UNIVERSITY OF WISCONSIN – MADISON
DEPARTMENT OF BIOMEDICAL ENGINEERING
BME 200/300 – DESIGN

Cartilage Bioreactor

Final Report

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Abstract

The field of tissue engineering is rapidly emerging as a method of promise for repairing damaged cartilage tissue. Research is ongoing to effectively grow cartilage tissue in vitro for implantation in vivo. From this research, it has been determined that a bioreactor enabling observation of how changes in compression affect the samples is needed to assess the strength of the tissue. The most effective method to observe this change would be to use high field magnetic resonance (MR) imaging. With this and other client requirements in mind, the team decided to pursue a final design made of medical grade polycarbonate with a threaded rod to provide compression. A biocompatible prototype of the final design was constructed that enables adjustable compression, MR scanning, and sterilization by autoclave. To ensure that the prototype met design specifications, a crude temperature test, compression test, and temperature decay test were completed.

Background and Motivation

Articular cartilage is an area of much interest in the field of tissue engineering and there has been a great deal of research to understand the function and structure of cartilage. In joints, articular cartilage covers the surface of bones to provide smooth movement and cushioning from impact. Cartilage consists of chondrocytes (cartilage cells), extracellular matrix molecules, and water. The extracellular matrix is composed of collagen fibers that contribute to overall form and stability [1]. Additionally, water accounts for 70-80% of cartilage by weight and is responsible for the mechanical properties of cartilage. Without this large water fraction, cartilage would deteriorate rapidly due the constant compressive stresses it sustains. The water of the tissue is pressurized by compressive loading so that it bears most of the load, preserving the solid tissue [2].

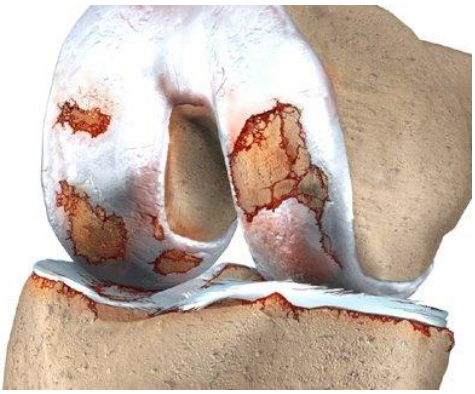

Figure 1 – Cartilage lesions and Damage due to Osteoarthritis of the knee [7].

One aspect of cartilage structure that poses a unique medical problem is its limited self-regeneration potential after damage. This is due to lack of blood vessels and nerve supply in the tissue. Furthermore, the small amount of healing that occurs creates fibrocartilage to fill in the lesion or damage. Fibrocartilage is inferior to the native cartilage because it lacks mechanical strength and it will deteriorate under repeated loading [1]. Unfortunately, cartilage damage is very common (affecting over 27 million Americans [2]) and is most often caused by traumatic injury or Osteoarthritis (OA). OA causes

the degeneration of joint cartilage due to age-related loss of ability to withstand normal forces or inability of the tissue to withstand overloading [5]. People afflicted with OA experience debilitating pain, swelling, and limited movement resulting in a reduced quality of life. Regrettably, the current treatments for cartilage damage are less than satisfactory and rarely restore long-term tissue function [6].

Available treatments include debridement, microfracture, total joint replacement, and autologous chondrocyte implantation. Debridement involves cleaning of the joint. Surgeons trim away damaged cartilage and smooth the remaining cartilage. This procedure is often coupled with repositioning of the joint to reduce the amount of force on the damaged area to offer temporary relief. Another option is microfracture which entails removing the damaged cartilage and introducing microfractures into the subchondral bone. This boosts cartilage regeneration but most of the new cartilage is fibrocartilage which is not as strong as articular cartilage. Thus, microfracture is only a temporary fix lasting, for 2-3 years. Eventually, patients will likely require joint replacement. Total or partial joint replacement often alleviates pain but has a long recovery time (3-6 months) and a risk of subsequent surgeries due to deterioration of the prosthetic. A newer method of repair, autologous cartilage implants, has recently been developed. In this approach, a small amount of articular cartilage is removed from a minimally weight-bearing area of the joint and the chondrocyte cells are separated out in vitro [1]. After these cells have proliferated, they are injected into the damaged area. Although this method has shown some potential, the cell source is limited so large lesions cannot be fully repaired [2]. As none of these methods are ideal, current orthopedic research has been aimed at finding new solutions.

