researcher during a tensile test. This would detract from the researcher's ability to conduct the experiment, record data, and make observations. Also, pouring the solution into the chamber is highly undesirable, as described above.

A second design alternative consisted of the same heating process as the original, using beakers and a hot plate. However, once the solution is at the desired temperature (i.e. 37°C), the researcher would transfer the solution to a polyethylene storage bottle. About 18 inches of cannula tubing would be attached to the outside of the storage bottle's cap, and by lowering the end of the tubing into the bottom of the chamber, the chamber could be filled without premature fracture of the gel specimen. The same draining mechanism as the first design would be used. This design was also discarded, as solutions would be transferred between three containers, instead of two, which further increases the risk of potential spills, and increases the time required for a single test. Also, as in the original design, the use of a hot plate is undesirable, as it is difficult to monitor the solution being heated up, while preparing the gel specimens, and recording results from the previous test.

Finally, a final design was selected, following suggestions from Paul Thompson (BME 400 Project Advisor) and Bill Hagquist, and design comparison analysis (Table 2). This design consists of a closed loop, and includes: a 10L carboy reservoir with a spigot; a three-foot length of cannula tubing; and a two-way ball valve. The researcher would prepare the desired volume of buffer solution, and transfer it to the reservoir. Once in the reservoir, the solution would be heated by an aquarium heater, or similar heating element, and by opening the spigot and valve,

and raising the reservoir so that its base is at a higher elevation than the fill line of the chamber, free flow from the reservoir to the chamber would result. Once a desired solution level in the chamber is reached (avoiding solution contact with Instron 1000, as described above), the valve would be closed off, the reservoir could be set down, and tensile test would be conducted. Following each test, the valve and spigot would be opened again, and the reservoir would be lowered so that the solution flows from the chamber back to the reservoir. The heater should maintain the unused solution at a constant temperature ($\pm 1^\circ\text{C}$), so that only a minor amount of heating time would be necessary to increase the temperature of the entire volume of solution to the desired temperature again.

	Original Design	Design #2	Final Design
Premature Gel Specimen Failure	-	+	+
Heating of Solution	-	-	0
Potential for Spills/Accident	-	-	+
Minimum transferring of solution	-	-	+
Overall Efficiency	0	-	+
Total	-4	-3	+4

Table 2. Decision matrix for solution filling/draining mechanism of tensile testing system.

4.3 Creep Testing

Over the course of the semester, the creep testing team researched creep testing components, considered alternative extensometers, performed preliminary tests, and chose a final design. A prototype was built from the selected final design.

4.3.1 Design Components

Creep testing will be performed in a creep chamber, which is separate from environmental chamber for tensile testing, and which allows the gel specimen to be tested in a solution of desired pH (4.5-8) and temperature ($37 \pm 3^\circ\text{C}$). The material of the creep chamber should be durable, non-corrosive, easy to manufacture, and transparent in order to observe the sample during testing. In addition, the creep chamber must maintain a relatively constant temperature ($\pm 3^\circ\text{C}$) over the course of a creep test, which may be more than 24 hours. Therefore, the creep chamber must also be insulating, and may require a heater and/or circulation mechanism to maintain an evenly distributed temperature. The pH should not vary by more than ± 0.5 pH units for the duration of a test. A system may be required to either change the buffer during testing, or adjust the pH to keep it at a constant level. The creep chamber must allow for researchers to adjust the sample inside the chamber prior to testing. Alternatively, a removable grip apparatus could be designed to address this requirement. The sample would be aligned outside of the creep chamber, and then secured inside the chamber just prior to a creep test. As mentioned earlier, a typical creep testing system is composed of 5 components: a chamber, a pair of grips, a loading apparatus, and an extensometer.

The grips, which are also separate from those used for tensile testing, function by holding the sample in place during testing. The samples should be gripped at the bottom and top of the gel specimen, in the same manner as the tensile testing system, and should allow for fracture at gauge length. The grips must not slip once they are attached to the gel, and they must remain in the same plane during testing, to prevent any unwanted torque to the sample. Because the grips will be immersed in pH solution during testing, they must also be made of a non-corrosive material, or be inexpensive, so they can be discarded before rusting occurs.

A constant load must be applied to the sample in order for creep properties to be measured. This can be accomplished by suspending weights from the bottom of the sample (Figure 10, left), or by loading a lever or pulley system, which then applies an upward force to the top of the sample (Figure 10, right). The interface between the weights and the grip must have small to negligible creep properties, and must not apply shear force to the sample. This will ensure that change in displacement is due to the applied load and properties of the gel alone, and will eliminate any confounding variables, that may skew experimental results.

The extensometer is responsible for measuring the creep displacement of the gel. It should be capable of taking measurements for more than 24 hours, and not interfere with the creep test itself. See Appendix E for complete product design specifications of the creep testing system.

