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DEPARTMENT OF BIOMEDICAL ENGINEERING
BME 200/300 – DESIGN

Cartilage Bioreactor

Mid-Semester 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 created three product alternatives and decided to pursue the Vertical Compression design made of polycarbonate. The next steps are validation and construction of the final bioreactor.

Background and Motivation

Articular cartilage, the cartilage between bones within joints, poses a unique medical problem because does not possess the ability to heal itself. This inability is due to a lack of blood supply. Hence, once damage is incurred, it can only be reversed through medical procedures. Lesions to cartilage can

be caused by traumatic injury or various joint diseases. Of these diseases, osteoarthritis is the most common, affecting nearly 21 million Americans. It causes the degeneration of joint cartilage resulting in debilitating pain, swelling, limited movement, and reduced quality of life [2]. Unfortunately, the current treatments for cartilage damage are less than satisfactory and rarely restore tissue function [4].

Available treatments include arthroscopy, osteotomy, and arthroplasty. Arthroscopy involves “cleaning” of the joint. Surgeons trim away damaged cartilage and remove loose debris. This may delay more serious procedures for younger patients, but it will not permanently fix the problem. An osteotomy entails repositioning of the joint to reduce the amount of force on the damaged area. While this relocation may hold for some time, the patient will eventually require an arthroplasty (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 [3]. As none of these methods are ideal, current orthopedic research has been aimed at finding new solutions.

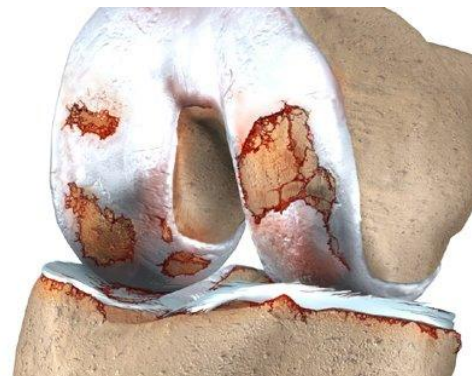


Figure 1 - The effects of osteoarthritis in the knee. [1]

Cartilage regeneration through tissue engineering is emerging as a very promising solution. This approach involves removing mesenchymal stem cells from the patient's bone marrow and placing them in a biocompatible scaffold that is equipped with specific bioactive molecules to promote differentiation and development of the cells [4]. Theoretically, the mature tissue could then be implanted into the affected area and integrated with the patient's existing cartilage in a less invasive procedure. The implantation step has not been performed in humans yet, because research is still ongoing in the development and differentiation stage.



Figure 2 - Cartilage tissue samples during development stage.

It is in this development stage, a bioreactor having the capability to apply compressive force while being imaged using MR would provide new insights. Currently, testing performed by researchers to track progress is destructive to the growing cartilage tissue. Thus the cartilage is no longer viable for future implantation, observation and analysis of the tissue's maturation is cut short.

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 magnetic 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 throughout the scan (the duration of which is estimated to be six to eight hours). Therefore a material with a low thermal conductivity would allow more time for scanning.

The bioreactor needs to fit inside of the 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 height, 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.

Designs

Vertical Design

The vertical design (figure 3) is the simplest design, both for fabrication and usage. 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. 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.

