Skeletal Organoid Bioreactor: Final Report

MEIE 4702: Capstone 2

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Skeletal Organoid Bioreactor: Design Review

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Abstract

When growing cartilage organoids in a laboratory, known as *in vitro* growth, conditions are mechanically different from those experienced in the human body, referred to as *in vivo* growth. This lack of external loading causes matured cells to exhibit worse mechanical properties than natural tissue. To address this issue, the skeletal organoid bioreactor aims to apply cyclic, hydrostatic pressure to cell cultures of cartilage organoids, mimicking the force loads and frequency that human cartilage endures when walking. Ideal conditions also require a temperature of 37 ℃ to be maintained for the cell culture to simulate body temperature, while a pressure of 10 MPa is applied at a frequency of 1 Hz through hydrostatic pressure.

Based on background research, the pressure vessel would require six primary components: the body, translational force mechanism, lid, seals, temperature control and sensors, and cell bag. Existing products, literature, and standards were researched for each part. Through this research, the team was able to design an initial prototype. The design differed from existing solutions primarily by placing the plunger inside the pressure vessel. Pressure vessel calculations and SolidWorks modeling were used to calculate appropriate dimensions for this unique tank. Additionally, a temperature control system was designed with heating coils, eliminating the need for an external heating system such as a water bath that previous solutions implemented.

Upon consulting industry professionals, the group honed in on the vessel's components and fabrication to bring it to its first prototype design. Design changes include allowing for the vessel and lid to be manufactured out of steel stock to avoid welds, adding a pressure-resistant thermistor to measure the water temperature, integrating a wire pipe heater into the vessel exterior, and including a pressure release valve and pressure gauge. The first physical iteration successfully achieved over 10 MPa with only minor leakage and high confidence in safe failure. Additional design changes will be implemented as continued testing is carried out in 2023, evaluating the design's capability to fully culture cartilage with improved mechanical properties.

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1 Introduction

Problem Statement

When growing cartilage organoids in vitro*,* conditions are mechanically dissimilar to those experienced during in vivo growth. This causes matured cells to exhibit far worse mechanical properties than natural tissue. The goal of this project is to apply cyclic, hydrostatic pressure (HP) of 10 MPa at 1 Hz to cell cultures of cartilage organoids in an effort to grow cartilage with stronger mechanical properties.

Background

To understand the goals and constraints of this project, it is necessary to understand what cartilage is and how it behaves. Cartilage has two general forms, articular cartilage and fibrocartilage. Articular cartilage is found at the ends of joints, comprising the bearing surfaces between them. Fibrocartilage is weaker, degrading much more quickly than articular cartilage under the same conditions, and is what the human body naturally grows to replace damaged articular cartilage [1]. This deterioration is one common cause of joint failure. Chondrocytes are the cells that grow cartilage, producing its two primary components. Collagen is a protein that forms strong cross linkages throughout the cartilage and makes up ⅔ of the dry mass of cartilage. Glycoproteins, namely glycosaminoglycan and aggrecan, form a network of linkages capable of holding water called the extracellular matrix (ECM). Collagen gives cartilage its tensile strength, keeping the structure together, while water held by the ECM allows the cartilage to withstand large and repeated compressive loads [2].

The ECM does not hold water statically, instead allowing water to move slowly throughout the joint to distribute the forces applied to the joint. Thus, compressive force is not cyclically bending a rigid structure, but rather compressing and displacing water through a resistive matrix [1]. This cyclic compression is also useful because it is also how nutrients reach the cartilage. Blood vessels cannot withstand the environment in cartilage, hence its white color, so the only way nutrients can reach the cartilage is through the synovial fluid, the liquid in and around each joint [3]. Many studies have also demonstrated that when cartilage is grown in vitro, such as in a petri dish with no gauge pressure applied, the strength of the cartilage grown is poor [1,2,3,4,5,6,7]. However, when HP, especially cyclic HP, is applied, the strength of cartilage increases, as shown in Figure 1 on the following page. mRNA signal levels, as shown in the figure, are positively correlated with cartilage strength.

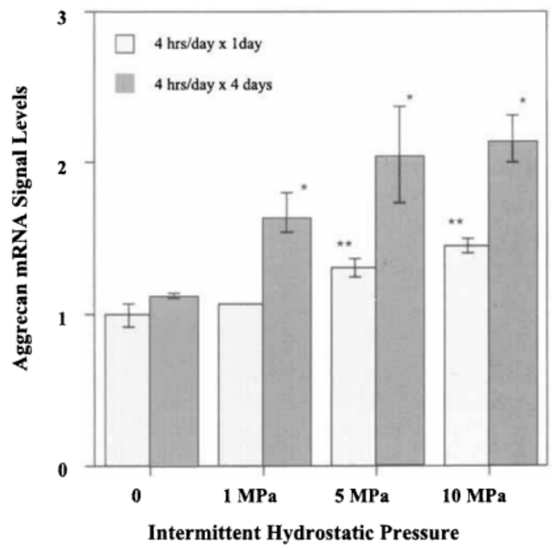

Figure 1: Graph Showing Correlation Between Cyclic Pressure and Aggrecan Levels [4]

The trend of increased strength when cyclic HP is applied has been shown by numerous studies [1,5,6,7]. There are alternative methods of increasing the strength of cartilage grown in vitro, such as lowering the oxygen content of the liquid from atmospheric concentrations or adding fetal serum, among others, but those are beyond the scope of this project [3].

In the researched studies, 10 MPa is the maximum pressure generally applied to in vitro chondrocytes, and it generally creates cartilage with the highest strength [1]. This pressure was chosen to be the pressure applied for this project, as well as a frequency of 1 Hz. This pressure and frequency was chosen because it is the pressure and rate that cartilage in a human knee is subjected to during normal walking. This 1 Hz, 10 MPa pressure will be done for four hours straight every day using the Bose dual-column Instron 3400 force machine at Professor Shefelbine's lab. Another requirement for the pressure vessel is that it remains at body temperature. In other words, the temperature of the chondrocytes within the pressure vessel must be kept within 36 °C to 37.5 °C. In addition, the bag containing the chondrocytes will be removed from the pressure vessel between cycling periods, so a quick, easy, and repeatable removal process for the bag is necessary. The bag will be 10 mL and be able to withstand the cyclic 10 MPa pressure, while not allowing any liquid or gasses to be exchanged across its membrane.

