# NOVEL BIOREACTOR DESIGN:
# DEVELOPMENT OF A BIOARTIFICAL LIVER FOR MULTIPLEXING WITH SERIAL ORGAN SYSTEMS FOR PHARMACEUTICAL TESTING

By
Vaibhav Hans

Honors Thesis
Applied Sciences in Biomedical Engineering
University of North Carolina at Chapel Hill

(April 10, 2014)

Approved By:

Jeffrey Macdonald

## 1. Introduction

Even in this age of medicine, acute liver disease is still primarily treated in the same way as a decade ago; through the use of a liver transplant. However, due to the increase in liver disease and the persistent shortage of donor organs, the development of a bioartificial liver (BAL) has been the center of many research projects around the world. Even so, in the last two decades, there has only been little advancement on the development of a suitable human liver representative bioreactor. Although tissue-engineering methods have improved, a bioreactor still needs to be developed that can emulate the liver in order to properly develop and test hepatocytes to be used in a bioartificial liver. This BAL model must be able to first of all maintain cell viability, as well as mimic gas exchange and nutrient delivery. However, the development of a suitable BAL is prolonged due to the financial aspect of pharmacology testing. The cost of testing a single pharmaceutical compound on hepatocytes in vivo can exceed millions of dollars in order to satisfy government regulations. The fact is that there are many new compounds that are synthesized each year that could fight liver disease, but the cost of *in vivo* testing is far too high [8]. By creating a BAL to model the *in vivo* pathways and mechanisms in the liver, an understanding of integration and the effects of consequent pharmaceutical compounds can be expedited and testing can be made more affordable.

## 2. Background

### 2.1 Acute Liver Failure and Methods of Treatment

According to the American Liver Foundation, over 45,000 people die of liver disease/failure annually [12]. Generally, liver disease is caused from the buildup of fats, sugars, and other metabolites due to some other underlying problem [2]. These buildups cause cirrhosis, which is a degenerative process in the liver that causes the destruction of hepatocytes (liver cells) and the eventual contraction of the organ [6]. Liver failure causes the disruption of the body's acid-base balance, metabolic instability, disruption of energy

supply, among others. Unfortunately, the mortality rate of acute liver failure is 80-90%, with death within a week or two. Even though technology today has improved in terms of success of treatment after transplantation, the rapid progression of the disease often makes this procedure unviable due to human organ shortages worldwide. To fill this deficiency, there are several alternative methods of treatment under exploration including tissue engineering implantable constructs, transgenic xenotransplantation, and extracorporeal bioartificial liver devices [1]. Although each method has its advantages and disadvantages, none have had consistent and absolute success in providing acute care to patients with liver failure. BALs have been researched for many decades, with a great deal of progress to show; however, even the commercially available products that claim to functionally mimic a liver are not without their flaws.

## 2.2 Hepatic Acinus

The hepatic acinus is the smallest functional unit of the liver [7]. To create an effective bioreactor, this unit of the liver must be modeled. In general, a liver lobule refers to the tissue that is centered on a single central vein, a branch of the hepatic artery. The hepatic acinus, however, is more along a branch of a portal triad and shows how well the hepatocytes in the area are oxygenated, along with the oxygen gradient. This unit is important as it shows us that the hepatocytes that are slightly closer to the central vein are less oxygenated than those right along the edge of the liver lobule.

## 2.3 Previous Prominent Bioreactor Designs

These extracorporeal devices utilize isolated hepatocytes in a chamber that allows the cells to survive and continue to provide functional support, similar to that in vivo. In order to create a bioreactor to emulate the hepatic function for pharmaceutical testing, it is important to see where previous designs have failed, as well as succeeded. Three prominent designs have emerged in the past 50 years of extracorporeal BAL history. Table 1 outlines these three approaches and highlights the advantages and disadvantages of each.

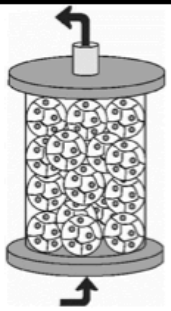
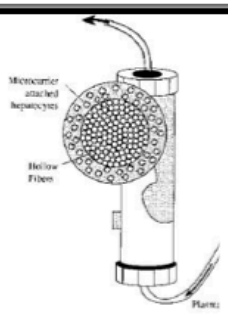
| Suspension | Perfusion | Hollow Fiber |
|---|---|---|
|  |  |  |
| Advantages<br>Versatility in operating conditions, easy harvesting, enhanced oxygen and nutrient transfer due to uniform environment, easily scaled-up | Advantages<br>Low resistance transport barrier, true 3D structure, cost effective in scale-up operations | Advantages<br>Structurally similar to hepatic acinus, large surface area to volume ratio, capable of high-density packing, lower chances of clogging. |
| Disadvantages<br>Degradation in long-term applications, shear force exposure, barrier for transport of pharma-chemicals | Disadvantages<br>Large shear stress exposure, nonuniform perfusion, clogging in long-term operations | Disadvantages<br>Nonuniform distribution of cells within hollow fiber. |

