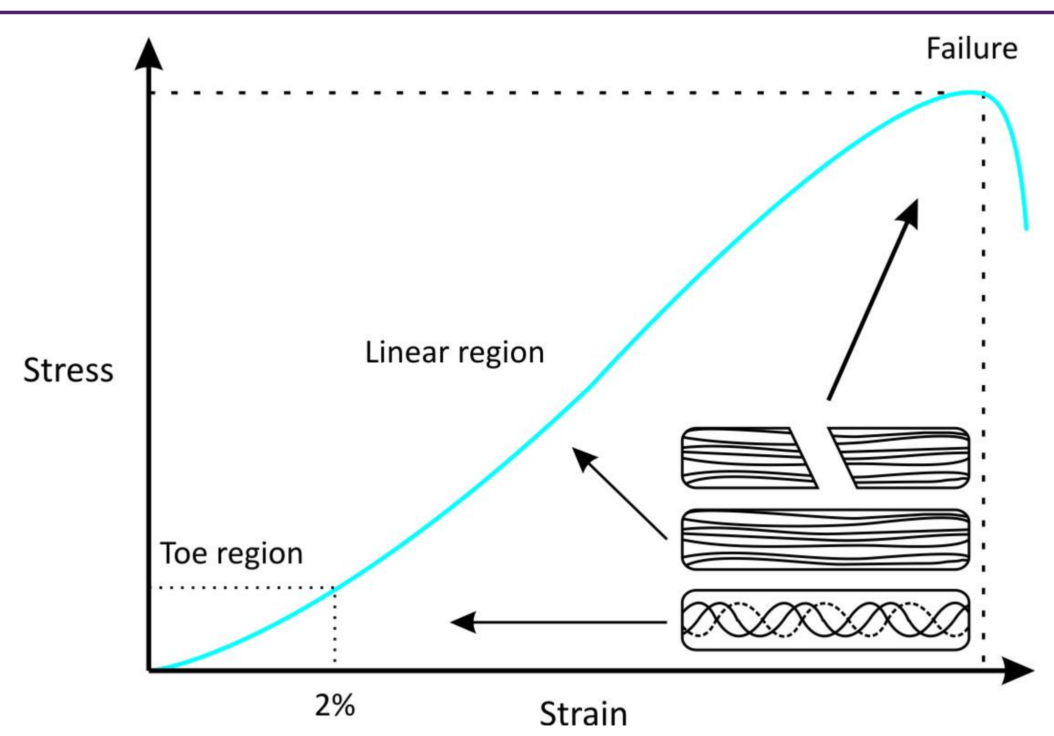
# Low-Cost, Reusable Bioreactor

Dillon Kayl, Sarah Saffer, Indara Garcia, and J'alyc Southern

## Project Description

A bioreactor mimics the natural cell environment to produce cell growth, and in this case, the focus will be on tendon cells. This particular bioreactor keeps a complete sterile field while maintaining a specified temperature, pH, and dissolved oxygen level, all while inducing biaxial loading onto a cell scaffold. It was found that tendons can only stretch up to 10% before yielding, therefore, the customized linear actuators were designed with a capacity to stretch the scaffold support up to 10% of the tendon's original length. The sterile field is maintained by keeping the tank completely sealed off from the outside environment. This was accomplished by welding around all of the valves and spargers that enter into the bioreactor. A reinforced glass window was attached with a silicone seal and enough pressure to completely seal it in. The temperature, pH, and dissolved oxygen levels are maintained by using autoclavable sensors and work off an Arduino, which is our designated power supply. The material used for this bioreactor is stainless steel due to its ability to withstand the autoclaving temperature of 121 degrees Celsius, its biocompatibility, and corrosion resistance so it will not harm the tendon tissues. Furthermore, this bioreactor is low cost because the entire manufacturing process cost less than $4,000, and therefore, the bioreactor can be sold at an affordable price. Ultimately, this bioreactor is reusable. Sterilization will occur by autoclaving after each use so the user can use this bioreactor multiple times.