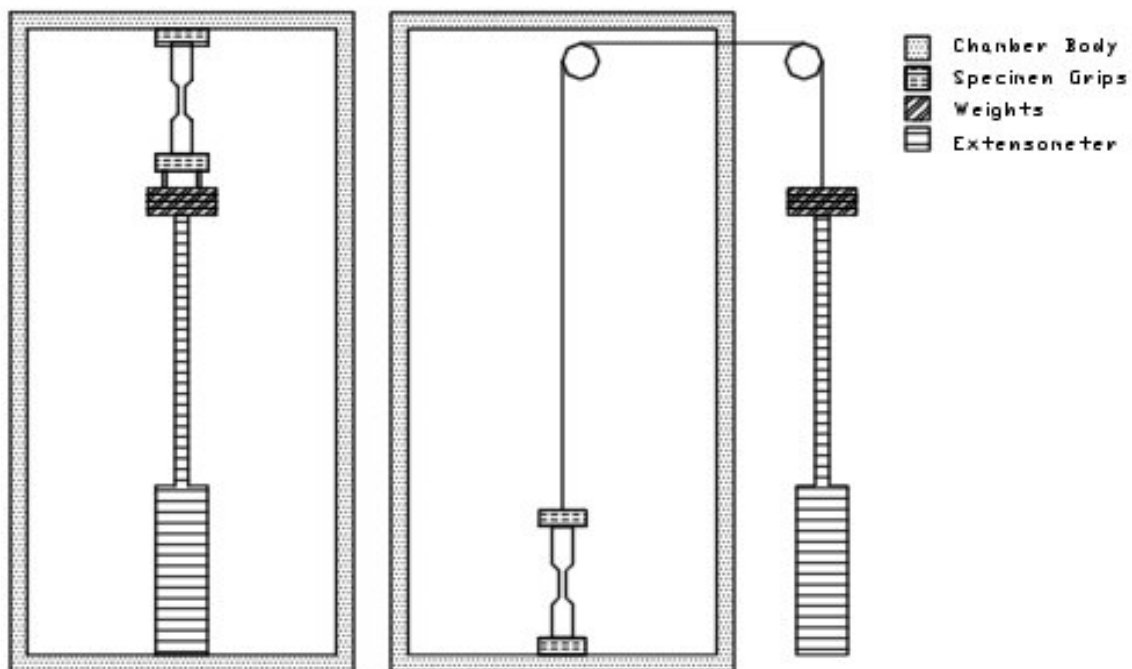


Figure 10. The components of the creep testing system: chamber, grips, loading apparatus, and extensometer. The sample in the left testing system is loaded from the bottom, whereas the sample in the right testing system is loaded from the top.

4.3.2 Preliminary Testing

Because no creep testing had been performed in Dr. Kao's lab prior to this project, the design team was interested in finding out details of testing, such as the approximate length of one test, amount of sample elongation, the approximate weights needed, and appropriate sample preparation before creep testing.

Nine PEG-dAc IPNs (Appendix F) were tested room temperature (23°C) and in distilled water at 40°C. A photograph of this testing set-up is shown in Figure 11. The sample was held with binder clips, and weights were suspended from the bottom of the sample. The testing set-up for the samples tested in distilled water at 40 °C is not shown. This set-up was the same, except the grips, sample, and weights were suspended into a 4L glass beaker (filled with distilled water) that was heated to 40 °C with a hot plate.



Figure 11. Preliminary Testing system. Binder clips hold the sample, which was loaded from the bottom by weights.

At room temperature, a 25g weight was applied to one sample, which immediately stretched 13 mm from its natural length, but exhibited no further creep for 1 hour. This test was terminated because after 1 hour, the sample was dry and brittle, and not characteristic of the initial state of the material being tested. “Maximum load tests” were done on two specimens, which consisted of loading the specimens with weight until fracture. The initial load applied was 85g, and the specimens withstood loads of up to >195g before fracture. However, these specimens were also dry and brittle at this time.

In distilled water at 40°C, two samples loaded with 5–10g fractured after five seconds. Four samples loaded with 3g fractured between five seconds and two minutes following loading. Five out of six samples tested fractured at the gauge length, indicating that the binder clips were satisfactory for creep testing use.

Preliminary testing showed that gel specimens can withstand higher loads in air than in water at 40°C. The wide range of applied loads in air (3–10g), and water (85–195g), suggest that the quality of the specimens varied and may have degraded prior to testing. To minimize these effects, future specimens should be made, by carefully following the IPN procedure, properly mixing the gelatin-polymer solution prior to photopolymerization, and using the same amount of solution to make each sample (Appendix F). Also, samples should be tested 24 hours after they are made to minimize material degradation. Furthermore, during testing many of the samples became dry and brittle during testing at room temperature, which may have altered the mechanical properties. A solution would be to place samples in aqueous solution for a specified length of time (0.5–2hr) prior to testing.

Because of drying or degradation of samples during this preliminary testing, it was difficult to determine the approximate length necessary for one test, the amount of sample elongation, and the approximate weight needed to induce creep. It is hypothesized that the

duration of the creep test will be shorter than 24 hours, although this is partly determined by the quantity of load applied. The amount of sample elongation is hypothesized to be less than 1000%, or approximately 4 inches. Finally, the samples will most likely be loaded with less than 10g. These hypotheses were considered during the design of the creep chamber and the choice of extensometer. In the future, further testing of these hypotheses will be conducted with adequate sample preparation.

4.3.3 Alternative Extensometers and Evaluation

Four alternative extensometers were considered for the creep testing system: a digital camera, an ultrasonic transducer, a resistive displacement transducer, and a linear variable differential transducer (LVDT).

A digital camera would photograph the sample at regular time intervals during the creep test. A ruler placed in the chamber, or on the sample itself, would allow the researcher to measure elongation by analyzing photographs of sample displacement relative to the ruler. This idea was rejected because of tedious data analysis, and human error due to “eyeballing” sample position.

Ultrasonic transducers, shown in Figure 12, were also considered as a means of measuring displacement. Ultrasonic waves emitted from a transmitter propagate through the desired medium to a receiver. The measured time difference is proportional to the creep displacement of the sample. For this design, the transmitter or receiver could be placed on the bottom of the hanging weights, and the other on the bottom of the chamber (for a bottom-loading system). This idea was rejected upon learning that ultrasonic transducers exhibit decreased accuracy when measuring distances of less than 5 inches, which was not suitable for this design [7].