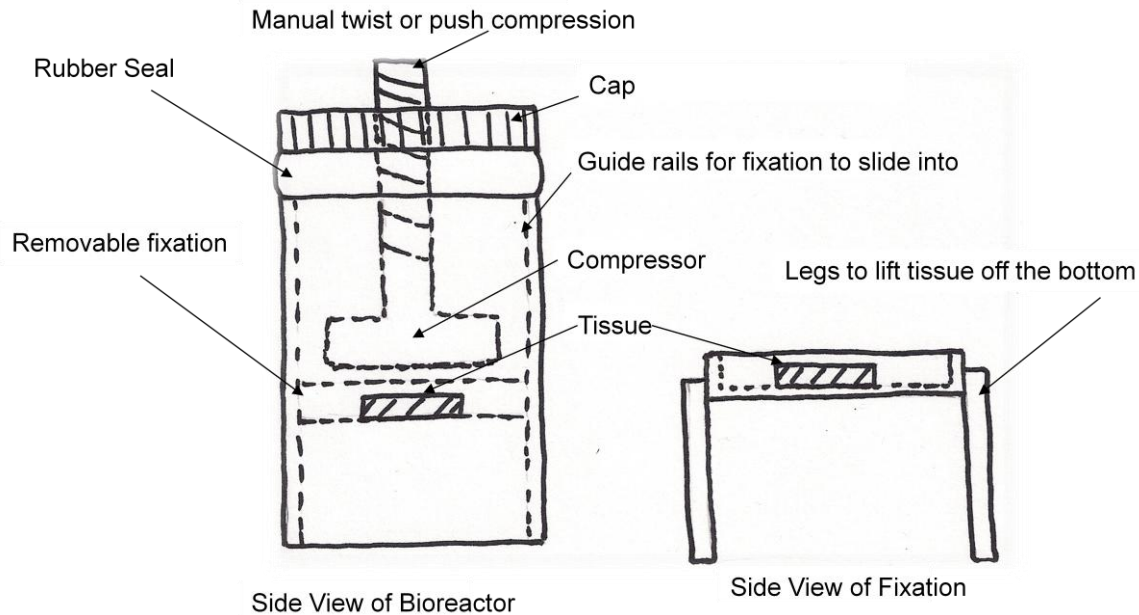


Figure 3 - Vertical Design

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 lever design (figure 4) is slightly more complex than the previous 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.