**Table 1.** Prominent types of bioreactors [9]

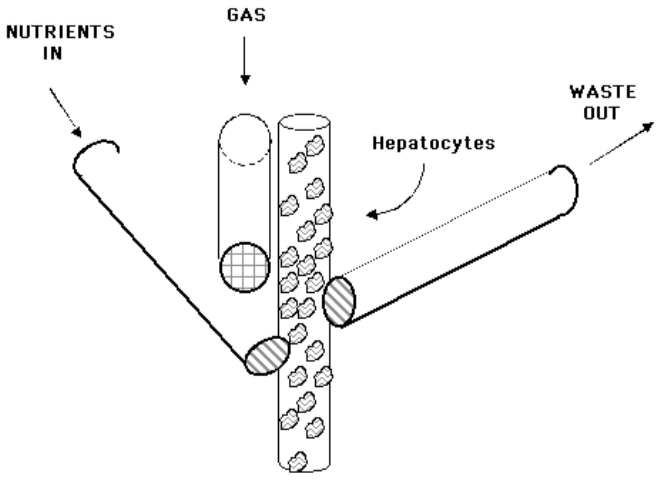
2.3.1 HepaAssist 2000

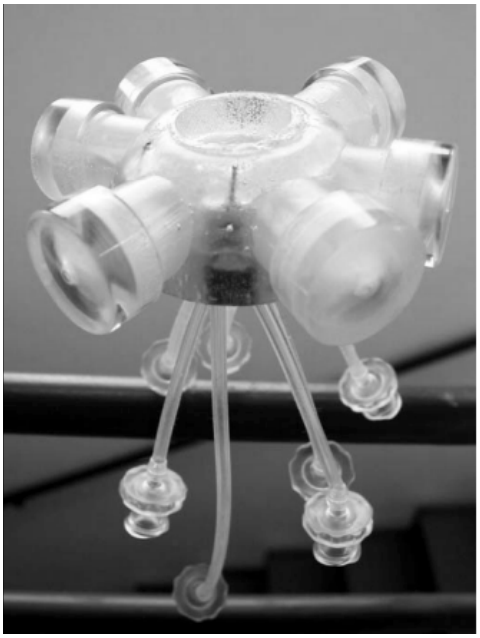
Circe Biomedical has a BAL System [4], termed the HepaAssist 2000, which is in its early stages of clinical trials. This liver support system takes a compartmental approach, along with the use of hollow fibers. To use this device for treatment, the plasma goes into a charcoal column, where it is pumped through two charcoal filters. These filters act as Kuppfer cells and filter the plasma from particulates and bacteria that might be harmful to the hepatocytes in the next compartment. After this basic filtration, the plasma enters the actual bioreactor, which contains a porcine-hepatocyte lined hollow fiber column. This membrane is porous with a pore size of 0.2µm, small enough to prevent the passage of whole hepatocytes cells, but large enough to allow protein bound toxins to travel between the plasma and hepatocyte chamber. After the plasma is run through this chamber, it is returned with the blood separated earlier and sent back to the body. Although this design shows results when used for a 6-hour treatment, the problem is that in order for pharmaceutical testing, 6 hours is not long enough to evaluate if the

results are significant. Hepatocytes in the hollow fiber chamber died out after 8-9 hours of experimentation.

2.3.2 Modular Extracorporeal Liver System

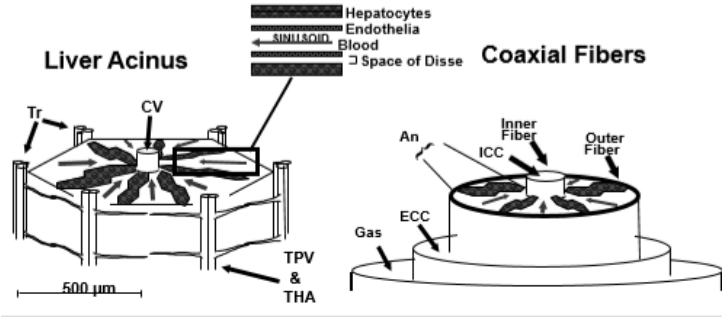
Dr. Gerlach's Modular Extracorporeal Liver System (MELS) consists of a 4-chamber design [10]. The four chambers include the cellular compartment and pathway compartments for nutrients, gas, and waste. The device relies on a network of thin hollow fiber membranes, in which hepatocytes are kept alive through the nutrients and gas pumped in. Inside the bioreactor, each of the functional units consists of a flow pathway formed by two plates. The upper plate consists of an array of gas filled hollow fibers, and the bottom plate is where the hepatocytes are attached. The separated blood plasma then flows through the channel formed and comes in direct contact with the hepatocytes. The flow through this device, depending on the number of channels available can reach 100ml/min, which is quite high, and the cells supposedly can survive up to one month. The problem with this bioreactor is that its media/biomass ratio is too high to mimic liver function for pharmaceutical testing.