Figure 12. Variety of ultrasonic transducers <http://www.piezotechnologies.com>

A resistive displacement transducer, shown in Figure 13 was also considered as a possible extensometer. This device consists of a moving core, which would be attached to one end of the sample, requiring the system to be top-loading. As the sample elongates, the core would make contact with a resistive coil, thus changing the resistance of the device. By Ohm's Law, this resistance change would produce a change in voltage, from which the change in displacement could be determined. This idea was rejected because the resistive displacement transducer is susceptible to the effects of contact friction. Since the forces applied to the gel specimens will be small ($>10g$), friction effects may produce notable error.



Figure 13. Resistive displacement transducers from <http://www.novotechnik.com/linear.html>.

A linear variable differential transducer, or LVDT, was also considered as a possible extensometer for the creep testing system. In its simplest form, an LVDT consists of a primary coil, secondary coils, and a magnetic core (Figure 14).

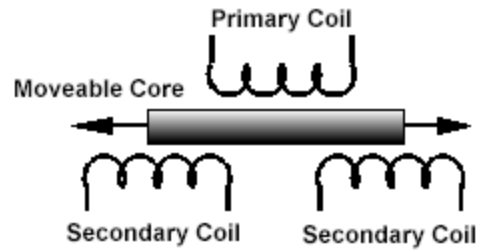


Figure 14. LVDT components http://bits.me.berkeley.edu/beam/lvdt_2.html.

An alternating current is produced in the primary coil, which produces a varying magnetic field around the core. This magnetic field induces an alternating (AC) signal in the secondary coils that are in proximity to the core. The voltage of the induced signal in the secondary coil is linearly related to the number of coils, as in any transformer. The basic transformer relation is:

$$\frac{V_{\text{out}}}{V_{\text{in}}} = \frac{N_{\text{out}}}{N_{\text{in}}}$$

where:

V_{out} is the voltage at the output,

V_{in} is the voltage at the input,

N_{out} is the number of windings of the output coil, and

N_{in} is the number of windings of the input coil.

The induced AC signal is then demodulated, so that a DC voltage is produced that is sensitive to the amplitude and phase of the AC signal. As the core is displaced, the number of secondary coil windings exposed to the core changes linearly, and the amplitude of the induced signal varies linearly with this displacement. The direction of displacement is indicated by the outputs of the secondary coils. These coils are connected in opposite orientations, with one clockwise and the other counter-clockwise. When the same varying magnetic field is applied to both coils, their output voltages have the same amplitude, but differ in sign. These outputs are summed together to determine the displacement. At the equilibrium position (i.e. zero displacement) a zero output signal is produced (Figure 15) [8].

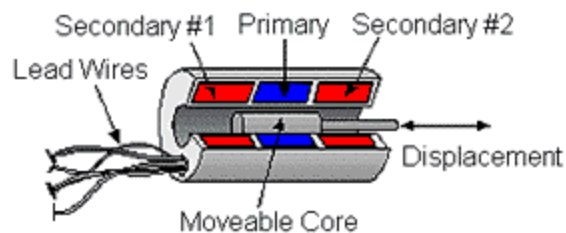


Figure 15. The zero position of the LVDT http://bits.me.berkeley.edu/beam/lvdt_2.html.

The LVDT was chosen as the extensometer of the creep testing system. The major advantage of the LVDT is that it is non-contacting, which means minimal friction. As discussed previously, this is very important for our design since such small loads (<10g) will be used. The only disadvantage of the LVDT is the larger size of the device (13 inches tall), which helps to avoid the non-linear range of the device at the extremes of operation. To compensate for this, it was decided that the chamber would be raised 12 inches above the lab-bench to accommodate for the longer device.

The factors considered for choosing the extensometer are summarized in Table 3. The LVDT received the most points, which lead to its use as the extensometer in the final design.

Alternative Extensometers				
	Digital Camera	Ultrasound	Resistive	LVDT
Measuring Range < 5 inches*	+	-	+	+
24 Hour Data Acquisition*	-	+	+	+
Ease of Data Analysis	-	+	+	+
Storage of Failure Time	-	+	+	+
No Extra Circuitry Required	+	-	-	-
Minimal Human Error	-	+	+	+
Cost < \$500	-	+	+	+
No Submersed Electronics	+	-	+	+
Height < 10 inches	+	+	+	-
Temperature Insensitive	+	+	-	+
Minimal Friction*	+	+	-	+
Total	7	10	10	12

Table 3. Considered characteristics of four extensometers. The LVDT received the most points, with 12, and was chosen for the final design. *Three factors were considered crucial for the creep testing system and were awarded twice as many points as the other criteria: could the device could measure within 5 inches; could it obtain data for 24 hours; and would it produce minimal friction.

4.3.4 Final Design

The final design of the creep testing apparatus consists of an LVDT, creep chamber, pulley assembly, analog to digital converter, a pair of grips, and weights. The LVDT will be located outside the chamber, and the sample will be loaded at the top, using a pulley system. The bottom grip will be attached to a 500g weight to oppose the theoretical maximum applied load, and prevent any displacement of this grip. The top grip will be attached to weights positioned outside of the chamber, via a pulley system. The core of the LVDT will be attached to the bottom of the weight rack. This design locates all of the electrical components of the system outside of the chamber. If the electrical components were positioned inside of the chamber, sealing or waterproofing would be necessary to prevent electrical shorts in the aqueous environment. Figure 16 is a three-dimensional rendering of the proposed design. A final drawing of the creep testing system, with appropriate dimensions can be found in Appendix L.