Clients' Motivation and Objectives

Cartilage has poor inherent repair capabilities and is subjected to wear and tear, injuries, diseases, and age related issues. Researchers at the MIT Shoulders Lab currently lack a model for understanding how cartilage cells grow and repair, and understanding the conditions for cartilage growth can be crucial in devising treatments and in engineering cells for cartilage regeneration. The clients are conducting research for improved conditions of growing cartilage cells and are looking to study how cyclic loading of HP can strengthen the mechanical properties of organoids. This project will serve to provide the client with a means of testing cartilage organoids samples under cyclic HP conditions.

2 Research

The vessel features six primary components: the body, translational force mechanism, lid, seals, temperature control and sensors, and cell bag. Existing pressure vessels and existing options for the individual components were researched to provide a basis for design and analysis.

General Considerations for Existing Solutions

Applying cyclic pressure to chondrocytes has commonly been done by using a pressure system to quickly compress and release a working fluid. One example of this is through the use of a force machine to apply force to a piston that acts as a pressure reservoir for the system. A screw driven force machine would be damaged if used cyclically for extended periods of time, and pressurized gas systems cannot create and drop pressure for 4 hours at this magnitude. All systems research had expensive and unnecessary features [8,9,10,11,12,14], none of which featured a single vessel design.

As a potential pathway to overcoming the costs and complexities of manufacturing a certified pressure vessel, commercially available pressure vessels made for lab scale bench top experiments rated upwards of 10MPa were investigated. One such device, with the desired housing and pressure rating, was the Parr pressure vessel, shown in Figure 2. This device, which was quoted at $3000, is built for static pressure applications, meaning a completely new lid would have to be designed and manufactured to implement cyclic pressure. Buying and then modifying this device was well beyond our budget, so the team chose not to purchase it. However, inspiration was taken from it for the design.

Figure 2: Parr Pressure Vessel [15]

Additional research was done by talking to pressure vessel manufacturers to determine who could manufacture a vessel of the appropriate size and pressure. These professionals, as well as the Northeastern machinists, pointed out that making welds that can withstand the necessary pressure for this project requires high-level certification. Furthermore, the manufacturers that could perform these welds had lead times in the magnitude of six months or more. The result of this research was that we decided the vessel had to be designed without welds.

Body

Research was first done to find the vessel's necessary cylindrical thickness, referred to as shell thickness. Research was done into how this dimension could be accurately calculated to ensure that it could withstand the necessary load applied to it without failing. Pressure vessel engineers were consulted initially. They recommended using and following the standards for ASME certified pressure vessels, which were then researched to determine the appropriate vessel shell thicknesses.

Translational Force Mechanism

In order to translate the force of a hydraulic force machine into pressure, common mechanisms such as pistons and pumps were investigated. Of these, the piston and plunger mechanisms commonly used in water pumps, shown in Figure 3 were considered likely options that could be adapted into the design of the bioreactor either as a standalone device or built into the pressure vessel as a single device. These mechanisms create pressure from linear displacement and could be designed to produce large pressures at small displacement ranges.

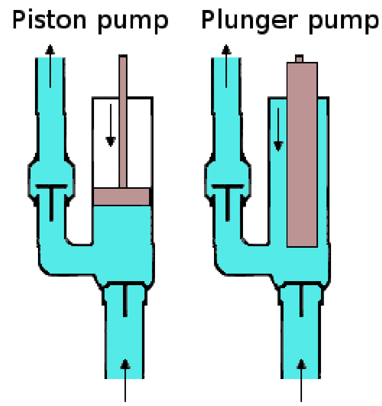

Figure 3: Types of Plunger Pumps [16]

Lid

The role of the lid is to house the force to pressure translation mechanism and to provide access into the vessel for loading cells. The lid must withstand the pressure criterion without leaks and also be readily and repeatedly removed in a way that is not too difficult for the operator. Research started with mechanisms used in other high pressure applications including screw clamps like in the Parr vessel, tri clamps, cam clamps, shown in Figure 4, and latched hatches, shown in Figure 5.

Figure 4: Cam Clamp [17]

Figure 5: Latched Hatch [18]

Seal*s*

The greatest challenge of implementing a translational force mechanism is making sure none of the pressure is lost. It is especially difficult because it is a dynamic system, meaning that a perfect seal must be formed at every step of the mechanism's movement. Seal research started with determining the types of seals that are commonly found in high pressure applications where there are moving components.

Of the systems studied, a stuffing box was presented as a possible solution. Shown in Figure 6, this device is used to seal a shaft against a fluid while still allowing the shaft to move freely. The primary components of a stuffing box are the casing, the throat bush and the packing rings. The casing houses the mechanism, both preventing external damage and keeping components under pressure and packed together. The throat bush is found at the pressurized end of the shaft, keeping it in place as well as preventing solids from reaching the packing rings. The packing rings are made of braided fibers, held tightly against the shaft. This prevents fluid from entering the stuffing box while still allowing the shaft to freely move.

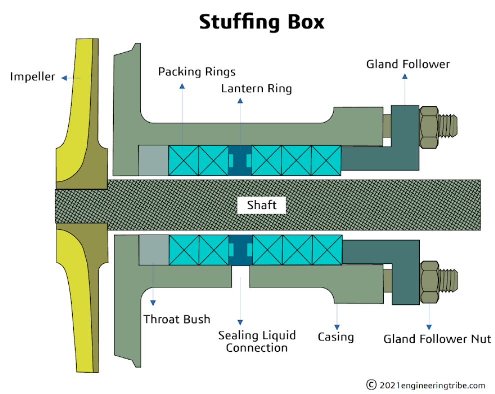

Figure 6: Stuffing Box Design [19]

The stuffing box is found across many applications including pumps, boat props, and pistons. However, should a stuffing box be used, it is likely that a custom product would be required to adapt it to the design and size of the bioreactor, as stuffing boxes are finely tuned and cannot be easily altered.