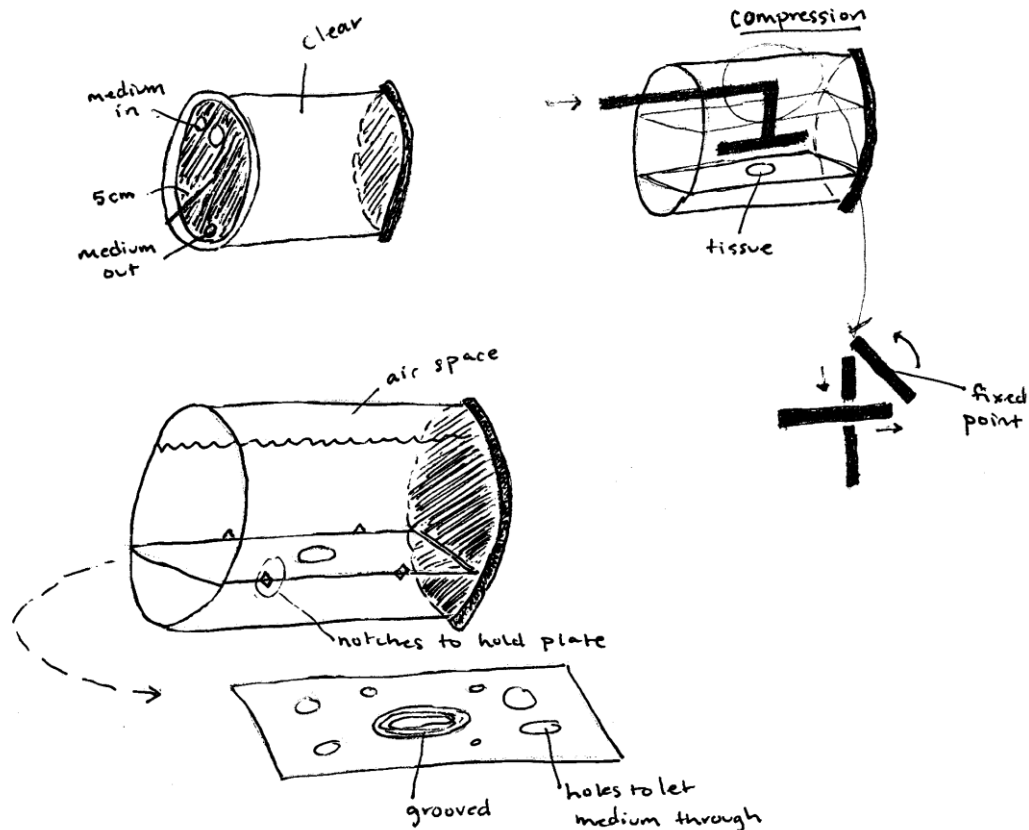


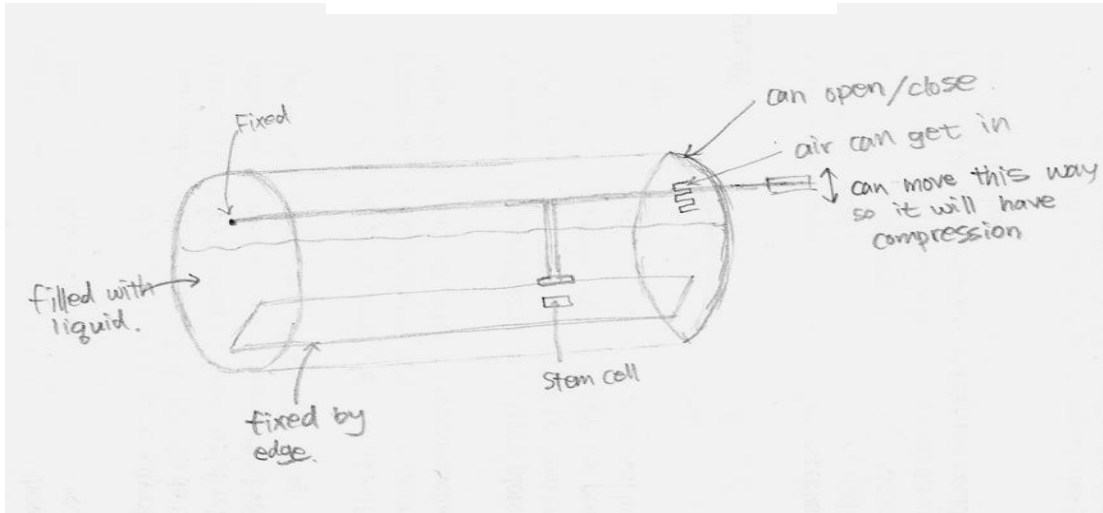
Figure 4 - Lever Design

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 Angled Design consists of a horizontally oriented cylinder with a removable plate. 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 - Angled Design



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

In order 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.

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, 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 and therefore will be pursued in the final product.

Proposed Design

As previously stated, the design that the team will pursue is the Vertical design. The team will continue to develop the specifics of the device. The main cylinder of the bioreactor will be made from clear polycarbonate plastic. The cap will be made from similar material. There will be a rubber seal around 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 will be constructed from a combination of polycarbonate and non-ferrous metal materials. All internal components of the bioreactor, including the removable fixation table, will be made from polycarbonate. So that the bioreactor can easily be inserted into the Varian 4.7T animal scanner, the cap will be no larger than 3" in diameter. The main cylinder will be 2.75" in diameter. The compression mechanism of the bioreactor will be constructed using a gear and thread mechanism. Compression of the cartilage will be changed by twisting the threaded component, causing the internal force applicator to move vertically. Because deformation of the cartilage sample will be small, in the range of 150 μm , the compression mechanism will be assembled such that a large twist of the threaded component will result in a small rotation of the gear. There will be a depression in the fixation table to ensure the sample does not become dislodged while it is being compressed. The depression will be 0.0625" in depth (1/16"), approximately half the height of the sample. This is so that deformation of the tissue can be measured through the desired 20% of its height.

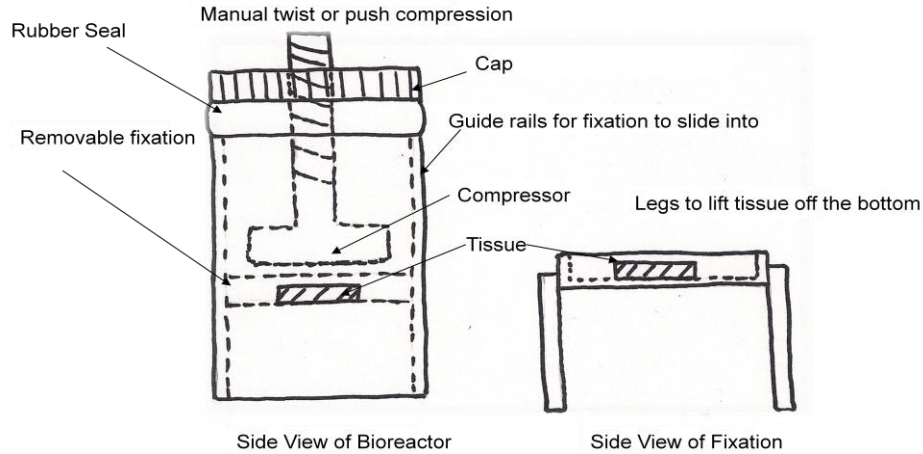


Figure 6 - Proposed Design (Vertical Design)