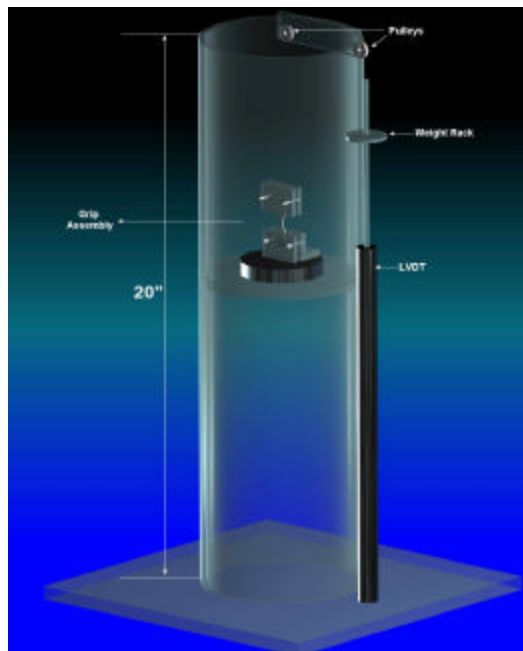


Figure 16. 3D rendering of proposed final design

4.3.4a LVDT

An LVDT, model number 75S2DC-2000SR, was selected and purchased from Sentech Inc. This transducer has a displacement measurement range of 0 to 4.000 inches and a DC output signal ranging from 1 to 10V. Complete specifications for this LVDT are available on the manufacturer's website at <http://sentechlvdt.com/pdf/75S2DC.pdf>. The main body of this LVDT is 13 inches tall. In order to eliminate friction from the travel of the core of the LVDT, it must be upright, as the core does not contact the transducer in this orientation.

4.3.4b Creep Chamber

The body of the creep testing chamber is constructed from a cylindrical shell of acrylic plastic purchased from www.mcmaster.com. The cylinder has an outer diameter of 6 inches and a wall thickness of 0.25 inches. Although a larger thickness is desirable to provide better insulation for temperature control, the next highest available thickness cylinder is roughly three times more expensive, and it was decided that the extra insulation was not worth the extra cost. In order to account for the length of the LVDT, the chamber must be raised from the level of the

bench-top. A cylindrical shell of the same diameter and thickness is placed beneath the chamber for this purpose.

4.3.4c Pulley Assembly

Two pulleys are designed into the system to translate the downward force of the weights to an equal upward force on the top grip, and hence the gel specimen. The pulleys used were also purchased from www.mcmaster.com, part number 3434T12. Bill Hagquist recommended a nylon sheave with ball bearings for this application. A nylon pulley will have a lower moment of inertia than a metal one, and the ball bearings will decrease friction. The lowest available diameter was chosen to minimize the force required to rotate the pulley. If any force is required to cause rotation of the pulley, the force translated to the specimen will be less than the load applied. Figure 17 shows an explanation of how the force required to turn a pulley is related to its radius. The two pulleys are “sandwiched” between two rectangular supports attached to the top of the chamber. A small screw between the two supports acts as an axle for the pulleys.

$t = F \cdot R = I \cdot a$	$a = \text{angular acceleration of pulley}$
$FR = \frac{1}{2}MR^2 a$	$a = \text{linear acceleration of edge of pulley}$
$F = \frac{1}{2}MR \left(\frac{a}{R} \right)$	$a = \text{acceleration of weights}$
$F = \frac{1}{2}Ma$	$I = \frac{1}{2}MR^2 = \text{moment of inertia of pulley}$
	$R = \text{radius of pulley}$
	$F = \text{force applied tangent to edge of pulley}$

Figure 17. Relationship between pulley force and its pulley radius. Since the mass is proportional to the radius (a bigger pulley will have more mass,) the force F is directly proportional to the radius of the pulley. Therefore a smaller pulley will require less force to turn.

4.3.4d Analog to Digital Converter

An analog to digital converter (ADC) was included in the design to facilitate computerized data acquisition and logging. Since creep typically occurs slowly, manual data logging is impractical, as it would require the researcher to be present for the entire duration of

the test (1hr – days). In selecting an ADC, four criteria were considered: resolution, conversion rate, signal range, and output type.

It was determined that a resolution of at least 10 bits is required to achieve a precision of 0.1 mm. There are 1016 divisions of 0.1 mm in 4 inches. A resolution of 10 bits gives 1024 measurement levels, which is satisfactory for the purpose of this system.

As previously discussed, creep processes are time dependent and usually occur very slowly. Therefore, the output signal of the LVDT is expected to have a very low bandwidth. Accordingly, measurements of displacement will only be taken at intervals of several minutes or more. Therefore, the conversion rate of the ADC is not required to be very high. Most commercially available ADCs have conversion rates that well exceed the requirements for this creep testing system, so this does not limit our selection. Beneficially, this allows us to sacrifice a higher conversion rate for an ADC at a lower cost.

The LVDT has an output of +1V to +10V. Therefore, the ADC would ideally have an identical input range. However, if the voltage range is smaller, the output of the LVDT could be scaled down by using a voltage divider. The output of the ADC was required to be compatible with an input port available on any computer in the laboratory. Therefore, ADCs with serial port and parallel port outputs were considered.

With these considerations in mind, the DI-154RS ADC from www.dataq.com was purchased. This converter has a 10 bit resolution, 240 Hz conversion rate, a $\pm 10V$ input, and a RS-232 serial port output. The input voltage range is almost twice as large as the output range of the LVDT (+1V to +10V). In order to have a precision of 0.1mm, the output of the LVDT will have to be scaled and adjusted to the range of the ADC.