Tendon Mechanical Properties Taken from "Tendon Biomechanics"

## Project Justification

Bioreactors are very high precision stainless steel and glass apparatus with specialized control systems, which are costly to both purchase and maintain. There is a new market quickly filling with low cost, single use bioreactors, but these lack customizability, scalability, and have low oxygen transfer coefficients. The solution to this problem is a state-of-the-art design for a low-cost, reusable bioreactor. This bioreactor will be able to be reused by being able to withstand the autoclaving process. Many bioreactors are stirred tank or rotating wall bioreactors, but this is a novel design that will induce biaxial loading on the cells by stretching the scaffold in which they reside on. The tank conditions will be completely customizable by the user based on what cells they are growing. It will be a fully autonomous machine by regulating temperature, pH, load, and oxygen levels. The main market is going to be aimed towards labs and universities. It was found that the bioreactor market is estimated to grow 15.5% between the years of 2019 and 2024.

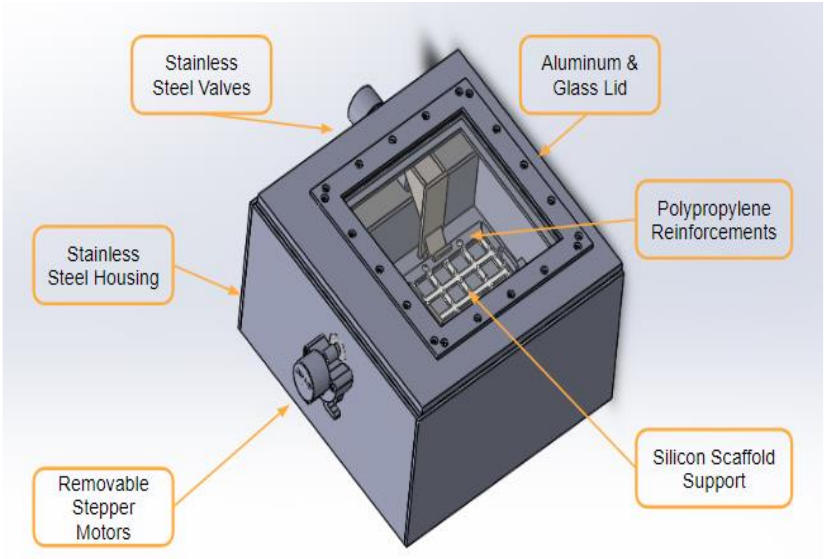
## Project Specifications and Standards

| User Requirements | Engineering Specification | Justification |
|---|---|---|
| Can be sterilized | ❖ Made up of material and seals that can withstand an autoclaving process of up to 60-90 minutes at 121◦C<br>❖ Dimensions of 9 x 9 x 4.5 inches | Specific dimensions and material properties will allow the bioreactor to withstand the autoclaving process. |
| Ability to hold a sterile field | ❖ Air-tight lid and valves for linear actuators, inlets/outlets.<br>❖ Syringe valve near scaffold support | With an air-tight environment, no bacteria will enter the bioreactor and compromise the sterile cell field throughout the entirety of the cell loading and growing process. |
| Biaxial loading | ❖ Load forces stretching up to 8% of tendon's original length in biaxial directions with actuators<br>❖ Flexible scaffold support | Biaxial loading can be achieved if load forces are created through actuators for loading and a plate/plunger system for compression. |
| Easy Monitoring | ❖ Clear viewing window<br>❖ Probes and user interface for monitoring | Clear material will allow the user to see cell growth/generation, and the sensors could display culture conditions for ease of monitoring. |
| Autonomous temperature control | ❖ Regulate environmental temperature within ±2ºC | Heating element, along with temperature sensor and Arduino, will control the temperature within the specified range set by the user. |