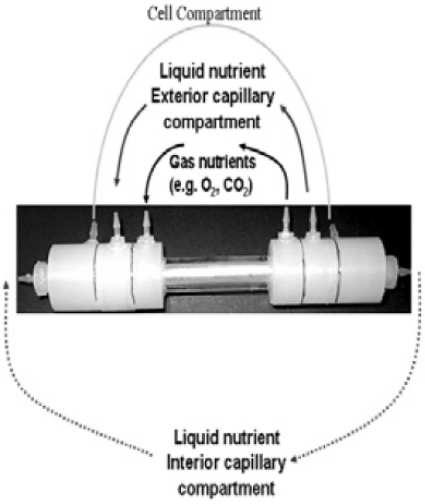


2.3.3 Multi-coaxial BAL

ADMET Technologies has been working on a multi-coaxial bioreactor to serve as a human bioartificial liver [7]. This bioreactor attempts to mimic the liver lobule/acinus in both structure and dimension, which had not been done before. The reason for its success is that the hollow fiber within hollow fiber design allows for precise control of the diffusion distances of nutrients to cells. In the case of testing pharmaceutical compounds, this is particularly important. Dr. Macdonald, a professor at UNC, was working on a modified version of this bioreactor with a gas chamber on the outside layer on which he did extensive testing which showed signs that this bioreactor was able to mimic liver function, as well as keep the hepatocytes alive for long periods of time, even up to 30 days. This bioreactor had a slightly larger media-biomass ratio than needed, due to the available hollow fiber membranes at the time. Hollow fibers are one of the more practical approaches to allow for the exchange of small molecules from one chamber to another.




## 3. New Bioreactor Design

3.1 Goals and Task

The Hamner Institute for Health Sciences in RTP, North Carolina is interested in Physiological Based Pharmacokinetic Modeling (PBPK) for various drugs and chemicals through the liver. PBPK is a modeling method to predict mathematically the distribution,

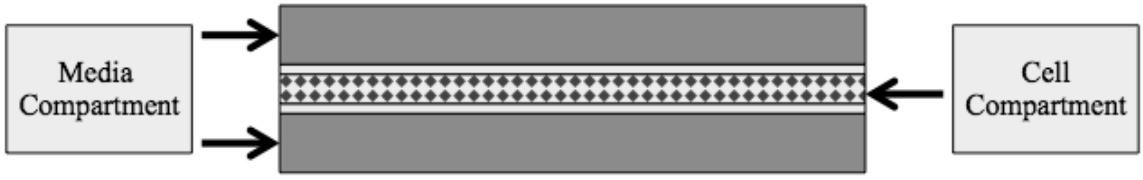
metabolism, and excretion of substances in the human body [3]. This technique is the prominent method in order to assess the risks and effects of a drug in the pharmaceutical research field. Even in five decades of extracorporeal bioarticial liver devices, there is still a need for a device to model human pharmacokinetics in the liver.

Based on the previous bioreactors out there, some goals can be set for an ideal bioreactor for the task at hand. First of all, for PBPK, the blood (media) to tissue (biomass) ratio needs to be optimized close to 1.34:1 [6], to accurately mimic the liver acinus. This is important as to not overwhelm the hepatocytes with a volume of blood that they cannot handle [6]. Looking at the previous designs, with the exception of the multicoaxial bioreactor, the blood-biomass ratio was too high. This high ratio could be the reason that the previous designs were not able to keep the hepatocytes alive for a long duration of time. Further, the oxygen gradient also needs to be properly optimized. When looking at the liver acinus, the central vein (deoxygenated) runs along the middle of a lobule, and the portal triad runs on the outside of the lobule. Then, as the blood travels through the acinus, it travels from the oxygenated vessel, through hepatocytes, to the deoxygenated vein. In a bioreactor, it becomes difficult to mimic this gradient. In the multicoaxial bioreactor design, the gas chamber is only on the outside of the media fiber, which removes any chance of an oxygen gradient. Also, for testing with pharmaceutical compounds, the scientists must have access to the cells to be able to run imaging and other tests. When looking at Circe's bioreactor model, the porcine hepatocytes are sealed inside the internal hollow fiber. If these cells were removed, the bioreactor would be rendered useless. Therefore, in order to provide access to the cells without destroying the function of the bioreactor, the bioreactor must be easily disassembled. Ease of inoculation should also be taken into consideration. Additional factors to consider for PBPK include accommodating for both alginate encapsulates and cell aggregates (spheroids), along with taking into account the material binding factors of Albumin and Alpha-1-glygoprotein (AAG). Albumin and AAG are both markers used in PBPK to gauge a quantitative understanding of drug action and distribution [5]. Last, for the experiments run for PBPK, long-term function is also a factor as these experiments can be run for up to 60 days. For