Cartilage regeneration through in vitro tissue engineering is emerging as a very promising solution. This approach involves removing mesenchymal stem cells (MSCs) from the patient's bone marrow and placing them in a biocompatible scaffold that is equipped with specific bioactive molecules (growth factors) to promote differentiation and development of the cells while providing structure [6]. During this development stage, mechanical stimulus is applied to the growing tissue to mimic body conditions. Experiments have shown that mechanical loading enhances maturation of the cells and increases production of the extracellular matrix. Finally, the mature tissue could then be implanted into the affected area and integrated with the patient's existing cartilage. The advantages of this type of stem cell therapy

Figure 2 –This Image of cartilage tissue cultures gives an idea of samples size and shape. The cartilage is housed in this bioreactor during the developmental stages to promote differentiation and maturation.

include less invasive laparoscopic surgery, a plentiful source of MSCs, and a reduced chance of rejection of the implantation by the body. While engineered cartilage has not been implanted in human subjects yet, animal testing has delivered encouraging results. For example, a study on rabbits showed positive cell arrangement and some integration of the engineered tissue with the native tissue after only six weeks and nearly complete integration after 12 weeks [4].

Although the results of the animal trials are optimistic, further research is still needed. The goals of such research are to determine the proper amount of growth factors to maximize cell differentiation and to assess the stability of the mature tissue compared to natural cartilage. Current testing used in this research is destructive to the tissue. Thus, the cartilage is no longer viable for future implantation or observation, and analysis of the tissue's maturation is cut short. Non-destructive methods that provide insight into properties such as mechanical stability and that enable researchers to track the full development of a single sample are necessary. One possible method would be to MRI the engineered samples in their compressed state. Water content and movement in the regenerated cartilage could then be compared to natural cartilage to determine if the regenerated tissue is mechanically stable enough for human implantation. Furthermore, the long-term mechanical development of cells under static compression could be monitored via MR imaging [3]. Currently, there are no devices that are MR-safe with the capability to compress samples. A few devices can create static and dynamic compression but are not MR-safe.

Problem Statement

The overall objective of this project is to produce a biocompatible chamber (bioreactor) to protect cartilage tissue cultures while causing a 5-20% height deformation to the tissue. Furthermore, this bioreactor will enable high field MR scanning of the tissue samples in their compressed state. This way, tissue samples can remain living throughout the development stage and reach maturity while researchers gain insight into their properties. This will be a great improvement over the current testing methods that destroy the tissue.

Design Specifications

As detailed above, there is a need for a non-destructive testing procedure for cartilage tissue. Therefore, the material used should be biocompatible with human tissue to keep the tissue healthy and must be capable of sterilization using an autoclave so the product can be used for multiple trials. The bioreactor will be MR scanner compatible, so it cannot include ferrous metal, as this would interfere with the MR imaging. Ideally the material used should also

be transparent to allow the researcher to view the sample throughout the testing process. The temperature of the medium surrounding the tissue cannot vary more than 5°C from body temperature throughout the scan (the duration of which is estimated to be six to eight hours). The ideal range is (32°C – 37°C).

The bioreactor needs to fit inside of the cylindrical bore of a Varian 4.7T MR animal scanner which is 3" in diameter. A cylindrical shape is needed to maximize the amount of space available. Access to the tissue is also important, so the bioreactor must include a removable, leak-proof cap. The cap cannot allow medium to exit the bioreactor and leave the tissue exposed to air. The tissue needs to be surrounded by medium, but it also needs access to air to receive the proper influx of oxygen. Therefore the bioreactor must include some amount of air exchange with the environment.

The type of mechanical loading that will be used with this bioreactor is a compressive force. To create this compressive force, the tissue must first be fixed in one place. The disk shaped tissue, of approximately 1.5 cm radius and 3 mm thickness, cannot become dislodged during transfer or scanning and it must also be elevated off of the bottom of the bioreactor. The fixation device must be contained inside the bioreactor to reduce the risk of leaking. Once the tissue has been properly secured, the compressive force can be added. This force should be adjustable by the researcher and should cause a 5-20% height deformation. The force must also be self-sustaining, as it must remain constant throughout the scan.

Design Alternatives

Vertical Design

The first design alternative (figure 3) is the simplest design, both for fabrication and usage. It is called the Vertical Design due to its initial vertical orientation. It consists of a vertical cylinder, a removable table, and a removable cap. The tissue would be placed on top of the tray, which has a circular lip with a slightly smaller diameter than the bioreactor to hold the tissue on the table. This table is then placed inside of the bioreactor, its legs providing elevation off of the bottom. The cap can then be placed on top of the cylinder and the compressive force can be twisted or pushed into position. This design requires that the compressive force be applied to the tissue to hold it in place and this compressive force must be applied in the vertical orientation. The bioreactor would then be rotated 90° and placed inside of the scanner. Both the table and the cylinder would be made of transparent plastic, while the remaining pieces would be made of opaque plastic.