4.3.4e Grips

Currently, small binder clips that have been modified for testing serve as grips for the creep testing system. However, because there is much variability in the stiffness of binder clips that are otherwise identical to each other, our client did not approve of this selection. An alternative to using binder clips would be to purchase a pair of the Instron 2711 grips, which were used in the tensile testing system. A less expensive solution will also be considered.

4.3.4f Weights

A set of laboratory weights ranging from 0.5g to 500g was obtained from the University of Wisconsin–Madison Physics department. The weights are circular with a radial slot on one side that allows them to be easily added to and removed from a rack. Figure 18 below, is a diagram of one weight. A thin rod of acrylic, with a diameter of 4.8mm (3/16”) is attached to the core of the LVDT. A small disk of radius 30 mm is attached to the rod, and will serve as a base upon which the weights will be placed (Figure 18).

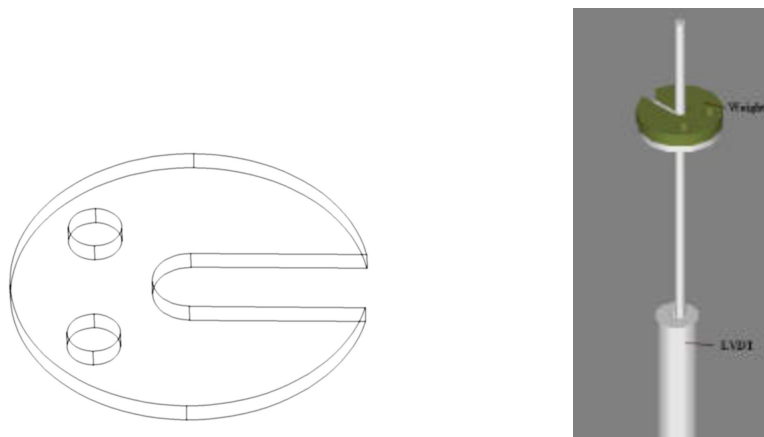


Figure 18. Left: Illustration of one weight obtained for the creep testing system. Right: The weight is added to the rack, which is attached to the LVDT.

5. FUTURE WORK

The updated stencil procedure will be used in ongoing research, and researchers will provide feedback, regarding its efficacy. The results of the tensile testing system validation will be discussed with Dr. Kao, and depending on his decision, further testing and or design modifications will be made. Should Dr. Kao wish for the team to continue testing of the Instron 1000, an aquarium heater will be incorporated into the tensile testing system, to maintain a constant temperature of the solution in the reservoir during testing. Heat transfer analysis would be performed in order to determine the necessary size of the heater, and whether any insulation would be needed for the outside surface of the reservoir.

As for the creep testing system, in the future, a heater, or circulating water bath may be added to maintain a constant temperature of the testing solution over the course of a creep test. The creep testing system will be validated, and will also be used to perform creep tests. More adaptations to the system will be made if necessary. Depending on the needs of the client, more chambers may have to be built so more than one gel specimen can be tested at a time.

Finally, in order to fulfill degree requirements, the required outreach project will be completed, and a publication will be discussed with our client during the forthcoming design course, BME 402.

6. ETHICS AND SAFETY ISSUES

In the cleaning process involved with the stencil procedure, it should be mentioned that hexanes are toxic, flammable, and can affect the skin if continuous contact occurs. For the tensile testing system, given that 37°C is normal body temperature, acute exposure to solutions with this temperature during setting up of the experiment, caused by any unforeseen spills, would not be harmful to the researcher. However, for longer exposure to solution at temperatures above 25.5°C, the body begins to absorb heat. It's important to mention that the body will only

adapt to changes as great as 20 % within the comfort range (mouth to foot, 26.7–37.8 °C) through evaporative cooling [9].

Safety aspects were considered when developing the design of the creep testing system. The specimen was loaded from the top via a pulley system, so that displacement could be measured outside of the chamber. This insures that the LVDT and additional circuitry will have minimal to no contact with the buffer solution. Other safety aspects should be considered when making the gel specimens. Gloves should be worn when handling, mixing, and heating reagents, and UV light exposure should be minimized (Appendix F).

All collaboration in this project was mentioned throughout the paper. Our design team was conscientious of other researchers and personnel on campus, including Prof. Wendy Crone, Bill Hagquist, and John Dreger that assisted us during the process of this design project. All team members always followed rules when using University and private lab equipments. Whenever a problem occurred, such as a chemical or water spill in any lab, the appropriate person was contacted and informed about the event. An open line of communication was maintained between our client, advisor, team members, and other collaborators, and all their support and guidance was appreciated and cited, where appropriate.

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APPENDIX A

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, Charlie Haggart, Michael Piche

Last Updated 12/06/02

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

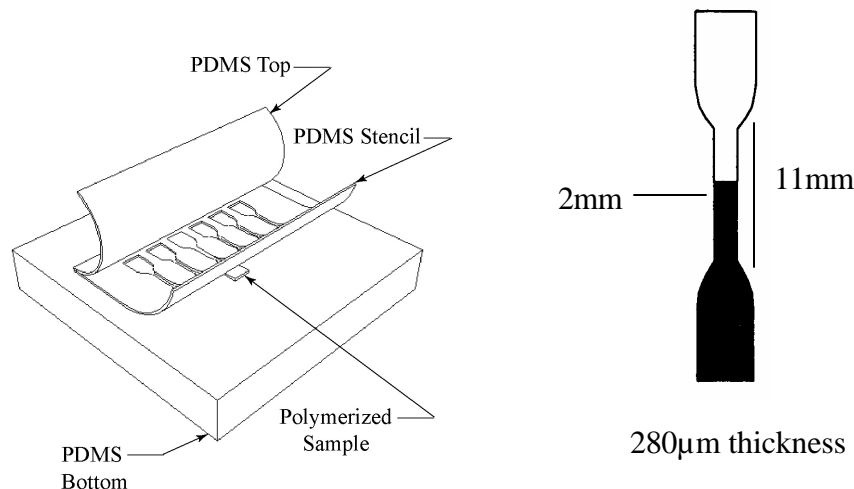
Client Requirements:

- Fast, exact procedure to make ASTM approved PDMS stencils.
- Method to ensure uniform thickness and area of stencils, reducing anisotropy in hydrogel samples.