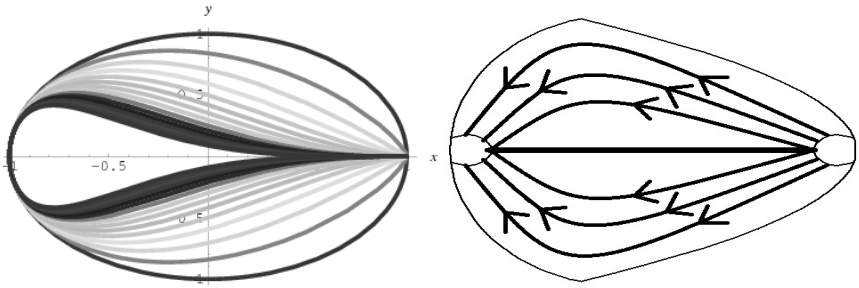
practicality's sake, the cost of the device must be reasonable for an honors thesis project, as opposed to Gerlach's device that costs $3000. From previous bioreactor designs, it seems that the use of hollow fibers seems to be the best way to house the hepatocytes. However, after some initial research on the availability of hollow fibers in the market today, an alternate solution is required. Hollow fibers are rarely sold independently today; they are only available as part of a pre-existing bioreactor. This option would be far too expensive. The question then arises as to how to build upon the success of the multi-coaxial bioreactor, but without the use of hollow fibers.

3.2 Design

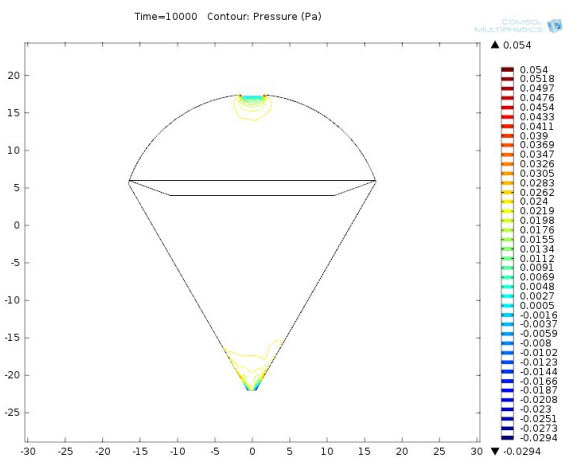
The new design is based on an unraveled multi-coaxial bioreactor. However, instead of using hollow fibers, flat membranes are used. These membranes come in a variety of pore sizes, and are easily available from multiple manufacturers at a very reasonable cost. The new design can be described as a flat [2] packed bed bioreactor with three compartments, separated by the PES membranes.

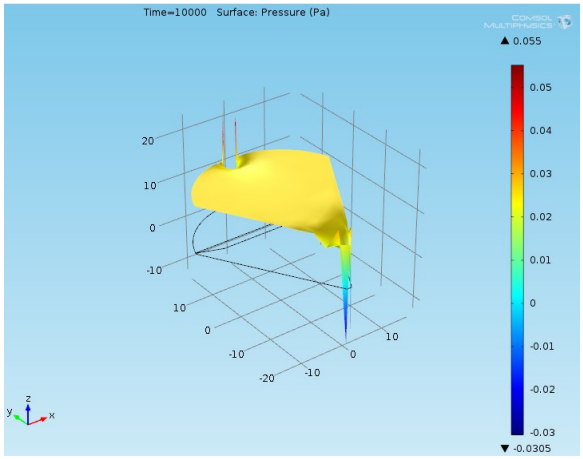


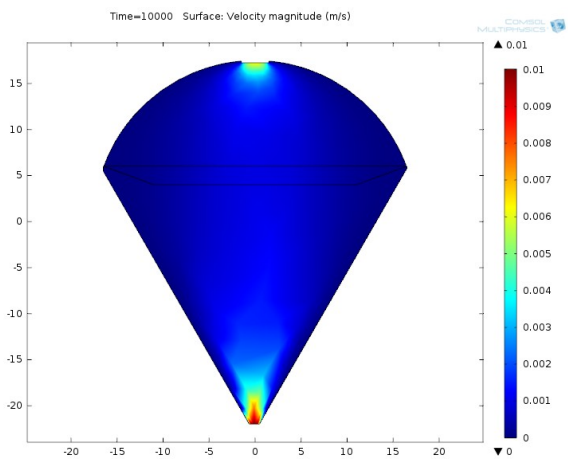

The middle compartment is where the cell culture will be housed, whereas the two outside compartments will where the media flow will pass through. The size and shape of the individual compartments is what is most important. In order to assure that the media will come into contact with the cells, the cross-sectional area of the media compartment is larger than the cell compartment. As far as the shape of the compartments is concerned, it can be described as a teardrop shape.

