Biaxial Loading Mechanobioreactor

Design Team
Abdulrahman Aba-Alkhail
Michael Dayton, Daniel Esposito
Anh Nguyen, Michael Tiene

Design Advisor
Prof. Jeffrey Ruberti

Abstract
Current biaxial mechanobioreactors provide mechanical stimulation over a period of time to a cell culture but lack the ability for microscope observation. Many also employ large fluid environments to grow and observe the cell-populated substrate, which is undesirable due to the high cost of perfusion fluids as well as the even higher cost of live cell stains, used to properly analyze changes in cell growth. To improve research on biaxially strained cell samples we have designed and constructed a bioreactor that has a working fluid volume of less than 2mL, a short working distance of 200 microns or less, a clear optical access to allow for high powered microscopy, and can apply up to 100% equibiaxial strain to a cell-populated substrate 6mm in diameter. This bioreactor includes a heating system and perfusion ports to ensure observation for up to two weeks. It employs a power screw mechanism with magnetic coupling to strain the cell-populated substrate without any moving seals. This biaxial mechanobioreactor allows a sample to be equibiaxially strained and observed over long periods of time with a very small fluid volume, which has never been examined previously.
The Need for Project

The emerging field of mechanobiology blends principles from molecular biology and mechanics to create a field of study based on the interaction between cells and their in vivo mechanical forces. The process by which this occurs is called mechanotransduction. While medical researchers in the past have targeted the genetic causes of a disease, research is shifting towards studying mechanotransduction, and the role it plays in the development of diseases.

The goal of this project is to develop a biaxial loading mechanobioreactor for high magnification imaging of cells, in real-time, and placed under a controlled equibiaxial strain. This product will allow researchers to apply a uniform biaxial load to a sample, culture that sample for a period of up to two weeks, and observe the sample in real time under a high magnification microscope.

The Design Project Objectives and Requirements

The design goal is to create an equibiaxial loading mechanobioreactor for high magnification imaging of controllable strained cells.

**Design Objectives**

The bioreactor design has two main critical success factors. First and foremost, the sample must be in an environment conducive to cell growth for periods of up to two weeks. Secondly, the design must be able to equibiaxially strain the sample, all while maintaining a working fluid volume of less than 2ml. If both parameters are met, the design must be considered a success.

**Design Requirements**

- **Controllable Biaxial Strain**
  
  The sample must be uniformly strained up to 100% within an accuracy of .25%. This is to ensure that we are able to precisely strain our sample under the desired loading conditions.

- **Direct Optical Access**
  
  The design of the bioreactor must maintain direct optical access to be compatible with a Nikon TE3000E 60x oil emersion microscope with a PFS stage and a short working distance of 200μm. This is the microscope that EMERL research laboratory uses, and our design must be compatible.

- **Sterile Culture Conditions for Periods of Up to 2 Weeks**
  
  Maintaining sterile culture conditions is of the utmost importance. The design must maintain sterility because if the sample is contaminated during an experiment, any data accrued will be compromised. This also means that the bioreactor must be kept at a temperature of 37°C +/- 1°C. The design must also include perfusion ports capable of delivering 400µl/hr of nutrient solution.

- **Working Volume ≤2ml**
  
  The dyes and perfusion fluids that are used within the bioreactor are very expensive. Minimizing the working volume (or the volume of fluid space within the design), will allow the experiments to be run cheaper and more efficiently. This will also increase the appeal and marketability of the product.

Design Concepts Considered

Alternative design concepts considered different actuating mechanisms to displace the grippers and strain the sample. Magnetic actuation was a focal point of the design because it would not require the moving seals that are associated with direct linear actuation of the sample grippers. Having a completely enclosed internal chamber reduced the risk of cell culture contamination and...
Electromagnet Design

Initially a large electromagnet was chosen to apply a pulling force from the exterior of the bioreactor to ferromagnetic material embedded in the gripper. The electromagnet was not used because it was unable to provide a constant force and reduced light available to the microscope.

Actuating Permanent Magnets Design

Another concept considered was the displacement of permanent magnets connected to an actuating beam on the exterior of the bioreactor. Having the magnets mounted to a beam located a distance away from the surface of the bioreactor improved the field of view and the light available to the microscope. The magnetic field of the magnets decreased exponentially from the surface of the magnet. When in contact, the magnets can exert strong pulling forces, but the force decreases significantly only millimeters away from the surface. To obtain the force required to displace the grippers, additional ferromagnetic material would be required in the system, which would increase fluid volume and surpass the maximum limit.

Recommended Design Concept

The recommended design concept uses a Power Screw Mechanism driven by a stepper motor and magnets coupled together through the wall of the bioreactor.

Design Description

The bioreactor consists of two main systems. The first consists of cartridge heaters, thermocouples, and fluid perfusion ports to ensure the cell culture is thriving in the proper environment of 37°C with plenty of nutrients. The second system consists of the power screw, magnetic couple, and glass strain piece. The system uses the magnetic couple to transfer torque into the reactor without the use of moving seals. The power screw then transforms rotary motion to linear motion. Since the glass strain piece remains stationary and the sample is attached to grips, the cells are forced to be equibiaxially strained across the bottom of the strain piece so it can be easily observed by a high powered microscope.