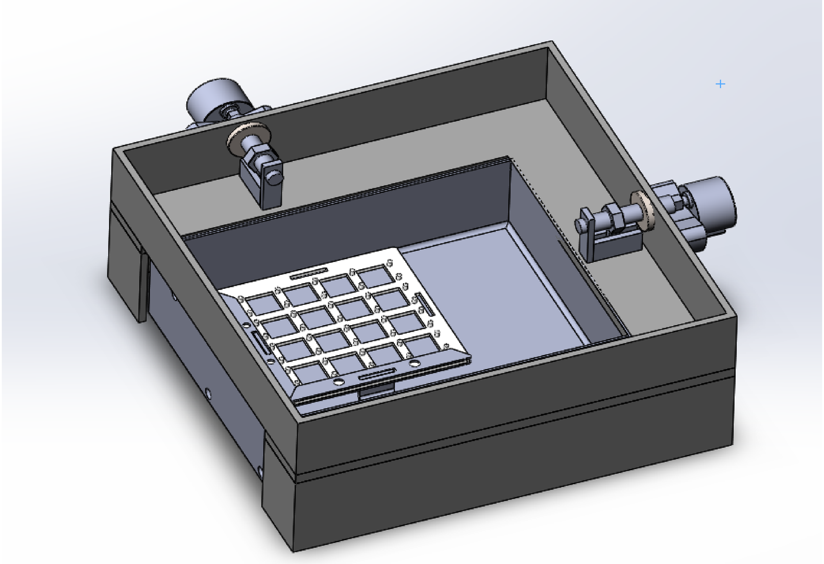
| Engineering Standard | Description | How Does it Apply? | How will it be Implemented? |
|---|---|---|---|
| ISO-11737-2 | Evaluates sterility of medical devices that have been exposed to a treatment with the sterilizing agent to ensure that the device is sterile. | The bioreactor needs to maintain a sterile cell field to ensure it is free from viable microorganisms. | An agar test will be conducted to verify that there is no bacterial growth |
| ISO-10993 | Entails a series of standards for evaluating the biocompatibility of medical devices. | The scaffold support must be made from a biocompatible material as it will be in contact with living cells. | An Agarose overlay test will be implemented. |
| ASTM F2315 - 18 | A list to properly characterize, assess, and ensure consistency in the performance of an encapsulation system using alginate. | This bioreactor will immobilize and encapsulate living cells or tissue in an alginate gel. | This bioreactor will be capable to run this test, but that is out of the scope of this project. |
| IEC 60364-4-41 | A parameter that requires there being some form of protection against electrical shock | The bioreactor is run by an outside power source. | It will be tested by running the machine for several minutes. |
| IEC 60364-4-42 | A parameter around thermal effects that can occur when mechanical devices are left running. | This bioreactor will be running for long periods of time. | Heat dissipation will be observed over time. |
| IEEE 2700-2017 | A common framework for sensor performance. | The bioreactor has three sensors: Oxygen, pH, and temperature. | A test run of the sensor's capabilities. |