Another solution was discovered upon studying hydraulic systems. Hydraulic cylinders are typically classified based on their duty levels which are referred to as light, medium, and heavy duty applications. Each duty level refers to a different pressure and temperature range. For an application of 10 MPa, the system falls under the light duty cylinder classification which has application for pressures up to 16 MPa and temperatures of 70ºC [20]. The types of seals commonly used in hydraulic cylinders are shown in Figure 7.

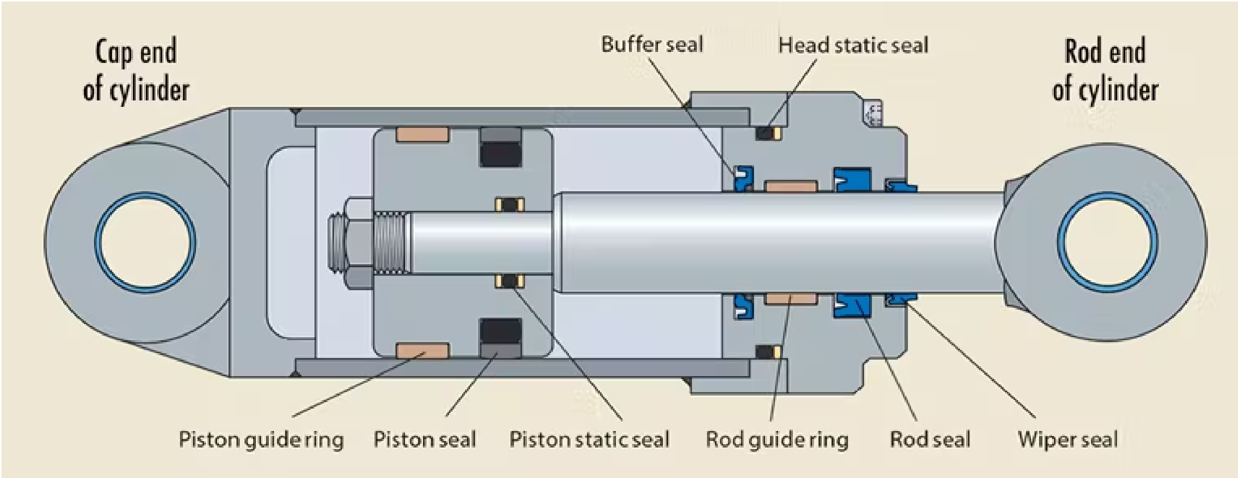

Figure 7: Hydraulic Seals [21]

Rod end seals include the rod seal, guide ring, buffer seal, and wiper seal. The rod seal serves as the primary pressure barrier and prevents the working fluid inside the vessel from leaking. The guide ring serves as an interface between the rod and the lid to prevent metal to metal contact that would otherwise cause galling in the absence of a proper lubricating media. While a buffer seal is not always considered necessary in light duty applications, research shows that buffer seals increases the lifespan and operating quality of a rod seal by acting as a barrier to pressure spike, a secondary barrier to the system's fluid pressure, and act as a vent for intermediate pressure that could otherwise be trapped in the rod seal [21]. Similarly, a wiper seal is not considered crucial in the operation of the plunger, since it does not act as a pressure barrier, but serves to keep contaminants, such as dirt, dust, and fluids, from the outside environment from entering the pressure vessel. Through this examination, the types of seals used in hydraulic systems match pressure requirements and many of the operating conditions that will be needed for the bioreactor.

Temperature Control and Sensors

The vessel must be maintained at a constant temperature of 36.5 ± 0.5 °C to promote proper cell growth. The first heating method found during research was a water bath, as shown in Figure 8. This device is commonly used to precisely control temperature in lab settings. The entire vessel would sit in the bath and be fitted into the grips of the force machine.

Figure 8: Off the Shelf Water Bath [22]

The second option identified was to wrap the vessel in an electrically resistive heating coil. Figure 9 shows a pipe wrapped in a heating coil, analogous to what would be done to the vessel. Ideally, the dial on the control box would be calibrated to the internal temperature of the vessel so that it could be set and not need manual interference throughout loading.

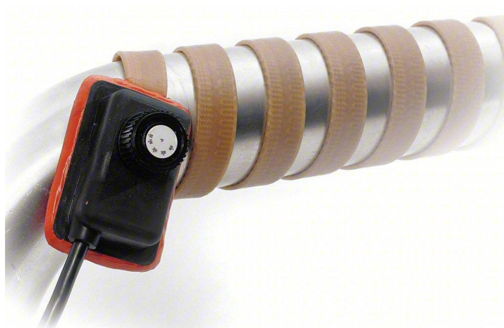

Figure 9: BRISKHEAT Heating Tape [23]

Both of these systems appeared to be possible with some modification, and did not require further research

To ensure accurate internal pressures are reached, a pressure sensor needs to be used. The one shown below in Figure 10, manufactured by Omega, is an example of a high pressure digital sensor. The primary goal of the pressure sensor is to calibrate the force machine to the appropriate pressure. In this specific application, the resolution of the pressure gauge does not need to be higher than 1 psi.

Figure 10: Omega Pressure Gauge [24]

To ensure safety, the pressure vessel must have a failsafe to avoid catastrophe. Should internal pressure exceed the safe limit, a mechanical release valve like the one pictured in Figure 11 would be activated, venting excess pressure. The upper cap can be twisted to the target pressure and calibrated with the pressure gauge.

Figure 11: Pressure Release Valve [25]

The various control systems here are crucial for the design, both for fulfilling temperature and pressure criteria as well as ensuring safety. The heating system provides a steady state for the cells to grow in, and the pressure valve and sensor ensure the accuracy and safety of the internal pressure.

Cell Culture Bag

There were characteristics the bag had to have to work as needed. The cell culture bag needed to isolate the cells from the pressurized water in the vessel while still allowing hydrostatic pressure to pressure its contents, so it had to be flexible while not allowing water to contaminate the cell solution inside or contaminate the cells itself. Thus, the bag had to be sterile and be able to withstand a compressive pressure of 10 MPa. Furthermore, the medium inside the bag will be replaced every couple of days, so there had to be a way to insert and extract the cell solution without removing the cells. In addition, it had to have a volume of 5-10 mL.