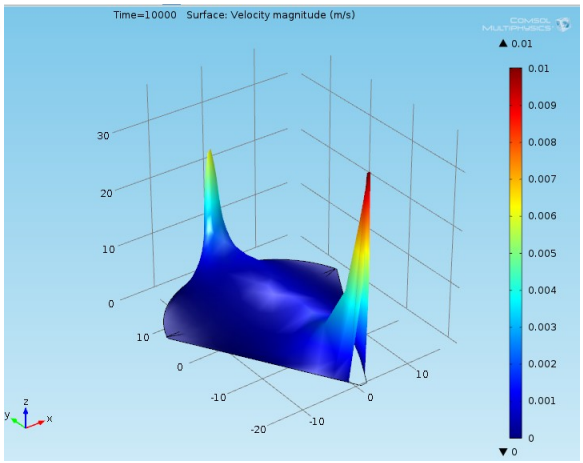



To determine the effectiveness of the teardrop based shape of the media compartments, fluid dynamics were analyzed using COMSOL Multiphysics, with an input velocity of 1mL/min.

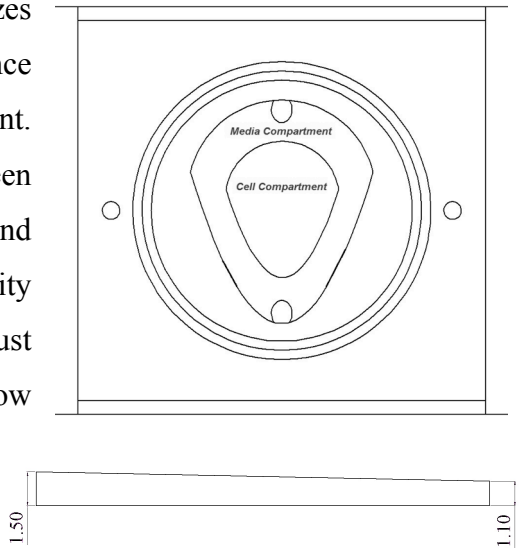


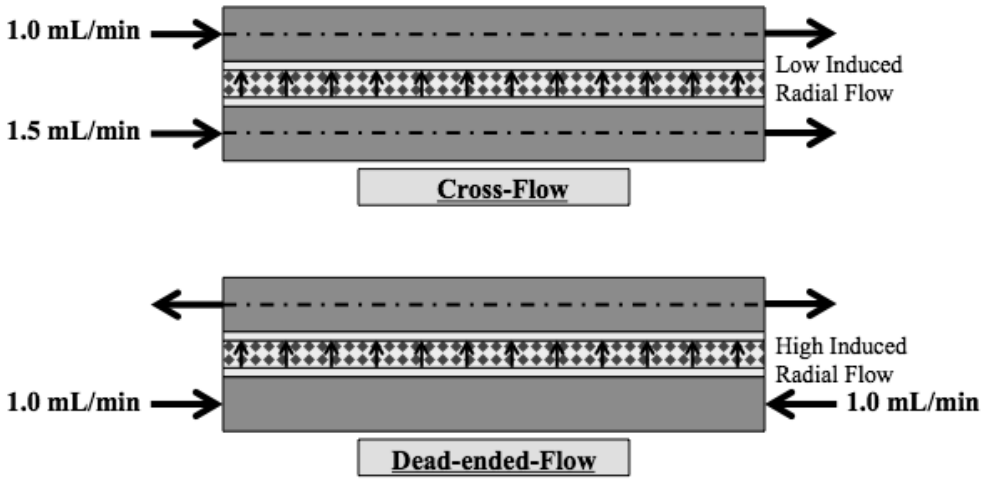
This shape ideally allows for a flow that minimizes dead flow areas and promotes uniform residence time within all portions of the compartment. However, after some initial analysis, it can be seen that there are pressure peaks near the two inlet and outlet ports, as well as nonuniform flow velocity down the center of the chamber. These issues must be addressed in order to maintain a uniform flow and pressure within the cell chamber. To address the issue of the high pressure points, the cell compartment is scaled down so that the cells themselves do not undergo a strong shear stress. As shown to the right, the cell compartment will maintain the teardrop-based shape, without coming into direct contact with the pressure points. When making this design change, it is important to keep in mind the blood to biomass ratio; to keep the ratio as close to 1.34:1, the cell compartment size must be maximized, without hindering on the pressure points. Further, the nonuniform flow distribution can be addressed using a tapered media compartment. Be gradually decreasing the depth of the compartment from the narrow end to the wide end, the flow can be better distributed. Due to the nature of laminar flow fluid dynamics in the compartment, it is difficult to gain even flow near the edges of the compartment; however, as long as there is a uniform flow within the cell compartment area, it is not of concern. At the narrow end of the compartment, a larger depth