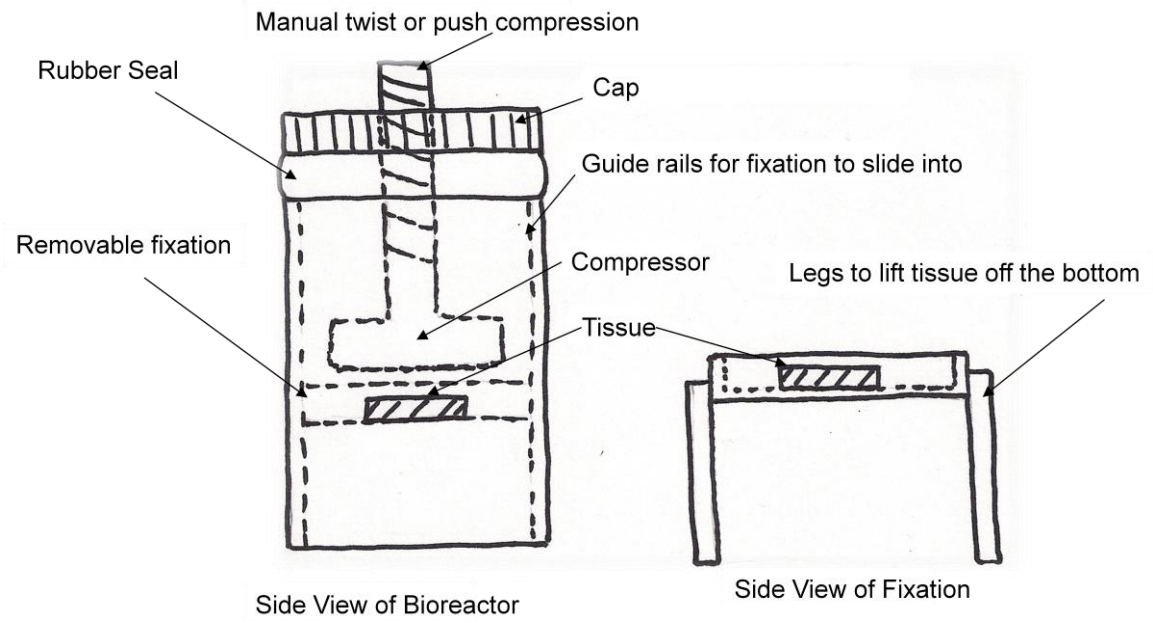

Figure 3 – Side view of the components of the Vertical Design. Notable characteristics include the compressor rod, removable table, and initial vertical orientation.

One advantage of this design is its simplicity. It would be very cost efficient, easy to manufacture, and straightforward to use since it does not include any complex pieces that may require a great deal of labor to create. It is also very easy to adjust the amount of compression desired. This design also has some disadvantages to it. Due to the method of force application, it is very difficult to quantify the exact force or percent height compression that is being applied to the tissue. Also, once the bioreactor is tipped onto its side, the tissue fall to the edge of the table due to gravity. This will create an additional normal force that may alter the findings.

Lever Design

The second alternative (figure 4) is slightly more complex than the previous design and is called the Lever Design due to its lever compression system. Like the Vertical Design, this design is also comprised of a transparent cylinder, detachable cap, and a removable plate to hold the tissue. The tissue is set into a grooved area on the plate and then slid into the cylinder (which is already rotated 90° from vertical). The compressive force is then added using a system of levers around a fixed point. The researcher should be able to push the end of the rod inward to create a downward compressive force. The cap must then be attached and the medium added through valves in the cap. The cap includes three holes: one for medium inflow, one medium outflow, and one for the compressive rod.