As a starting point, medical-grade cell culture bags were investigated due to their sterility and durability, and because they often require addition and removal of fluids. The group investigated multiple options for the bag. Ultimately, three bags potentially fulfilled the design requirements mentioned above. A visit to the MIT Shoulders lab was helpful in gathering information about ways to extract the cell solution without chondrocyte cells. A female Luer-Lock syringe is typically used to extract and insert the cell growth medium for the chondrocytes. A filter with a male Luer port on one side and a needle port on the other side, shown in Figure 12, is used along with the syringe to prevent the chondrocyte cells from also being extracted with the medium when the medium is changed.

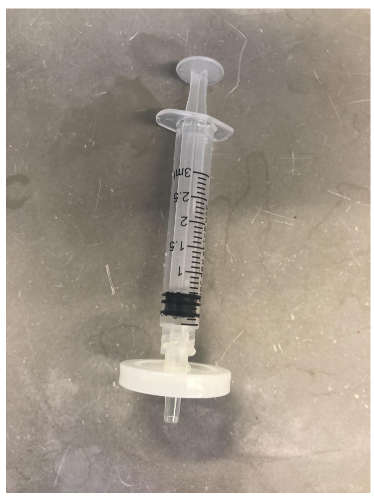

Figure 12: Female Luer-Lock Syringe with Filter

Because the syringe has a female Luer Lock, the group determined that a male Luer-Lock port will be required on the bag. A filter with a male Luer-Lock port on one side and a female Luer-Lock port on the other side will be necessary to attach it between the syringe and the bag. This allows the bag to be used with or without the filter in between, depending on the size of the chondrocyte cells. An example male to female Luer-Lock filter is shown below:

Figure 13: Male to Female Luer Lock Filter [26]

First, the Sartorius Flexsafe 2D Bag is a medical grade bag that is built for reproducible cell growth. It is opaque, sterile, 20mL, and has an inlet and outlet Luer-Lock port [27]. The Origen Permalife Cell Culture Bag has a luer-actuated adapter for one opening, and a normal inlet. The first allows gas but no liquid through, and the second allows flow of liquid and gas [28]. The smallest volume for the Origen bag is 10mL. Lastly, the Sartorius Flexboy Bag is also a medical grade bag that is sterile, 5mL, and has one Luer-Lock port [29]. These three bags are shown in Figures 14-15 below.

Figure 14: Sartorius 20mL [27] **Figure 15: Origen 10mL [28]** **Figure 16: Sartorius 5mL [29]**

Summary

The research as described above for the general vessel and each component proved to be essential for the design of the vessel. ASME research and calculations were vital parts of the body and lid design. Outreach to professionals in the pressure vessel space was helpful for affirming what was needed to design the shell, lid, and seals most of all. The research into the bag and sensors were directly implemented into the design with little modification needed, while the research into the temperature control system served as the basis for the existing design. Thorough research prior to the design process enabled the design process to be finished completely and have confidence that it would meet all design constraints.

3 Design

Design 1

The original design, shown in Figure 17, featured two separate vessels: one to create the pressure, and one to house the cells. The pressurized water would be transferred between the vessels using a high strength metal hose. The biggest advantage of this design was that each vessel only had to achieve one task. All the piston vessel had to do was ensure the piston moved perfectly along the axis, and the cell vessel only had to withstand the pressure and be easy to handle. With this approach, the lid could have a very simple design making it easy to open and close.

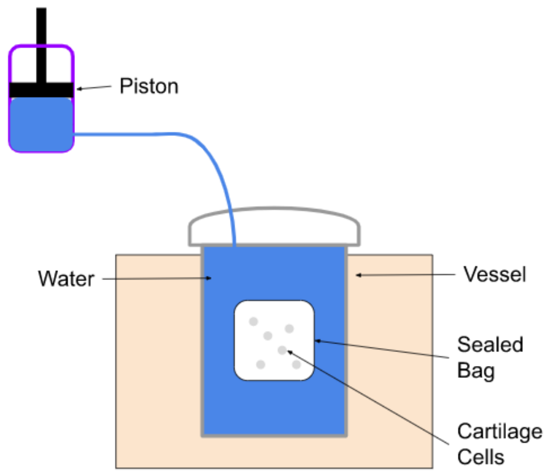

Figure 17: Two-Vessel Design

Furthermore, only the piston vessel would have to fit into the Instron. The operator could choose to use a long hose and have the cell vessel and water bath sit wherever they would like without worrying about the Instron damaging the water bath.

On the other hand, the biggest problem with this approach was that it required designing and manufacturing two vessels instead of one; Rodrigo called it two projects in one and advised against it. Additionally, the hose to transfer water required fittings to be welded to both vessels. The capstone machine shop is not fit to make high pressure welds, so this design would have to be outsourced, costing the group precious time and money.

Design 2

To overcome the difficulties of the first design, the group combined the two vessels into one. Although this decreased some of the usability, it greatly improved manufacturability and increased the likelihood of having it ready for Capstone Day. The design was intended to combine both the pressure and heating capabilities in one vessel, while still housing the cells inside. The advantages to this design were a significantly reduced cost as it roughly halved production and material costs, as well as decreased the complexity. The overall design may be seen in Figure 18 below.

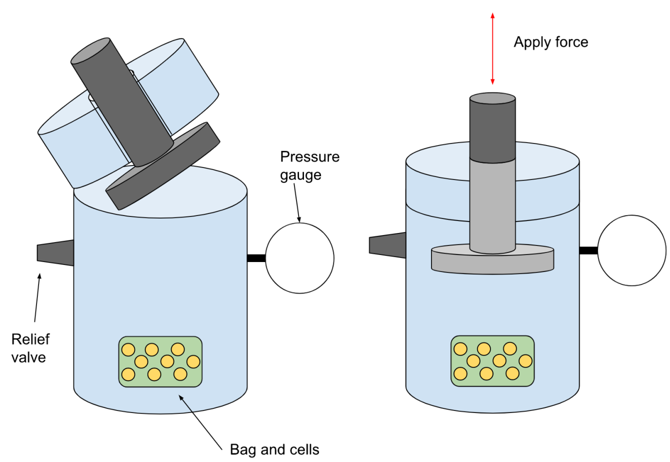

Figure 18: Initial Single-Vessel Design

Body

The necessary width of the vessel was the primary dimension to determine. As such, it was calculated in multiple ways to validate the result. The maximum stress was calculated using fatigue analysis, assuming infinite cycles, a machined finish, bending, and reliability factor, found to be 19 ksi =130 MPa. This was input into the hoop and longitudinal stress equations, with known internal pressure and radius, and the minimum thickness was found to be 3/16”.