For the force/strain component, the design will have a stepper motor that rotates, spinning an adjacent gear attached to a driver. This driver has magnets embedded within it, which are coupled to identical magnets located on the opposite side of the glass strain piece (the glass strain piece is fixed in place and does not rotate). These identical magnets are embedded within the threaded chamber piece. When the stepper and gear rotate, the force is transferred to the threaded chamber piece which has a power screw thread on its inside diameter. The grip assembly is threaded on the outside diameter to fit perfectly into the threaded chamber piece. When the stepper motor is turned, it results in the movement of the grips along the center axis of the chamber, displacing them and stretching the sample (which is clamped between the grips) over the strain ring (see figure on the next page). By providing extra room for the grips to displace upward, the bioreactor has the ability to strain the sample to more than 100% strain, exceeding the design requirement.

To maintain cell growth, the design has a heating and fluid perfusion system. As was previously mentioned, the internal chamber has a fluid volume of .844ml and is made of 316L stainless steel. This fluid volume is well below the required fluid volume of the chamber.
The fluid perfusion system, which circulates a nutrient solution at 400μ-liters/hr, is integrated into the bioreactor chamber through two tubes, while the heating mechanism surrounds the chamber to keep the sample well within the required temperature. The internal chamber also houses the strain mechanism.

**Analytical Investigations**

In order for cornea cells to grow, they need to be in a sterile environment maintained around body temperature (approximately 37°C). A typical method to obtain consistent heat control is to use cartridge heaters. Four eighth inch Watlow Cartridge heaters were chosen to heat the chamber of the bioreactor design. These heaters were chosen because of their high watt density, ease of use, and integration. An initial hand calculation was performed to estimate the time for the system to heat to 310K. Using $Q=mc\Delta T$, the preliminary calculation estimated a time to heat of less than three minutes.

The design was modeled in a FEA program and the appropriate boundary conditions were applied. Using a thermal contact resistance of ~1E-4 m²K/W for stainless steel to copper, ~5E-4 m²K/W for stainless steel to stainless steel, ~15E-4 m²K/W for stainless steel to glass, and ~30E-4 m²K/W for copper to polycarbonate. A free air convection coefficient of ~10 W/m²K was also applied to the entire design. The simulation was run with the cartridge heaters at 50% power. The time calculated to heat up the bioreactor to 310K was approximately 2 minutes. Since the heat distribution was uneven, the simulation was repeated with the cartridge heaters at 8% power. This simulation proved that even heating could be achieved within approximately 10 minutes.

In order to prove 100% biaxial strain was achievable, the design was modeled once again in a FEA program. The appropriate degrees of freedom were applied to our sample and the maximum thickness of the material was assumed (500μm), as well as the maximum elastic modulus (1Mpa). The simulation showed that even with our over estimations, the design is able to produce 100% biaxial strain.

**Experimental Investigations**

Introducing the neodymium magnets into the bioreactor chamber has increased the proximity of the tissue sample to the magnetic field, thus increasing the likelihood of undesirable affects during cell growth. The manufacturer provides magnetic field data of each magnet in free space.

To further investigate the effects of the magnetic field on the samples, a gaussmeter was used to measure the field at the minimum distance between the tissue sample and the magnets. The gaussmeter confirmed that the maximum field seen by the sample at such a distance was less than 500mT. This is well below .5T (the field shown to influence cell mechanics).

**Key Advantages of Recommended Concept**

Each system and mechanism was chosen after a thorough testing and analysis. The design concept had several key advantages, such as:

- Applying a uniform constant force on the sample
- Ensuring a closed system
- Ease of installation and operation
- Minimal fluid volume
- Accuracy

The recommended design concept strives to meet the specifications in the simplest, most effective and economical way.
Financial Issues

The benefits of having a biaxial mechanobioreactor far outweigh the monetary cost. There was a strong and consistent effort to reduce cost at every stage of the design. Custom parts were designed to be easily manufactured, and compatible with off-the-shelf products.

Although this project did not start with a budget, it was known that decreasing the cost of the reactor would greatly increase its marketability to research labs. Due to the size and custom design of the drive system, the largest cost during manufacturing is part machining. To reduce this cost, special attention was paid to the manufacturability of each part, and the overall design.

To further reduce the cost of the final product select sensing equipment was removed from the base design while still allowing reintroduction later. This reduced the base price of the reactor without limiting its uses. If the additional sensors are required, they can be easily integrated without any drawbacks. Additional sensors include Fiber Bragg Grating strain elements to calculate force on the sample and optical distance sensors to calculate sample strain.

The overall cost to Northeastern will be slightly more than the product would cost if sold commercially. This is mostly due to the fact that each part purchase and manufacture is done on a one-off basis. As orders for units increase the overall sales price will reduce even further as multiples of parts are much cheaper to purchase and machine since the tooling has already been fabricated.

Recommended Improvements

Aspects of the design that were limited by lead times, but could easily be incorporated into future revisions include sensors, an adjustable bottom slide, bearings, and better magnetic coupling.

Key areas that could be improved with additional time primarily focus on the magnetic coupling and the addition of a bearing. A magnetic coupling with trapezoidal magnets would optimize the amount of transferred torque by increasing the available magnetic area. Bearings would greatly reduce the thrust and radial friction, which increases the amount of torque available to the power screw. However due to material selection both of parts have lead times exceeding the time frame of this project. Other possible areas of improvement include the addition of an adjustable bottom slide to accommodate for different sample sizes and more direct sensing equipment to get a better understanding of the forces and strain experienced by the sample.