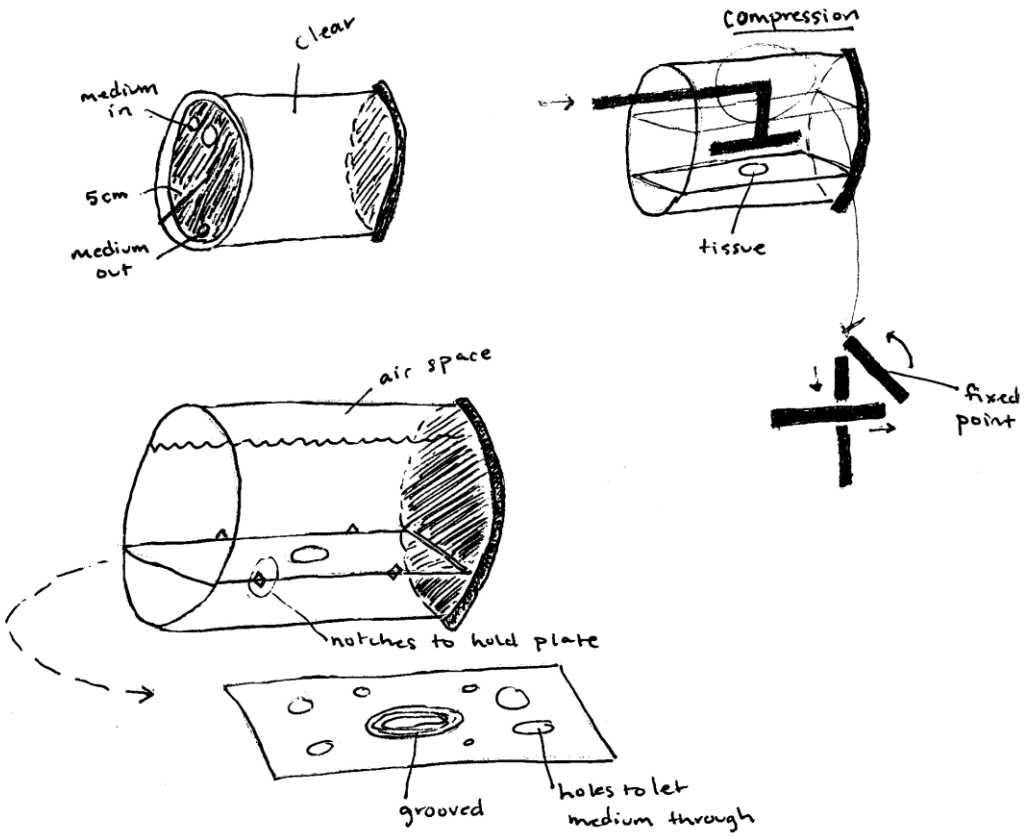

Figure 4 – Schematic drawings of the Lever Design components and operation. Notable characteristics include the horizontal orientation, valve for filling and removing medium, and lever controlled compression.

An advantage of this design is that the tissue is securely fixed due to the presence of the grooved area in the plate and its horizontal orientation. It is also set up to allow for the perfusion of the medium. With valves for medium transport, it could easily be adapted to continuously filter the medium through the bioreactor. Unfortunately this design has many disadvantages as well. The complex system of levers would be extremely difficult to fabricate and maintain. A slight disturbance to the bioreactor could easily offset this system. It would also be complicated to maintain the applied force during scans.

Angled Design

The third and final alternative consists of a horizontally oriented cylinder with a removable plate and is called the Angled Design because of its adjustable, angled compression rod. In this design, there is a rod running nearly parallel to the plate. The tissue is fixed to the plate. One end of the rod would be fixed to the edge of the bioreactor and the other end would

be vertically adjustable. By altering the height, the researcher can change the amount of compression delivered to the tissue.

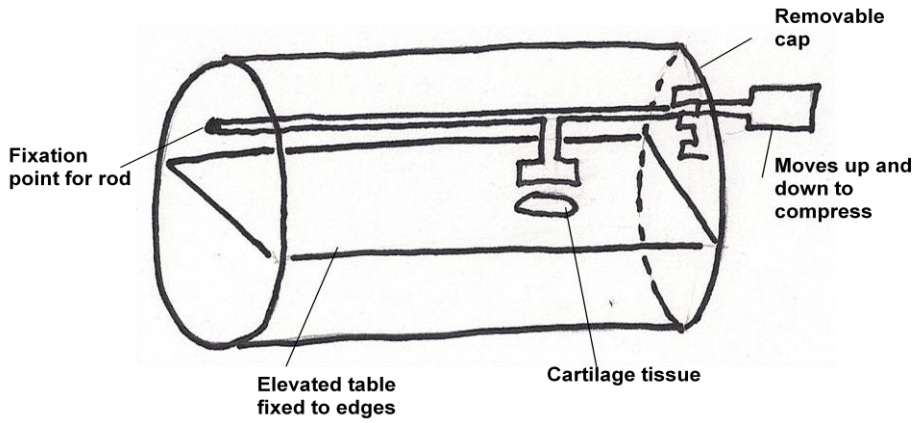

Figure 5 – Schematic drawing of Angled Design including notable components such as the fixed table, horizontal orientation, and adjustable compressing lever with notches.

A major advantage to this design is that it would create a more reliable way to measure the force delivered to the tissue. Since the heights of both ends of the rod are known, an angle and a force can be calculated. Another advantage to this design is that it would be relatively cost effective since there are not very many pieces to the design. The prototype could be created with a minimal amount of plastic. The major disadvantage to this design is that the adjustable end of the rod introduces a huge risk of leakage. It would be challenging to allow the rod to move without allowing any medium to leak out. Also, this design does not include a mechanism for adding or removing the medium. That would also introduce another area for potential leaking.

Design Matrices

To decide which design concept to pursue, the alternatives were evaluated using design matrices. For the design matrices, two important categories were chosen: materials and methods for compression application. In the matrices, some columns were weighted greater because they are imperative to sustaining the life of the cartilage tissue, as described in the Problem Statement section.