This design served well to approximate dimensions and required Instron forces and displacements, but had some downsides. The most major flaw was the weld needed to attach the vessel’s base to its body, as this required sending the design to an external manufacturer. By this point, the team had discovered that external lead times were over 15 weeks long and that most manufacturers were unable to fabricate a vessel of this size.

Seals and Translational Force Mechanism

An additional feature of this design was the switch from a piston system to a plunger system, which eased the seal requirements significantly. Instead of needing a seal all along the entire outer face of the wall, the plunger only needed to have a seal at its interface with the lid. It was unclear how this was to be done, but a stuffing box was proposed to be placed on top of the shaft. The rest of the vessel was to be made from three pieces of steel stock, a disc for both the lid and base, and a hollow cylinder for the body.

Temperature Control and Sensors

Temperature control was thought to be most achievable by placing the vessel in a store-bought water bath, which would provide a cheap way to regulate temperature without having an internal temperature probe. This was a hugely advantageous solution, as thermistors are difficult to mount inside a pressurized chamber, and pressure resistant ones are expensive. Unfortunately, further research into water baths revealed that they were unable to withstand the 10kN forces required to be applied to the cylinder, so work began on manufacturing a water bath in-house.

In this design phase, very little was decided. It was determined that a single vessel would be made, with a plunger to apply the pressure within the vessel, but the thickness of the vessel, lid type and thickness, temperature control device, sensors, and cell bag were still in question.

Design 3

As conversations with industry professionals progressed, the group honed in on the vessel's components and created the model below. To circumvent welding due to the issue mentioned previously, the body and base were made to be manufactured from a single solid steel cylinder. The Instron's jaws would be responsible for supporting the entire system, so fins were added to the top of the plunger and bottom of the vessel. These fins were designed to match the geometry of the jaws to give the system the most contact and stability. This iteration of design is shown in Figure 19 on the following page.

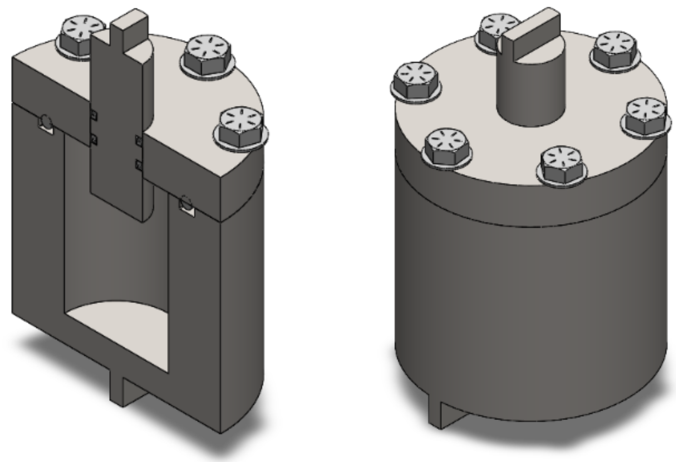

Figure 19: Updated Single-Vessel Design

Body

The ASME standards for pressure vessel thickness, found in the ASME Boiler and Pressure Vessel Code Section VIII: Rules for Construction of Pressure Vessels (2021) standards [30] were used to calculate the necessary shell thickness to obtain a more precise and corroborating answer. The equations in UG-27(c) were used to calculate a minimum thickness of 0.11”, assuming a maximum tensile stress of 19 ksi and outer diameter of 2.9”.

Lid

This was the phase in the design process in which the lid type was chosen. The mandatory functionality was that it would not leak or break, that the plunger could be inserted through the middle, and that it could be opened and closed. Once that was met, the priority was to design the lid to be opened and closed as quickly as possible. The lid or hinging mechanisms discussed in research such as the hatch would have added weight and significant complexity to the lid. These alternatives were also more difficult to attach (requiring welding), and their strength was much more difficult to quantify. Bolts and threaded holes were easily manufactured and their strength could be confidently calculated.

The lid was to be bolted into a flange wrapped around the shell of the pressure vessel. As such, both lid thickness, bolt size, bolt durability under fatigue loading, and bolt pretension were calculated. A pressure vessel manufacturer was contacted to determine the best way to calculate lid thickness and ensure the vessel would not fail. The website called PV.eng was recommended, which uses ASME specifications to calculate given dimensions. This website had a calculation for flat lid thickness, and was the basis for the initial lid thickness estimation of greater than 0.5”. This thickness was validated using FEA.

The initial design used 4 ¼-20 grade 8.9 bolts. The safety factor for axial fatigue loading was calculated in the bolts using the frustum method. The safety factor was just under 1, so the bolts were upgraded to ⅜-16, achieving a safety factor of 1.3. It was then decided to use six bolts and to extend their length to account for a thicker lid, raising the safety factor of bolt failure to over 2.

In a discussion with an expert from a pressure vessel fabrication company, it was recommended that socket head bolts be used in place of hex bolts or to incorporate washers with the hex bolts to avoid damaging the lid surface. Due to the lack of availability for the socket head bolts at the sizes needed, hex heads were kept in the design, but washers were incorporated as suggested.

Seals

Following investigation into hydraulic systems and discussions with experts in hydraulic cylinder design, plans to incorporate a stuffing box in the design of the plunger mechanism were foregone in favor of reciprocating seals typically found in hydraulic systems. Reciprocating seals in hydraulic systems, such as piston seals, are relatively inexpensive and much less complex than mechanisms like a stuffing box. The sealing mechanisms chosen for Design 3 were piston seals placed in grooves on the plunger rod itself, as well as an O-ring placed in a groove between the lid and the body. The team anticipated further research to determine whether using seals from the dynamic component or the static components of a standard hydraulic system would be more appropriate for this application.

The pressure ratings and operating characteristics on rod-end seals used for light duty cylinders encompasses the application of the plunger for the pressure vessel. Incorporating all of the aforementioned rod-end seals creates the desirable operating conditions inside the vessel by preventing inherent problems such as pressure spikes, pressure leakage, metal to metal contact, or contamination in the vessel content.