Design Requirements:**1. Physical and Operational Characteristics**

- Performance Requirements:* The stencil made should be disposable. The procedure should allow multiple stencils to be made at one time.
- Safety:* Be careful when using the hot plate because it is very hot. Although, the materials used are nontoxic, gloves should be worn. Hexanes are used to clean any residues of PDMS and it is very toxic, volatile, and flammable. All cleaning shall be performed under a chemical hood.
- Accuracy and Reliability:* The mixture of silicone elastomer and curing agent must be homogenous and properly degassed.
- Life in Service:* Ideally, the stencil shall be used only once. However, if the stencil is to be used more than once, the researcher must use their best judgment in assessing the integrity of the stencil following each usage.
- Shelf Life:* The stencil should be stored in the petri dish in which it is made.
- Operating Environment:* The procedure should be carried out at room temperature and normal humidity.
- 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. Each stencil, at least requires the use 10 g of silicone elastomer and 1 g of curing agent.
- j. *Materials:* The following materials should be used: Sylgard® 184 silicone elastomer, Sylgard® 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, and transparency sheets.
- k. *Aesthetics, Appearance, and Finish:* N/A

2. Production Characteristics

- a. *Quantity:* One procedure is needed. Number of stencils required will be determined by individual researchers' needs. Tensile and creep testing for client's current research will require approximately 100 stencils.
- b. *Target Product Cost:*
- a. PDMS Kit – \$40.00/per kit
 - b. EPON masters – donated by Prof. Crone's lab.

3. Miscellaneous

- a. *Standards and Specifications:* ASTM guidelines need to be followed.
- b. *Customer:* Dr. Weiyuan John Kao, UW-Madison School of Pharmacy & Dept. of Biomedical Engineering.

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

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

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

Gabriel Martinez-Diaz, Darcee Nelson, Charlie Haggart, Michael Piche

Last Updated 12/06/02

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

Client Requirements:

- Maintain the pH of a solution determined by experimental design, which will include pH 7.4 and 4.5.
- Maintain the temperature of a solution at 37 (± 3) degrees Celsius.
- No interference with the dynamic stress-strain relationship, ultimate stress and strain, and yield stress and strain tests.
- Compatibility with the Instron 1000 mechanical testing system.

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, basic, very hot, 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. This is especially important during the preparatory phase of each test, when the researcher is to align the samples between the testing system's grips.
- o. *Life in Service:* The chamber should maintain the temperature (± 3 ° C of the desired temperature) and pH (± 0.5) of a solution over a time period ranging from 0.5 to 5.0 minutes, the length of each individual mechanical test, which follows ASTM standards.
- p. *Shelf Life:* The chamber should be stored clean and dry in a cool and dry environment.

- 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 varying temperature and pH, as indicated above.
- r. *Ergonomics:* The chamber should be easy to transport, and lighter than 33 lbs. [9].
- s. *Size:* Instron 1000 dimensions : 25.4 cm between two columns for load cell travel. The chamber should be compatible with this machine.
- t. *Weight:* The chamber should be less than 33 lbs, as recommended by the Human Factors Design Handbook [9].
- u. *Materials:* The materials of the chamber should be durable, transparent, easy to manufacture, affordable, insulating, and able to withstand changes in temperature from 20 - 40 degrees Celsius and changes in pH from 4-8.
- 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:* Appendix I

3. Miscellaneous

- e. *Standards and Specifications:* None.
- f. *Customer:* See client requirements.
- g. *Patient-Related Concerns:* N/A
- h. *Competition:* There exist no environmental chambers designed for use with the Instron 1000.

APPENDIX C**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 30 min.
 - a. Make sure all bubbles are gone.
 - b. For larger volumes to make more than 1 stencil and 1 base, it may longer than 30 min.
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 °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 hexanes. This must be performed under a chemical hood.