Table 1 - Materials Design Matrix

	Resistance to Heat	Transparency	Durability	Cost	Ease of Fabrication	Total (35)
Bioceramics	10	2	1	1	1	15
Teflon	10	2	5	2	2	21
PVC	4	10	4	3	5	26
Poly-carbonate	6	10	4	5	5	30

The first design matrix assesses biocompatible materials. It is very important to choose the right material for the final product to function correctly—keep the cartilage sample alive and be compatible with MR imaging techniques. Commonly used materials such as bioceramics, Teflon, PVC, and poly-carbonate were compared in the categories of resistance to heat (necessary for autoclave), transparency, durability, cost, and ease of fabrication. The chemical properties of the materials were determined after some research. This information was used to score the options. The first two categories, resistance to heat and transparency, were deemed more important than the other categories because they are essential for prolonged tissue life. Therefore, these categories were weighted by a factor of two and the scores totaled. Since poly-carbonate received the highest score and thus it will be the material used in prototype production.

Table 2 - Design Alternatives Matrix

	Risk of Leakage	Fixation	Maintain Force	Durability	Ease of Fabrication	Cost	Total (50)
Vertical	8	6	8	4	5	3	38
Lever	6	8	4	3	2	1	28
Angled	6	8	6	4	4	3	35

The second design matrix assesses the previously outlined methods of compression: Vertical, Lever, and Angled designs. Each method was evaluated in the categories of risk of leakage, fixation, maintenance of force, durability, easy of fabrication, and cost. The first three categories were weighted (by a factor of two) because they are vital to the function of the final product, whereas the other categories pertain to the construction of the final prototype. The vertical compression method received the highest score. This outcome confirms the team's initial thoughts. The Vertical Design appeared to fulfill the design specifications the best and be the most feasible prototype to build as mentioned in the design alternatives section. Thus, the Vertical Design was pursued in the final product.

Final Design

As previously stated, the design that the team pursued is the Vertical design. The main cylinder of the bioreactor was made from clear polycarbonate plastic. The cap was made from polycarbonate as well. There is a rubber gasket on and around the interior of the cap to prevent leakage of medium. To ensure survival of the cartilage inside the reactor, the rubber will never come into contact with the sample. The compression mechanism was constructed entirely from polycarbonate. All internal components of the bioreactor, including the fixation table, were made from polycarbonate. So that the bioreactor can easily be inserted into the Varian 4.7T animal scanner, the cap is exactly 3" in diameter. The main cylinder is 2.5" in diameter. The compression mechanism of the bioreactor was constructed using a simple thread mechanism. Compression of the cartilage is changed by twisting the threaded component, causing the force applicator to move vertically. Because deformation of the cartilage sample will be small, in the range of 150 µm, the compression mechanism was assembled such that only a fraction of a turn of the threaded compressor rod is needed to produce the needed vertical movement. A detachable dial can be placed on the top of the bioreactor cap so that the rod can be accurately twisted to every 1/8 of a turn. There is a depression in the fixation table to ensure the sample does not become dislodged while it is being compressed. The depression is 0.125" in depth (1/8"), approximately the height of the sample. The compression rod fits evenly into the depression in the table. This is so that deformation of the tissue can be measured through the desired 20% of its height. The table is

Figure 6 - Final assembled prototype in the compressing position.

fixed to the bottom of the bioreactor to ensure that the sample does not move during compression.

Testing

To ensure that the bioreactor meets the standards presented in the product design specifications, a series of tests were completed on the material and the final prototype.

Oven Test

The final product has been designed to be sterilized using an autoclave, so it is vital that the plastic and adhesive can handle the heat used during this process. Autoclaves use excessively high heat to kill any bacteria that may be living on the surface of the object being sanitized. It has been determined that temperatures in an autoclave reach 130°C or approximately 270°F. To observe whether or not the plastic can handle the heat, two small pieces of plastic were adhered together using the medical-grade adhesive used in the bioreactor. This plastic was allowed to dry and then placed into an oven at varying degrees. The plastic was clear and firm at 200°F and 250°F, but became slightly cloudy, yet still firm at 300°F. The adhesive was strong at 200°F and 250°F, but became slightly pliable at 300°F. Thus, the plastic and adhesive exceeded the specification of 270°F, but began to fail around 300°F. To compensate for this potential failure, all joints on the prototype were given a depression to fit into, providing extra security.

Compression Test

A very important part of the bioreactor is producing a measurable compression on the cartilage tissue. Therefore, it was imperative to quantitatively test how far the compressor moved per turn. Based on the equation given in Equation 1, it was calculated that to produce the desired 10% compression (0.3 mm) about a quarter of a turn would be needed. It was also expected that the compressor was moved the same amount despite the stiffness of the material.