Temperature Control

While buying a water bath off the shelf proved infeasible because anything below the vessel would be crushed by the Instron, work began on designing one to be built from a cheap steel pot. The intended method was to lathe out the bottom of a large pot, weld it around the pressure vessel, fit it with a heating coil and temperature gauge, then fill it with water while on the machine. This method seemed feasible until it was realized that gauges had to be mounted to the side of the vessel, which would be impossible to see from the inside of a pot. The upside to this realization was that a temperature sensor could be added without much additional effort, due to already mounting other sensors in the pressurized system.

The next solution was the heating coil, which would allow force to be sent straight through the vessel and forego the need for an additional support like in the case of the water bath. With the heating coils the team would also need to implement a temperature sensor as well as a controller for the heater and temperature sensor. The temperature sensor would need to be accurate to 0.5 °C and be able to withstand 10 MPa. The design would also have to incorporate a hole and seal in the vessel for the temperature sensor to connect to the controller. This logic would be fairly straightforward and the power is low enough so that an Arduino could be used. The entire vessel would then be insulated so that the user could hold the vessel without making direct contact with the coil. A simulation of this design done in SOLIDWORKS, shown in Figure 20 showed that a coil applying 5 Watts to an insulated vessel provided an appropriate thermal distribution at the required temperature.

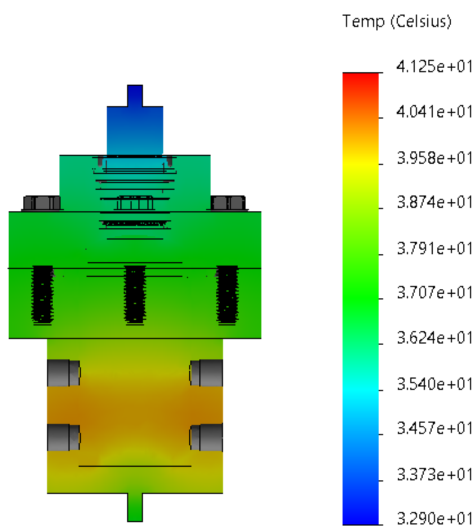

Figure 20: Thermal Distribution from Heating Coil

This design phase resulted in a choice of lid, a disc attached with bolts to the vessel's shell. Significant progress was made into designing the seals around the piston and between the lid and the shell. In addition, a temperature control system was determined, and additional calculations were made to determine a minimum vessel shell thickness.

Final Design

The significant changes made in Design 4, shown in Figure 21, are a result of updated calculations made for the bolts on the lid, the vessel size and wall thickness, the lid thickness, added NPT holes, and use of rod seals. Similarly, considerations regarding the fabrication of this device along with incorporating components such as sensors and controls required changes to Design 3. The following subsections will dive into each change in the design components.

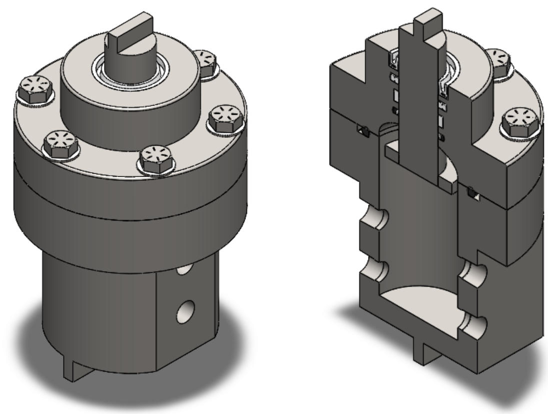

Figure 21: Design 4: Final Design

<u>Body</u>

After it was deemed critical to have access into the vessel for gauges and sensors, four standard ¼”NPT holes were added to the body. Previous thickness calculations did not account for the stress concentration created by the NPT holes. It was conservatively assumed that the stress concentration around the holes could at worst increase the stress by a factor of three. Therefore, the new maximum was calculated as .33”. This gave a safety factor of 1.7. This modification for stress concentration was not part of the ASME calculations, but was an addition the group deemed prudent for the the second minimum shell thickness calculation. The outer diameter of the lid and the vessel body was increased from 4 to 4.5 inches to add more thickness to the walls around the bolts and reduce the stress concentration at the bolts. Subsequently, in order to reduce the weight of the device, the body section of the vessel underneath the bolts was trimmed down to a diameter of 3.4 inches.

Another section of the ASME code, UG-23, specifies the shell thickness of a pressure vessel. The calculation performed found that the wall thickness of the bioreactor, being 2.3 times thicker than the required thickness, yielded a safety factor of 2.3. Having designed our pressure vessel in line with standards from ASME, we were confident that our vessel would not fail due to the shell thickness being too small. The last corroborating analysis was done by putting the final CAD model through an FEA analysis with hydrostatic loading at 10 MPa applied to all inner walls of the vessel. As the bioreactor is unique in its shape and does not fit the standard calculations made for pressure vessels, the FEA proved to be crucial to finding these stresses occurs.The von Mises stress plots shown in Figure 22 indicated that the maximum von Mises stress of 41.29 MPa occurs at the inner edge of the o-ring groove on the vessel body. Overall, these plots demonstrate that in its current dimensions, about 9/16" thick, no parts of the tank would exceed the maximum allowable pressure of 19 ksi (130 MPa). Together, these four types of analyses demonstrate that the vessel wall thickness is sufficient and will not fail during operation.