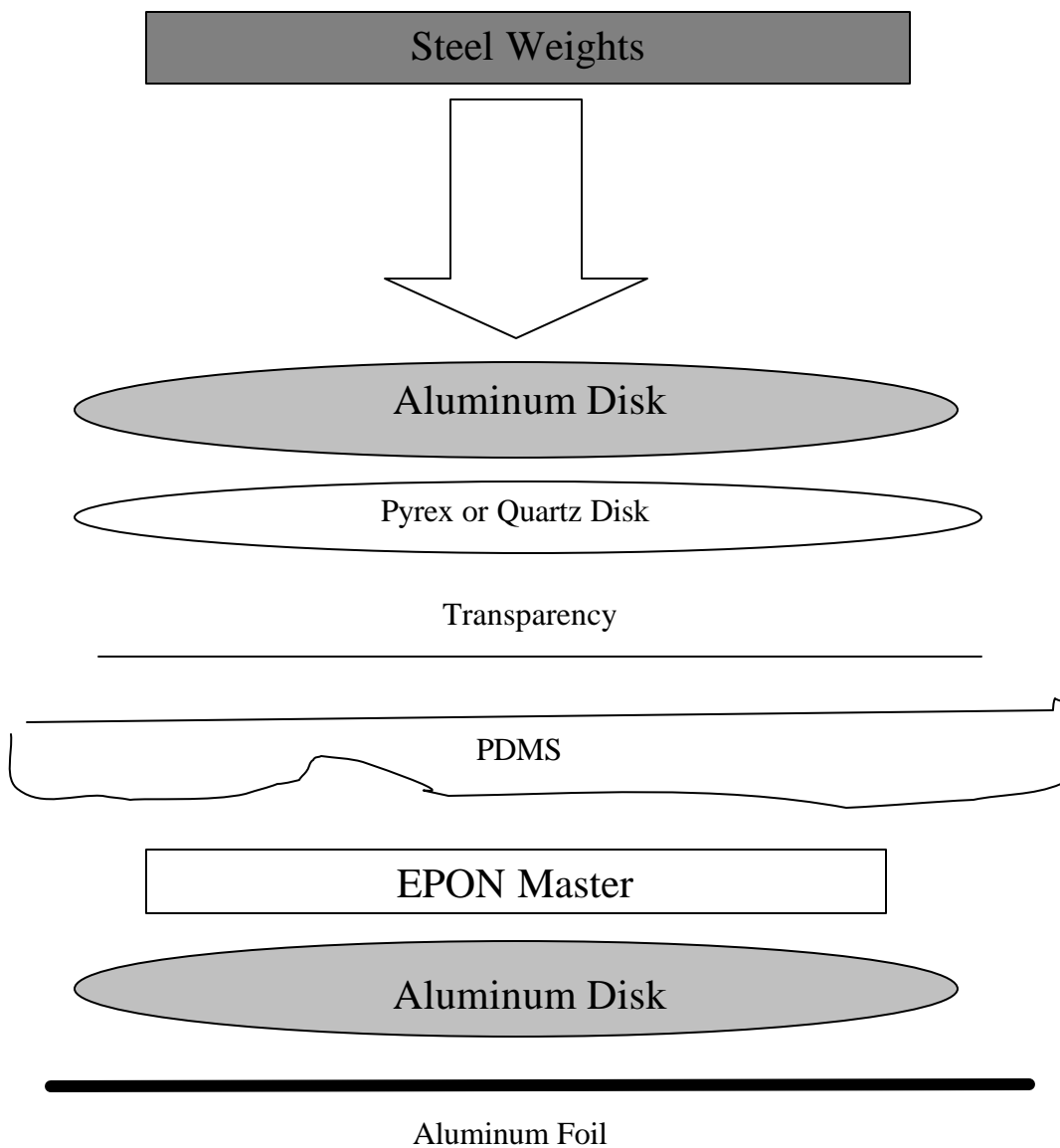


Figure 19. A cross-sectional representation of the PDMS tension stencil procedure assembly.

APPENDIX D**Base 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.
 - b. The cover is used during the photopolymerization of IPNs (Appendix F).
7. Bake in an oven for 3 hours at 80 °C.

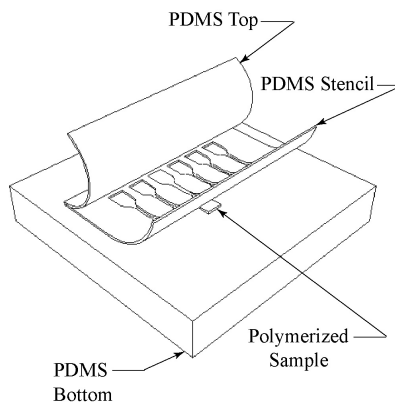


Figure 20. A 3D representation of the PDMS tension stencil with base and cover slip.

APPENDIX E

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

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Last updated: 12/6/02

Function: To measure creep properties of hydrogels in a pH and temperature controlled environment.

Client Requirements:

- Maintain the pH of a solution from 4.5-8.
- Maintain the temperature of a solution at approximately 37 ± 3 °C.
- Chamber should not interfere with creep testing.

Design Requirements:

1. Physical and Operational Characteristics

- w. *Performance Requirements:* The creep testing apparatus should be capable of being used for consecutive and possibly simultaneous tests.
- x. *Safety:* The chamber should be securely sealed to prevent potentially acidic, basic, very hot, 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.
- y. *Accuracy and Precision:* The temperature should not vary more than ± 3 °C of the desired temperature, and the pH should not vary by more than ± 0.5 pH units. Extensometer must be as accurate to 1 mm and precise as possible.
- z. *Life in Service:* The chamber should maintain the temperature and pH of a solution over a time period of one creep test (1-2 days).
- aa. *Shelf Life:* The chamber should be stored in a cool, dry place.
- bb. *Operating Environment:* The chamber is to be used at room temperature, atmospheric pressure, and normal humidity. It will be exposed to solutions of 4.5-8 pH and temperatures of 37 ± 3 °C.
- cc. *Ergonomics:* The chamber should be greater than 7 inches in diameter, so hands can be placed inside to adjust the sample prior to testing [9].

Alternative solution would be to create a system to adjust the sample outside of the chamber and then place the sample inside.

- dd. *Size*: The chamber should be of appropriate height to accommodate for tripling of hydrogel sample length (4 inches) during creep testing.
- ee. *Weight*: The chamber should be less than 33 lbs, as recommended by the Human Factors Design Handbook [9].
- ff. *Materials*: The materials of the chamber should be durable, transparent, easy to manufacture, affordable, insulating, and able to withstand changes in temperature from 37-40 degrees Celsius and changes in pH from 4.5-8.
- gg. *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

- e. *Quantity*: One creep testing apparatus is needed.
- f. *Target Product Cost*: Appendix I.