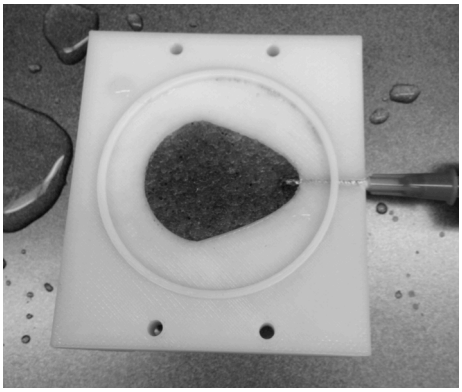



When looking at the maximum diffusion distance, there are two factors that must be balanced: maximum known diffusion distance for hepatocytes, and achieving a proper blood to biomass ratio. From past experiments, the maximum diffusion distance depends on how the hepatocytes are encapsulated [13]. It was found that if encapsulated in spheroids with a diameter of 500µm, a maximum cell compartment width of 2mm can be achieved. On the other hand, the minimum width required is based on structural integrity

limitations. Initially, the cell compartment width was set to 1.8mm; however based on final material choices and initial experimental data on hepatocyte function, this can be changed. In order to minimize the blood-tissue ratio for the proper metabolism ratios, the media compartment widths can be determined using the width of cell compartment. This is the area that the aforementioned bioreactors lack the most. Therefore, for initial testing, the media compartment was designed to be 2mm in width as well. Including both media compartments on either side of the cell compartment, this allows for a blood-tissue ratio of roughly 1.97:1. This ratio is far more representative of the blood-biomass ratio in the liver acinus than any other prominent BALs.

Due to the nature of the flat packed bed bioreactor, there are also multiple flow configurations that can be reached; Cross-Flow and Dead-Ended-Flow. Although this functionality adds new application possibilities, after examination of prominent research in the field, dead-ended flow eventually results in the clogging of the filter pores with albumin and other byproducts.



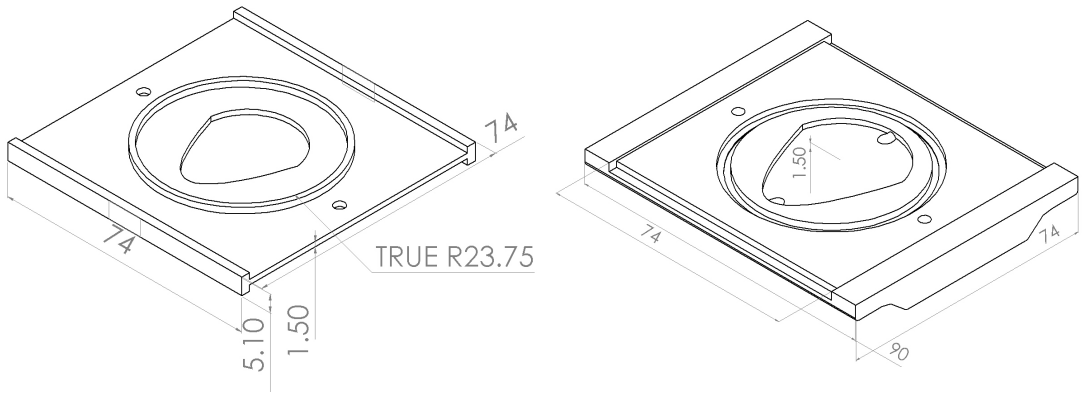


The ability to run experiments in different flow configurations is not vital, however, it allows for more flexibility in the types of experiments that can be run. The ability to pressurize one side of the bioreactor at a time also allows for better inoculation technique. By attaching a lab vacuum to both inlet and outlet of one media compartment while the other side lies open allows a vacuum-sealed inoculation; as the mixture of media and cells is placed into the cell chamber, the excess media is suctioned out through the PES filter. This method could be done without the use of a vacuum, but it would take considerably longer as the media would have to diffuse out on its own. Nonetheless, with this technique, the chamber was inoculated very evenly and fairly packed.

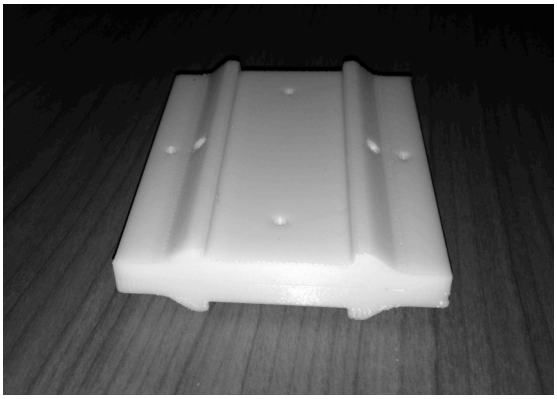
Further on, this new design provides easy access to the cells to perform metabolic analysis. The snap-close and screw-seal mechanism can easily be detached, allowing for the three compartments to come apart. Unlike previous bioreactors, even after disassembly, the bioreactor is still functional. By just replacing the PES membranes, the bioreactor can be reset and used again. The main benefit of using a flatbed design is that flat membranes are relatively inexpensive to their hollow fiber counter parts. The membranes that were used initially had a 0.47μm pore size and cost roughly $1 each. Filters are usually where the bulk of the cost lies; by minimizing that cost, the remaining parts of the bioreactor are relatively affordable.

### 3.3 Prototype

Taking all factors above into consideration the following design was finalized. This design has a cell compartment volume of 1.39mL, and a media compartment volume of 1.37mL. Therefore, overall, the blood to biomass ratio for this flat bed packed bioreactor is 1.97:1. The cell compartment is positioned and sized just small enough to maximize distribution of flow and to avoid the pressure points.