Future Work

Before fabricating the final prototype, it is first necessary to perform some preliminary testing. The team will build a mock-up of the prototype consisting of the outer framework. This device will be made from the same materials as the final prototype. The first test will determine the thermal conductivity of the imaging coil. The Varian 4.7T animal scanner implements an external magnetic coil for imaging that is inserted into the bore of the machine. All objects to be scanned are then placed inside the coil. In order for the cartilage sample to remain living throughout scans, there cannot be a large temperature variation. The initial temperature of the cartilage will be approximately 37° therefore the variation in temperature cannot exceed 5°. Using the mock-up, tests will be conducted to observe the temperature decay after it is inserted into the scanner. If the variation proves to be too large for proper maintenance of the sample, insulation methods will be implemented to reduce the variation. Some preliminary ideas include surrounding the cylindrical portion the device in a thin insulating material—similar to beverage cozies.

A second test will be performed to determine the necessary volume of medium for imaging. MRI systems are quite sensitive to air space surrounding imaging samples. To generate a high quality image, there cannot be large amounts of air space in close proximity to the portion of the sample being scanned. To ensure tissue survival at the end of the scanning session, there must be a small amount of airspace in the bioreactor. To determine the amount of allowable air space, pilot scans will be taken of the mock-up with various amounts of medium.

After testing, the team will then proceed to fabrication of a final prototype. Parts will be ordered, primarily polycarbonate plastic and non-ferrous connectors, from McMaster-Carr and various other retailers.

References

Figures

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

Text

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[3] "Surgical Treatment of Osteoarthritis of the Knee." *Orthopaedic Connection*. 01 Jul 2003. American Academy of Orthopaedic Surgeons, Web. 16 Oct 2009. <<http://orthoinfo.aaos.org/topic.cfm?topic=A00385>>.

[4] Tuli, Richard, Wan-Ju Li, and Rocky Tuan. "Current State of Cartilage Tissue Engineering." *Arthritis Research & Therapy* 5.5 (2003): 235-238. Web. 11 Oct 2009. <<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=193737>>.

Appendix A – Product Design Specifications

Product Design Specifications – October 9, 2009

Cartilage Loading Project (Project #47)

Sarah Czaplewski, Sarah Springborn, BeomKang Huh, Luisa Meyer

Problem Statement:

The goal of our project is to develop a chamber specifically designed to secure cartilage tissue during magnetic resonance (MR) scanning and provide mechanical loading during this scanning. The current method for this type of research is to grow the cells in one container and then transfer them to a different container for the scanning. This process of transferring from one container to another increases the chance for contamination significantly. This product is intended to eliminate that step, thus aiding in advancing osteoarthritis research!

Client Requirements:

- Biocompatible
- Ability to be sterilize
- Can be used in magnetic 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 for long periods of time as it is meant to simulate human internal conditions.
- b. *Biocompatible:* The chamber will be holding living tissue, so it must be biocompatible with human tissue.
- c. *Sterilize:* The chamber should be able to withstand any type of sterilization that is currently used, including autoclaving and chemical sterilization.
- d. *Insulation:* Ideally the chamber 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 metal involved in the construction of this device because this will alter the results produced by the scanners.
- 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 chamber cannot leak any of the medium that the tissue is growing in.

- c. *Cap*: There must be a cap on the chamber to allow researchers to monitor the tissue grow and to replace the medium.
 - i. This cap must be secure and not leak while closed.
 - ii. This cap needs to be large enough to insert the sample.
- d. *Not Air Tight*: Cells need to breathe, so the chamber should have some sort of air exchange with the surrounding environment.

3.) **Fixation Characteristics**

- a. *Movement*: The tissue cannot move at all during chamber transfer or scanning.
- b. *Height*: The tissue must be elevated off of the bottom of the chamber to all room for the mechanical loading.
- c. *Internal*: The fixation should be on the inside of the chamber 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 chamber 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 an 8 hour scan.