3. Miscellaneous

- i. *Standards and Specifications*: Testing system should accommodate hydrogel samples of ASTM approved dog-bone shape.
- j. *Customer*: See client requirements.
- k. *Patient-Related Concerns*: N/A
- l. *Competition*: None commercially available.

APPENDIX F**Procedure for synthesis of PEG-dAc and IPN specimens**

PEG-dAc Synthesis. PEG-diol (*Aldrich*, M.W. 2k Da) was reacted with acryloyl chloride (*Aldrich*) and triethylamine (*Aldrich*) in a respective 1:4:6 molar ratio at room temperature in tetrahydrofuran under Argon for 3 hr, precipitated in cold hexanes, filtered and vacuum dried to produce PEG-dAc. The final PEG-dAc purity was quantified with a reverse phase HPLC system (10% to 100 % acetonitrile at a flow rate of 1ml/min in 60 min with Jordi 500 Å column on a Gilson system coupled to UV-Vis and evaporated light scattering detectors). The elution time of PEG-dAc was approximately 13.2 min with a purity of 100 wt.-% PEG-dAc.

IPN Synthesis. IPNs were synthesized with gelatin, PEG-dAc, and photoinitiator (2,2-dimethoxy-2-phenylacetophenone, DMPA). Specifically, gelatin (i.e. G) was dissolved in deionized water with heat (80 °C) to form a 20wt.-% solution (0.2 g/ml). PEG-dAc was dissolved in deionized water, without heat, in an aluminum foil wrapped glass vial to form a 100 wt.-% solution (1 g/ml). The gelatin solution was vortexed thoroughly. DMPA was added to this mixture, vortexed and heated to 80 °C in the remaining parts of the procedure. The final gelatin/PEG-dAc/DMPA mixture was injected with a Pasteur pipette into a poly (dimethyl siloxane) mold (Figure 2) to fashion IPN final dimensions of 280 µm thick, 11 mm gage length, and 2 mm neck width (ASTM D 638-98 type IV dog-bone specimens) [6, 10, 11]. The mold/IPN mixture assembly was then irradiated with UW ling (long wavelength (365 nm) with 21,700 µO/cm² at 2" away from the sample) from the top and bottom simultaneously for approximately 3 minutes. The mold/IPN was allowed to cool before the IPN was removed from the mold. IPN nomenclature was based on the type of gelatin and weight percent of gelatin (i.e. 4G6P indicates IPNs containing 40 wt.-% gelatin and 60 wt.-% PEG-dAc). Once removed from the mold, each individual sample was placed into a conical tube and equilibrated in an incubator at 37°C with 95% relative humidity for 24hrs.

APPENDIX G**Procedure for Preparation of Dulbecco's Phosphate Buffered Saline [DPBS] Solution**

Components	g/L
Potassium Phosphate Monobasic	0.20
Potassium Chloride	0.20
Sodium Chloride	8.00
Sodium Phosphate Dibasic [Anhydrous]	1.15

Instructions

1. Measure out 90% of final required volume of water. Water temperature should be 15-20°C.
2. While gently stirring the water, add the powdered medium. Stir until dissolved. Do NOT heat.
3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
4. While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
5. Add additional water to bring the solution to final volume.

APPENDIX H

Prof. Crone's Procedure for making Polydimethyl Siloxane (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.

APPENDIX I

BME 301 Cost Summary	
Environmental Chamber - Materials	
Polycarbonate rods	\$50.00
Clear PVC Hose	\$10.00
Acry-Fab (includes labor)	\$250.00
PDMS Stencil Procedure - Materials	
PDMS Kit	\$160.00
Weights for PDMS-stencil	\$300.00
Labor	
ME Shop Labor	\$700.00
Total Costs	<u><u>\$1,470.00</u></u>

APPENDIX I

BME 400 Cost Summary	
Tensile Testing System - Materials	
Polyethylene Carboy	\$45.00
Steel Shaft Collar (2)	\$19.02
Steel Knob (2)	\$6.14
Nylon Tie-wrap (2)	\$0.10
Aluminum Grip Adaptor Assembly	
6" alum. cylinder (2"F)	\$6.47
0.5"F x 2.5" steel dowel pin	\$0.99
3/8" flat washer	\$0.02
1/8 sheet rubber	\$0.25
spring	\$0.25
	\$7.98
Ball Valve (1/4 x 1/4)	\$5.99
Brass Hose Barb (1/4 x 3/8" ID)	\$0.54
Galvanized Pipe Nipple (1/4 NPT)	\$0.35
Miscellaneous	\$1.14
Tensile Test Materials Subtotal	\$41.26
Tensile Testing System - Labor	
ME Shop Labor	\$150.00
Tensile Testing System Total	\$191.26
Creep Testing - Materials	
LVDT displacement transducer	\$400
Acrylic pieces	
2 6" F x 12" cylinders	\$46.42
6"Fx1/4" thick disc	\$5.58
12"x12"x0.5" sheet	\$10.07
3/16"Fx6' long extruded rod	\$0.57
3/16"x1"x6' rectangular bar	\$3.25
polypropylene 1/4"x1"x4' rectangular bar	\$1.16
stainless steel 3"F disc	\$7.58
foam strips for grips	\$4.78
100 plastic set screws	\$4.98
2 pulleys	\$13.12
Analog to Digital Converter	\$24.95
Fishing Line	\$2.00
Creep Testing System Subtotal	\$524
Total Costs	<u>\$715.72</u>

APPENDIX J

Aluminum Grip Adaptor (Dimensioned by Bill Hagquist)