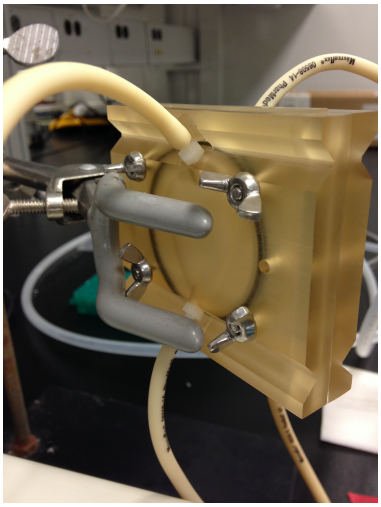

With the final design completed, a sample prototype was printed using FDM and a high precision 3D printer.

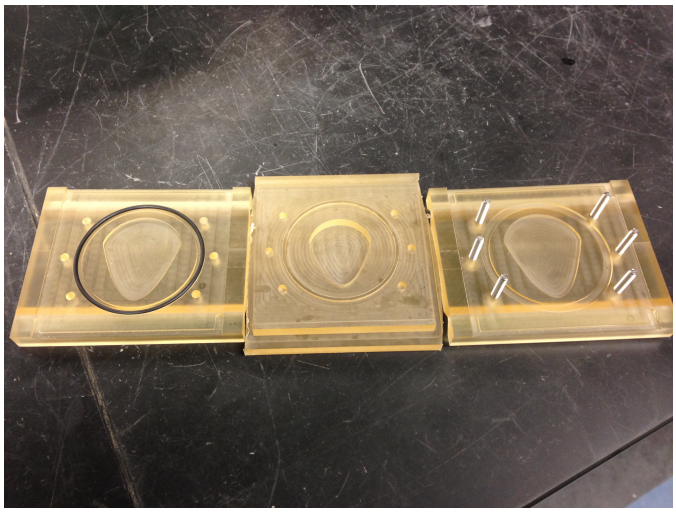


3.4 Manufacturing


The 3D printed material is not suitable for a final product due to drug binding and liquid permeability reasons. A chemically inert, rigid elastomer is needed for the final build material. After some examination of the materials available within a reasonable price range, polysulfone is chosen. Since the bioreactor is to be used in a PBPK modeling system, drug-material binding is a important factor to consider. Polysulfone is known to be chemically rigid, along with a low 0.3% water absorption rate. Another important consideration when choosing the

build material is its rigidity to manufacturing techniques. Polysulfone has a tensile strength of 10,200 psi and impact strength of 1.3 ft-lbs/in, which are both greater than most other elastomers that are chemically rigid. As far as the O-rings used to seal the 3 chambers, EPDM (ethylene propylene diene monomer) rubber O-rings were used to provide a strong seal to water. The polysulfone was milled using a CNC mill, and a variety of mill and drill bits based on individual sizes and depths. Overall, the process took around 30 hours of milling. For future iterations of this bioreactor, the manufacturing time would be greatly decreased as now I have some experience with how to mill polysulfone, and take into account its material properties. Throughout the manufacturing process, I learned a great deal about different manufacturing processes and how to better design the bioreactor in the future to allow for easier manufacturing. Overall, the final product was near perfect, with very few inconsistencies from the Solidworks design to the final product.



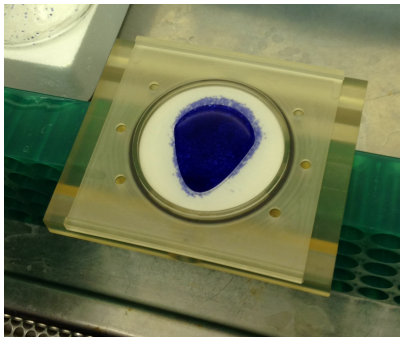


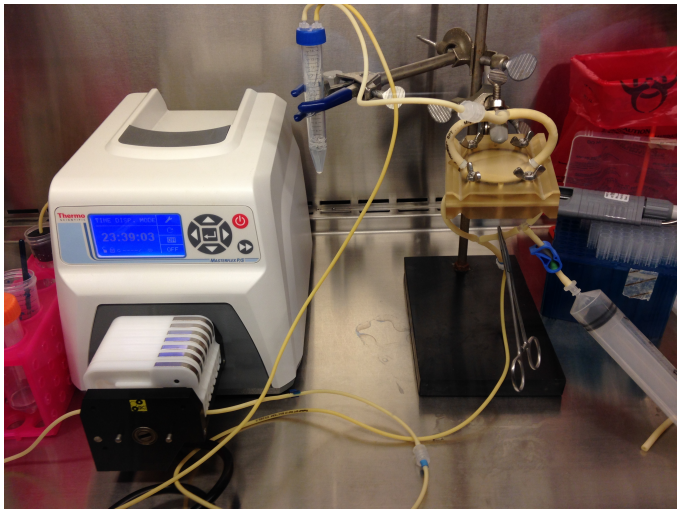
**4. Initial Experimentation and Next Steps**