## Design Process

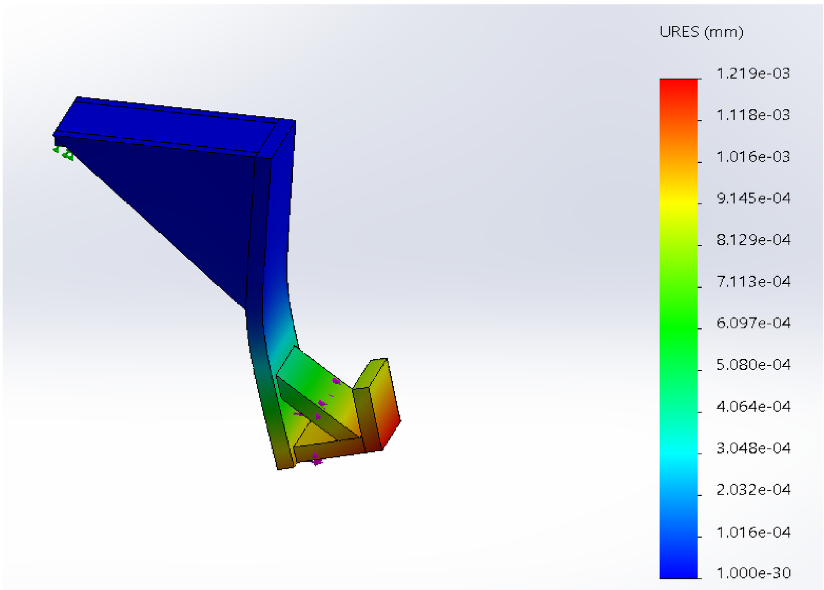
The process for developing the design specifications for the bioreactor began with customer feedback. The functional constraints determined that it needs to regulate the enclosed environmental conditions such as temperature, pH, and dissolved oxygen levels, it needs to induce biaxial loading on tendon cells to ensure proper growth and functionality, and it also needs to maintain a sterile environment. Probes and sensors were incorporated into the tank design with air-tight seals so that the user can set the upper and lower parameters and monitor real time conditions inside of the tank. To create biaxial loading, the design consists of a flexible material that will support the cell scaffold and be stretched in small increments with custom linear actuators controlled by an Arduino. To achieve a sterile environment, stainless steel was chosen as the material for the tank and aluminum was chosen for the lid, because it is lighter and cheaper. These materials were deemed to meet the constraint due to their high melting points and biocompatibility. Modern engineering tools, such as 3D printers and a CNC laser cutter, were essential and applied to the development of the design. As stated above, the bioreactor required custom fittings and attachments for the probes, sensors, and the linear actuators. It was decided that this type of additive manufacturing would be more reasonable for these applications rather than traditional manufacturing, because it would be much harder to get precise measurements. All components were not only designed on a computer-based program, but their geometries and structural integrities were evaluated using simulated conditions as well. A stress analysis of the scaffold support was used to ensure that a uniform stretch would be induced across the structure, and it was found that plastic attachments had to be made to increase rigidity at the outer edges. Finally, a thermal study on the assembly was completed to ensure that the design was optimal for autoclaving.