Equation1 – The dimensional analysis shown explains how far the compressor was expected to move in one turn. To reach the 10% compression (0.3mm) that was given in the product specifications, this was divided by the millimeters per turn expected.

$$\left(\frac{1thread}{1turn}\right)\times\left(\frac{1inch}{20threads}\right)\times\left(\frac{25.4mm}{1inch}\right)=1.27mm/turn$$

$$\frac{0.3mm}{1.27mm}=0.236turns$$

To test this property, agarose gels were created in different concentrations (5% and 10% by weight) to simulate the cartilage tissue. The gels were then loaded into the bioreactor and the compressor moved down until it just touched the agarose gels. The height from the table to the compressor was then measured using a caliper. One full turn of the compressor was then completed and the height from the table to the compressor was again measured. This was repeated for each gel. The distance compressed was computed by subtracting the compressed height from the original height and then this number was divided by the original height to find percent deformation.

Table 3 – This table shows the average percent deformation for each of the different percentages of agarose (simulating different stiffness). These results turned out much different than expected from the calculations.

Percent Agarose	Average Height (mm)	Average Final Height (mm)	Distance compressed (mm)	Percent Deformation
5%	2.636	2.441	0.195	7.551%
10%	2.299	2.038	0.261	11.126%

The experimental values for compressed distances turned out to be very different from the calculated values. The actual distances compressed were significantly less than the calculated 1.27 mm. The calculated 1.27 mm should have given approximately 42% compression but the experimental values showed approximately 10% compression. This discrepancy could be caused by fabrication error or imprecise measurement tools. Furthermore, the cap and cylinder used for this test were not the same as the final prototype, therefore it is likely that the cap did not secure as tightly as desired. Thus, when the compression was added, it pushed the cap upward thereby skewing the results. In addition, user error could have had an effect on the precision of the measurements since it is difficult to measure very small increments, on the order of a tenth of a millimeter. This combined with the likely case that the cap was moving during testing accounts for the discrepancy between the calculated deformation per turn and the experimentally determined deformation per turn.

Despite the fact that the calculated values and the experimentally derived values varied, the results were fairly consistent. All of the 5% agarose gels compressed about the same

amount, as did the 10% agarose gels. More agarose gel samples are needed for more complete data. See Appendix A for raw data.

Temperature Test

This bioreactor will be placed inside of an MR scanner for hours at a time. During this time, the living tissue needs a steady temperature to be maintained between 37°C and 32°C. If the temperature drops significantly below this range, it is possible that the tissue may die during the scan. Thus, it was important to determine how long the polycarbonate prototype could remain in the specified temperature range and to ascertain if an insulating layer may be needed in the future. Using the formulas and constants given in Equation 2, it was determined that the time constant for this system was 129 minutes and that the temperature would remain between 37°C and 32°C for 37.5 minutes.

Figure 7 - Temperature testing with fiber optic probe.

Equation 2 – This shows the equations used (a) and the constants used (b) to find the time constant of 129 minutes. The A is the surface area of the cylinder, R is the R-value of the polycarbonate at 0.125" thick, C is the thermal capacity of the water in the cylinder, T_0 is the initial temperature of the liquid and T_{air} is the ambient temperature of the air surrounding the cylinder.

$$T(t) = T_{air} + (T_0 - T_{air})e^{-kt} \qquad k = \frac{A \cdot R}{C}$$

(a)

$$A = 0.0304\,m^2 \qquad R = 8.65\,W/m^2K \qquad C = c_{water} \cdot volume = 2017.4\,J \qquad T_0 = 310K \qquad T_{air} = 290.2K$$

(b)

After completing the calculations, a physical temperature test was run. The prototype was filled with water that was approximately body temperature. A fiber optic probe was placed into the water and the temperature was recorded every 30 seconds. The data was then plotted and an exponential trend line was approximated. The results are shown in Figure 7. The time constant according to this graph is 200 minutes (calculated from 0.005^{-1}) which is similar to the calculated results. Also, when looking at the raw data, the temperature was between 37°C and 32°C for 39 minutes. This is very similar to the calculated results of 37.5 minutes.