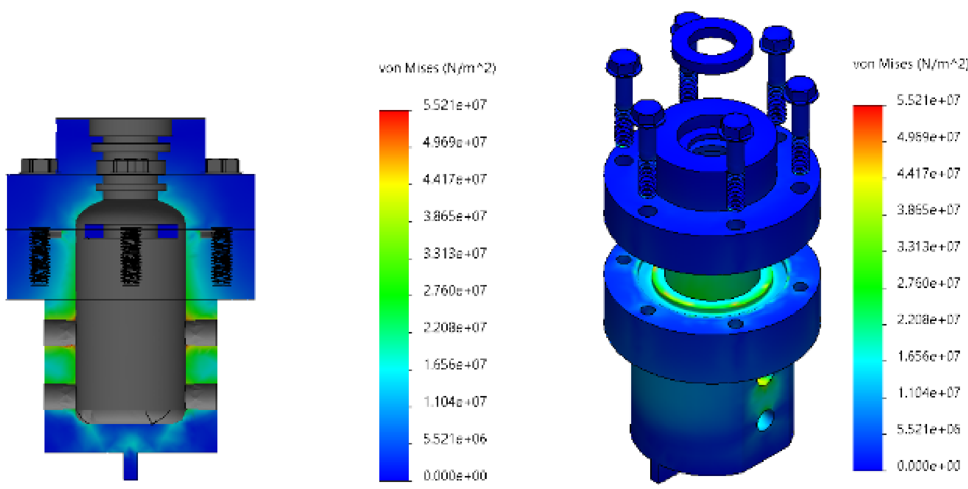

Figure 22: FEA Section Plots, Front (Left) and Isometric (Right) View

One notable feature of the body in this iteration is that two sides are designed flat in order to accommodate various sensors into the design. These flat edges have NPT threads machined into them, a standard tapping to make the vessel able to interface with a variety of high-pressure sensors.

<u>Lid</u>

For this design, the lid thickness was increased from 1 to 2 inches in order to incorporate grooves that will house the rod-end seals for the plunger mechanism and reinforce the lid around the hole. The section of the lid surrounding the seal grooves were also trimmed down to 2 inches to reduce the overall weight of the device. ASME Chapter VIII pressure vessel standards section UG-34 was used to find the thickness of the lid, then section UG-39 was used to find the area of reinforcement necessary to address the stress concentration surrounding the hole for the plunger. The lid thickness calculation yielded a safety factor of 1.9. The reinforcement calculation gave a safety factor of 6.1. These calculations, along with the FEA analysis in Figure 22, were enough to be confident that the lid was of sufficient thickness not to fail.

Seals

The investigations into hydraulic systems and the subsequent discussions held with experts in hydraulic cylinders and seals manufacturing led to the decision of using rod-end seals for the plunger mechanism in the final design, as shown in Figure 23. Compared to the piston seals chosen in design 2--which were placed on the plunger (the dynamic component of the plunger mechanism)--the rod-end seals are housed in grooves made in the lid. This sealing mechanism mimics that of rod-end seals found in hydraulic cylinders, which are designed to always stay in touch with the static and dynamic components of the vessel. To select the appropriate rod-end seals, as discussed in the Background Research section, properties such as operating pressure, temperature range, speed, fluid compatibility, and groove dimensions must also be taken into account. However, of these, the most important will be to determine the seals' material for fluid compatibility. Because hydraulic cylinders are designed to work with a particular operating fluid, most often oil, seals are designed to utilize the system's same operating fluid as lubrication. Since the pressure vessel will use water, a poor lubricant, as the sole operating fluid, the seals' material compatibility must satisfy a low friction requirement. Because the bioreactor content cannot be contaminated with another lubricating fluid, the seal materials must allow the plunger to operate in a non-lubricated environment, otherwise known as the dry run condition. Based on this factor, the seals chosen for this design are made of bronze filled PTFE which has a low coefficient of friction and are designed to withstand dry runs at high frequency [31].

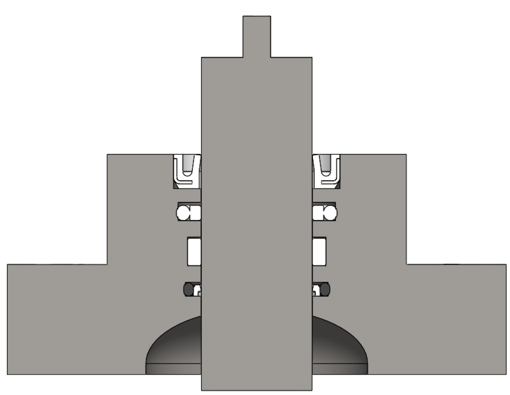

Figure 23: Lid and Plunger Seals

Temperature Control and Sensors

Having decided on a resistive heating element, a pipe heater was purchased with its own temperature control system. Once it and the pressure vessel was received days before Capstone Day, it was tested, only to find that the temperature control system did not work as believed, and couldn't be adapted to heat the vessel to within the required range. A new heating system was quickly developed. With the option of using NPT fittings to collect sensor data from inside the vessel, it was possible to place a pressure-resistant thermistor inside the vessel and wrap a resistive heating coil tightly around the outside to heat the water to a uniform temperature. The temperature would be read from the thermistor and controlled via an Arduino and a solid state relay to maintain a steady internal temperature. This was then wrapped in an insulating element to prevent heat loss, a custom, hand-crafted (purple) crocheted sleeve created by Damon Artis.

In choosing a release valve and pressure gauge, it was most convenient for them to have the same NPT fitting. Thus, a Swagelok pressure gauge and Swagelok release valve were picked, both having a MNPT-¼" threaded fitting. The group was assured by the manufacturer, Swagelok, that wrapping the NPT threads in Teflon tape and torquing them into the tapped holes will be enough to prevent leaking.

The relief valve is purely mechanical and the upper cap can be twisted to the target pressure and calibrated to the pressure gauge. Should pressure exceed the preselected threshold, the exhaust port on the right will open and release excess pressure from the system. Because of this, the group must ensure the valve is aimed at an area that can take this pressure or fit a hose to transfer it elsewhere.

It was decided that one of the holes of the pressure vessel would be used as the inlet for filling the vessel with water so water level in the vessel could be topped off precisely. This is necessary to ensure the piston does not have to move far or bottom out when oscillating. The NPT hole was to be filled with an NPT cap. During testing, NPT caps were observed to leak, and upon further research, it was learned that NPT caps can only be taken out and reinserted two to three times because their threads are deformed in the process. This led us to modify the design and decide to have an inlet valve that would be permanently inserted into the vessel.

Cell Culture Bag

The 5mL Sartorius Flexboy Bag, shown in Figure 24, was the first bag to be tested. The group went with the 5mL bag to limit budget usage and limit cell culture medium use. Initial testing of the 5mL Flexboy bag was performed by Professor Kathryn Marie Yammine at the MIT lab. She performed a quick cell culture test to determine the ease of use and capability of the bag. Her work is shown in Figure 24, where she can be seen holding the bag with cell growth medium (the red fluid) and chondrocytes (the white dots in the red fluid) inside.