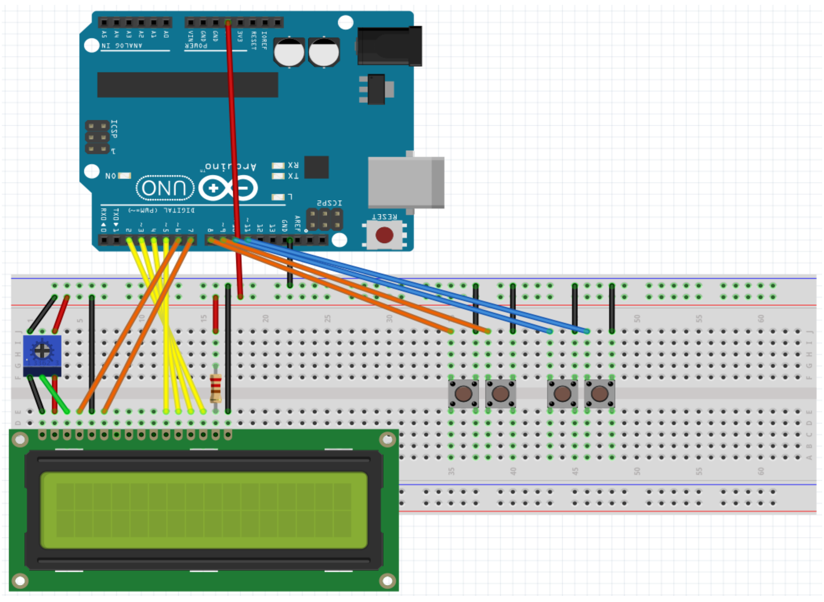

CAD Model of Bioreactor Assembly



CAD Model of Bioreactor Media Bed



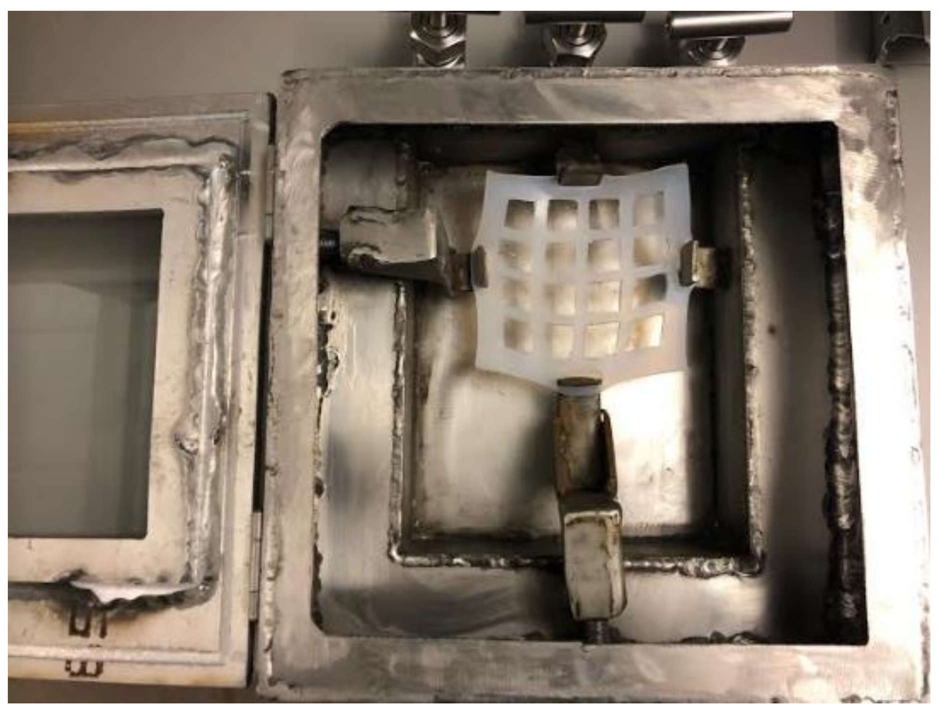

Stress Analysis on Actuator Attachment

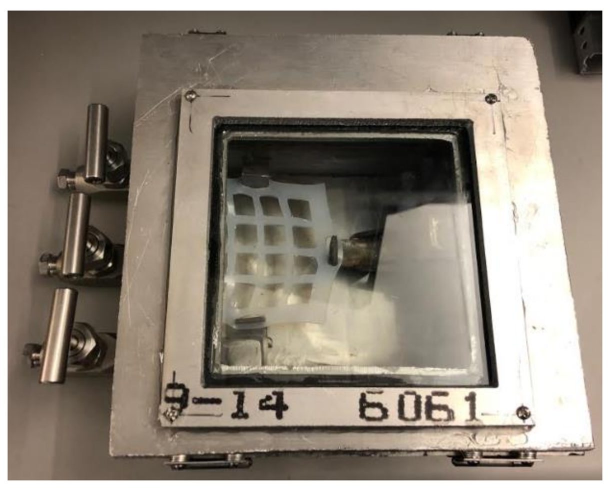


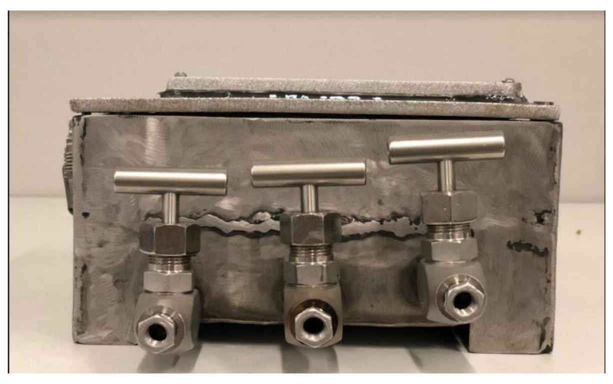
Schematic of LCD Circuitry

## Final Product and Results

The main source of data in this project came from simulations performed using SOLIDWORKS. These simulations allowed the team to access the stretch that the scaffold support, coupled with the linear actuator, would provide on the specimen. These results showed that the scaffold support needed reinforcements around the edges in order to provide an even stretch to the specimen. The stress analysis on the actuator attachment confirmed that they were rigid enough to withstand the loads from the scaffold support. The rest of the data would come from the verification process which the team was not able to accomplish this semester. It was expected that the bioreactor would withstand the autoclaving process and pass the sterility test with no sign of bacterial growth to ensure it was free of microorganisms throughout the entirety of the cell loading and growing process.





Final Bioreactor Prototype

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