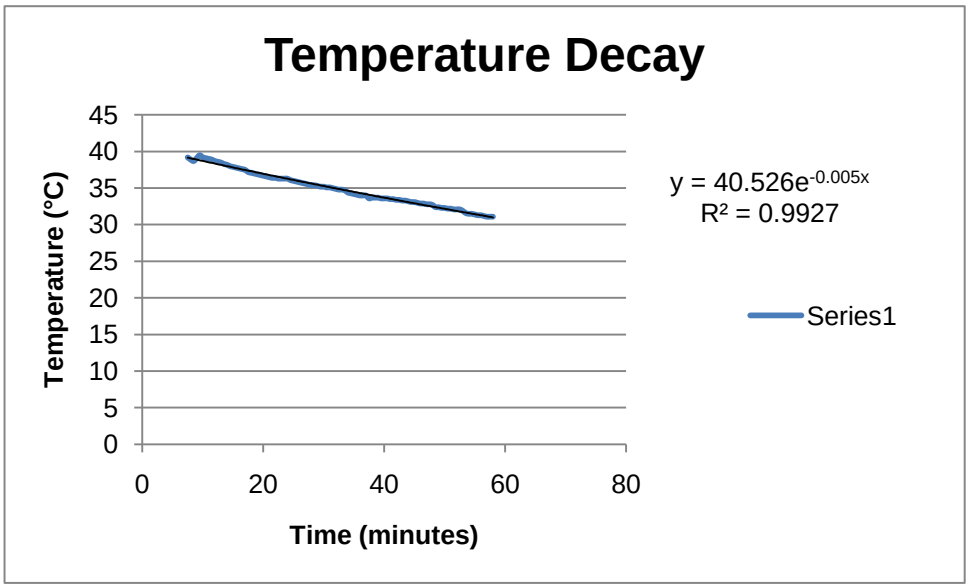

Figure 8-This graph shows the temperature decay of water inside the polycarbonate prototype. According to this decay, the temperature would remain above 32°C for 39 minutes.

Both the calculations and the test showed that the temperature will remain between 37°C and 32°C for less than 40 minutes. Therefore, the longest scan that could be run on the tissue using the current setup would be 35 minutes long, allowing time for setup and removal of the cartilage tissue. This does not provide much time to get a comprehensive scan of the cartilage tissue. Additionally, the ambient temperature during testing was 21.85°C but the ambient temperature of the 4.7T Varian animal scanner is 17.2°C. Thus, the time in the target temperature range during a scan may be a little smaller than the experimental values. To prolong the time that the temperature remains above 32°C, an insulating sleeve can be placed around the bioreactor during the scan. Another option would be to introduce an automatic perfusion system. An external heating apparatus used in conjunction with an automated perfusion system could maintain a constant body temperature for longer periods of time. Either of these two options could be researched if a scan of longer than one hour is needed.

Future Work

In the future, further compression tests will need to be conducted to gain more accurate deformation data from more samples. More precise testing equipment and a well-fitted testing environment to ensure data significance will be necessary. In addition, compression tests could be conducted on materials of different stiffness since percent deformation would vary depending on the stiffness of the testing sample. Also, more samples from each stiffness (i.e. percent agarose) should be tested to gain more comprehensive data. A method for determining the

magnitude of the applied force could then be developed since this value would also vary with the material stiffness. To maintain body temperature inside the bioreactor, an automated perfusion system could be implemented. The constant flow of medium through the bioreactor would allow the cartilage temperature to remain constant throughout scans as well as promote oxygen and nutrient exchange. If an automated system is not feasible for fabrication, a simpler solution would be the design of a stationary, external sleeve of insulating material that would encase the bioreactor preventing rapid temperature decay. Finally, to make the bioreactor design more adaptable to different scanner types, the design could be scaled up or down to increase its range of practical applications.

References

Text

[1] Atala, Anthony, Robert Lanza, James A. Thomson, and Robert M. Nerem. *Principles of Regenerative Medicine*. Boston: Academic Press, 2008. Print.

[2] Athanasiou, Kyriacos A., Eric M. Darling, and Jerry C. Hu. "Articular Cartilage Engineering." *Synthesis Lectures on Tissue engineering* 1 (2009): 1-182. *PubMed*. Web. 23 Nov. 2009.

[3] Herberhold, C., S. Faber, T. Stammberger, R. putz et al. "In situ measurement of articular cartrtilage deformation in intact." *Journal of Biomechanics* 32 (1999): 1287-95. Web. 30 Nov. 2009.

[4] Levicar, Natasa, Nagy A. Habib, Myrtle Y. Gordon, and Ioannis Dimarakis, eds. *Stem Cell Repair and Regeneration*. Vol. 3. London: Imperial College Press, 2008. 269-90. 3 vols. Print.

[5] Risbud, Makarand V., and Michael Sittinger. "Tissue Engineering: Advances in In Vitro Cartilage Generation." *TRENDS in Biotechnology* 20.8 (2002): 351-56. Web. 23 Nov. 2009.

[6] Tuli, Richard, Wan-Ju Li, and Rocky S. Tuan. "Current State of Cartilage Tissue Engineering." *Arthritis Research & Therapy* 5.5 (2003): 235-38. *PubMed*. Web. 27 Nov. 2009.