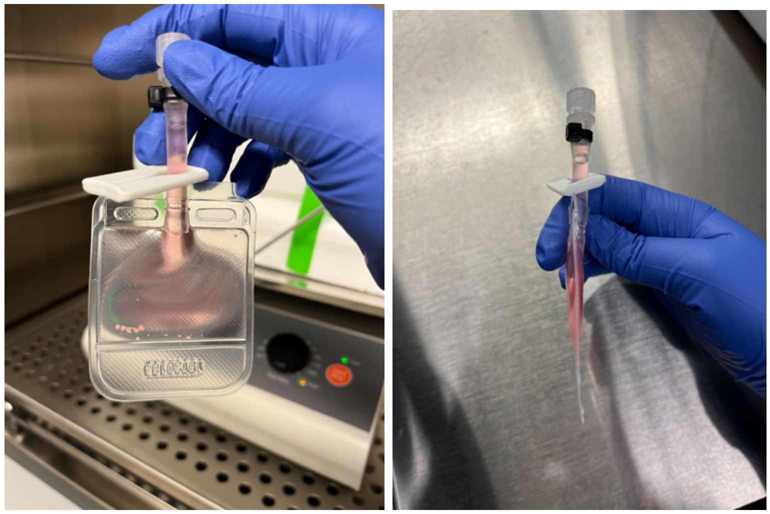

Figure 24: 5mL Flexboy Bag Being Utilized to Culture Cells in the MIT Lab

Professor Yammine reported that the bag was easy to use as she was able to successfully add cells and medium to the bag as well as change the medium, and that the Luer-Lock system made this process easier, allowing her to use the bag alongside the syringe. However, Professor Yammine stated that the bag was thin, resulting in the chondrocytes not moving around when placed on an orbital mixer. A possible solution for this would be to switch to an end-over-end mixer to fully invert the bag to ensure the chondrocytes move around. Moving the chondrocytes around is important to ensure that the cells do not stick together and eventually merge into a bigger cell during growing phases. In addition, she stated that some cell culture medium would spill out occasionally when releasing the syringe. This issue can be addressed by adding more air into the bag, which is acceptable since the stem of the bag is rigid and less susceptible to compression.

Execution of Final Product

After CAD models were finalized, the files were sent to Xometry, a third-party machining specialist, for manufacturing. This incurred significant costs, due to the need for expedited lead times, but resulted in a well-made product that met all of the team's requirements. Other components were confirmed to fit inside the CAD model, then ordered and assembled by the team. A full image of the completed product may be seen in Figure 25 below.

Figure 25: Final Product Under Pressure

<u>Material Sourcing</u>

Material for both the vessel and lid was left to be sourced by Xometry. Most other materials were sourced from McMaster-Carr, with the exception of the solid state relay and thermistor, which were sourced from Amazon and Grainger, respectively.

<u>Machining</u>

While the Capstone lab had the means to machine this vessel, it was unable to be made in-house due to logistical issues. Machining of the vessel and lid were instead outsourced to Xometry. The plunger was made by the capstone lab, as it was a relatively simple job. All parts of the device were designed for manufacturing easily on a lathe and CNC router.

Final Capabilities

It was learned two weeks prior to Capstone Day that the Instron force machine, meant to be used for the full cyclic loading tests, broke. There is not a machine capable of applying the necessary cyclic pressure on campus so the full cyclic load test of the device at 1500 psi will depend on fixing or replacing this machine. There is still work to be done in the meantime. The manufactured vessel was received a week before Capstone Day, and the pressure gauge was inserted into an NPT hole, while the other three holes were filled with NPT caps. The vessel was filled with water and pressure tested to 1500 psi using an arbor press. The vessel remained fully intact and the seals did not leak. However, the NPT fittings did. This was not a major concern, as they will be replaced with the thermistor, pressure release valve (which currently lacks a component to make it operational), and inlet valve. Furthermore, the intended temperature control system incorporating the thermistor, Arduino, and solid state relay was not fully made. Due to time constraints, a safe and lasting circuit could not be built in time. However, the group is confident that this temperature control design will work once made. In sum, the vessel body, lid, fasteners, and seals successfully held pressure, while the accessories for sensing, safety, and temperature control were not able to be implemented in time. Once finished, and with the Instron force machine fixed or replaced, testing of the device at full cyclic load must be conducted.

4 Discussion and Conclusions

The largest obstacle in this project was the high pressure specification. None of the group members had worked in high pressure systems, but the success of the seals and plunger mechanism has inspired the confidence that we are capable of taking on new challenges and learning something we are completely unfamiliar with.

With regards to safety, specifically the lid and tank design, the team had to determine how to not only perform calculations, but be confident that the calculations were accurately describing the design. The team had to be confident that when a calculation said the vessel had to be a certain thickness, that the value could be trusted beyond doubt. Calculations began with simple equations learned in class, verified with FEA, and then back with calculations based on ASME pressure vessel standards. With all calculations giving similar results, the team is confident that the chosen thicknesses will be sufficient.

Throughout the design process, the team worked to understand how pressure vessels are designed, manufactured, and tested. Safety has been a primary concern since the beginning of this process and it was critical to have insights into what makes pressure vessels safe. Designing the plunger mechanism was the other major concern throughout the design iterations, as pressure vessels with plunger designs like these were not commercially available or commonly built. Getting feedback and suggestions from experts in hydraulic systems, seals, and pressure vessel fabrication was an important factor in determining how such a system can be built and operate as intended.

While the vessel has succeeded in holding pressure, it must still be made to maintain constant temperature. This is a relatively straightforward task that involves building the circuit relay and calibration of sensors, and will be completed by some members of this team who are continuing with this project in the coming semester. The following steps for this device will be to use it for culturing cartilage cells under pressure, which will also be performed by these team members in Professor Shefelbine's lab when the Instron is fixed. All relevant hand-off documents are being written to ensure none of the hard work this team did is lost. Professor Shefelbine is happy with the current state of this project and the team is more than satisfied with the results.

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