Figures

[7] “Knee Osteoarthritis.” <http://www.eorthopod.com/images/ContentImages/knee/-knee_osteoarthritis/knee_osteoarth_intro01.jpg>

Appendix A – Raw Data for Compression Test

Sample #	Height (in)	Height (mm)	Height after 1 turn (in)	Height after 1 turn (mm)	Distance Compressed (mm)	Agarose Concentration	Percent Deformation
1	0.1	2.54	0.093	2.3622	0.1778	5%	7.00000000
2	0.098	2.4892	0.091	2.3114	0.1778	5%	7.142857143
3	0.096	2.4384	0.089	2.2606	0.1778	5%	7.291666667
4	0.071	1.8034	0.063	1.6002	0.2032	5%	11.26760563
5	0.138	3.5052	0.129	3.2766	0.2286	5%	6.52173913
6	0.118	2.9972	0.11	2.794	0.2032	5%	6.779661017
7	0.097	2.4638	0.091	2.3114	0.1524	5%	6.18556701
8	0.099	2.5146	0.091	2.3114	0.2032	5%	8.080808081
9	0.117	2.9718	0.108	2.7432	0.2286	5%	7.692307692
10	0.076	1.9304	0.069	1.7526	0.1778	10%	9.210526316
11	0.116	2.9464	0.102	2.5908	0.3556	10%	12.06896552
12	0.088	2.2352	0.074	1.8796	0.3556	10%	15.90909091
13	0.082	2.0828	0.076	1.9304	0.1524	10%	7.317073171

Standard Deviation for 5% agarose gels: 1.5070669

*was not calculated for 10% agarose due to limited number of samples

Appendix B – Budget

At the request of the client, total must not surpass $500.

Price	Description
$10	Gasket material and Teflon tape
$70	Material for cap and labor
$ 85.19	All other plastic components
$ 23.89	Medical grade adhesive
$189.08	Total

Appendix C – PDS

Product Design Specifications – December 3, 2009

Cartilage Loading Project (Project #47)

Team Members:

Sarah Springborn – Team Leader

Luisa Meyer – Communicator

Sarah Czaplewski – BWIG

Beom kang Huh (B.K.) – BSAC

Advisor: Professor Block

Problem Statement:

The goal of our project is to develop a bioreactor specifically designed to secure cartilage tissue during magnetic resonance (MR) scanning and provide mechanical loading during this scanning. This will allow researchers to look at the percent water composition of the cartilage under different loading. This is also a non-invasive method that could prove to be an accurate measure of cartilage maturity in the future.

Client Requirements:

- Biocompatible
- Ability to be sterilized
- Can be used in magnetic resonance (MR) scanner
- Apply mechanical load to tissue samples

Design Requirements:

1.) **Material Characteristics**
    a. *Temperature:* The materials must not deteriorate when held at temperatures near 37°C indefinitely as it is meant to simulate human internal conditions.

b. *Biocompatible:* The bioreactor will be holding living tissue, so it must be biocompatible with human tissue.
c. *Sterilize:* The bioreactor should be able to withstand any type of sterilization that is currently used, including autoclaving and chemical sterilization.
d. *Insulation:* Ideally the bioreactor will be able to keep its internal temperature at 37°C for 6-8 hours, but it must not lose more than 5°C in that time.
e. *Metal:* There cannot be any ferrous metal in close proximity to the cartilage because this will alter the results produced by the scanners. Ideally, no metal will be used during the construction process.
f. *Transparence:* The tissue should be visible from the outside of the container to allow for frequent visual checks on the tissue.

2.) **Physical Characteristics**
   a. *Size:* Must fit inside a 3-inch diameter scanner (with a cylindrical shape).
   b. *Leaking:* The bioreactor cannot leak any of the medium that the tissue is growing in.
   c. *Cap:* There must be a cap on the bioreactor to allow researchers to monitor the tissue grow and to replace the medium.
      i. This cap must be secure and not leak when closed.
      ii. This cap needs to be large enough to insert the sample.
   d. *Not Air Tight:* Cells need to breathe, so the bioreactor should have either some sort of air exchange with the surrounding environment or a medium exchange apparatus to ensure adequate oxygen during the scan.

3.) **Fixation Characteristics**
   a. *Movement:* The tissue cannot move at all once it has been loaded in the bioreactor specifically as it is transferred into the scanner.
   b. *Height:* The tissue must be elevated off of the bottom of the bioreactor to allow room for the mechanical loading.
   c. *Internal:* The fixation should be on the inside of the bioreactor as to not introduce possible sites for leakage.
   d. *Sample size:* Ideally the fixation device should be able to adjust to different sample sizes, but at a minimum it should hold a disk shaped sample of proportions: 1.5 cm radius and 3 mm height.

4.) **Mechanical Loading**
   a. *Compression:* The bioreactor should have a mechanism for providing a compressive force on the tissue sample up to 20% compression.
   b. *Adjustable:* The force should be able to easily adjustable by the researcher.
   c. *Measurements:* The force should be deliverable from 5-20% in 5% increments. This amount should be visible and easily set.
   d. *Longevity:* The compressive force must remain at the same pressure throughout a 6-hour scan.