Initial experiments were run using inoculated alginate beads. The objective of this initial experiment was to gauge an understanding of if the bioreactor was capable of performing long-term tests and if the filter pore sizes were adequate for various flow configurations. The first aspect to test is the flow and if it is equalized throughout the

whole cell compartment; in other words, optimize the radial flow. To do so, first empty alginate encapsulates will be made and injected into the cell compartment. By varying the input and exit tubing, the linear flow rates can be modified. If the flow through one media compartment is held constant, and the exit flow of the second compartment is modulated, the effective radial flows can be analyzed by studying the encapsulated beads. After initially attempting this with alginate beads, it seemed that the radial flow was plenty, in the dead ended flow configuration (input flow at 1mL/min).

Therefore, the chamber was then inoculated with 1:1 Alginate: Rat Hepatocytes. To this mix, trypan blue was added in a non-lethal dose (100 ppm) in order to increase visibility of the cells when inoculating. The oxygenation/perfusion tubing was setup in such a way that the incoming and outgoing media was oxygenated over the course of 20 cm, with an aeration tube. This setup was allowed to run for 3 days with 2mM $CaCl_2$ DMEM based media. The $CaCl_2$ was added in order to keep the alginate beads intact.





Unfortunately, this initial experiment resulted in the clogging of the filter pores. In the dead ended flow configuration, there was too much albumin produced in the cell chamber, with very little time to diffuse out. However, this is not a major set back, as this just shows that in the dead-ended flow configuration, it is required that the filter pore size be much larger to accommodate for the induced radial flow. Initial results from the first

24 hours of the run show that the cells were alive and consuming glucose and producing lactate. Overall, the experiment still supports the success of the bioreactor.

From the first experiment, I have gauged an understanding of what needs to be tested next. First, the pore size of the filter needs to be increased. This is a inexpensive change, and if needed, nitex flat sheet membranes can be used. For the next experiment, the cross-flow configuration will be used in order to maximize the concept of pressure induced radial flow. The data collection will involve testing for the following quantitative measurements: Glucose consumption, lactate production, enzyme leakage (Lactate Dehydrogenase, ALT and ASP rates. The cells will also be analyzed for its metabolic fluxome [11] as well as analyzed under a microscope for structural integrity and viability.

**5. Conclusion**

The need for a pharmacokinetically compatible bioartificial liver was the primary motive for this project. After looking at all the methods of current treatment and solutions for acute liver disease, it becomes apparent that there are very few pharmaceutical based solutions available now. The only way to expedite this process is to allow scientists to have a way to test their drug without the expensive process of live testing. Obviously, to get the drug approved, it must go through clinical trials, but what if there was a precursor available that mimicked the body and liver in function. This, the serial organ system, will represent the human body in a functional ideology. After examining the various types of hollow fiber bioreactors available in the present and the past, it becomes apparent that the Multi-Coaxial bioreactor has the greatest promise. It has a low blood-biomass ratio, and has proved to be able to keep cells alive for long durations. However, since hollow fibers are no longer readily found, I took the design of the hollow fiber and modeled an unraveled form; the packed flat bed bioartificial liver. After modeling the fluid dynamics and doing extensive research on prior bioreactors, and where they have succeeded and failed, a design was formed. After some initial printed builds, it became apparent that the bioreactor needed some improvements, mainly in sealing better and minimizing the blood to biomass ratio. After some improved versions of the design, a second prototype was

finalized on, which had a blood to biomass ratio of 1.97:1, which is within reason. This prototype was then manufactured from polysulfone with the use of CNC technologies. After some initial tests at the Hamner Institute, the bioreactor shows great promise, and indications that it is capable of sustaining cell life. I plan to further research and test the bioreactor, as well as create an improved version based on my experiences post-construction. This process of designing a bioreactor was far more involved and critical than I imagined. However, I gained invaluable experience in the field of bioreactors and bioartificial systems.

## 6. Acknowledgements

This project was supported by the Kimball King Undergraduate Research Fund, administered by Honors Carolina.

Dr. Jeffrey Macdonald for his invaluable guidance and support.

Steve Emanuel for aiding in the design tuning and machining the final product.

Andrey Tukunov for being a great resource throughout this project.

The Hamner Institute for Health Sciences for presenting me with such an opportunity.

## 7. References

[1] Allen, Jared, and Sangeeta Bhatia. "Improving the next generation of bioartificial liver devices." *Cell & Developmental Biology* 13 (): 447-454. http://lmrt.mit.edu/publications/2002/Allen2002_CellDevBio.pdf

[2] Bartalo, De, and A Bader. "Review of a flat membrane bioreactor as a bioartificial liver."*Ann Transplant* 6 (): n. pag. Print.

[3] Bader, A.. "A Novel Full-Scale Flat Membrane Bioreactor Utilizing Porcine Hepatocytes: Cell Viability and Tissue-Specific Functions." *Biotechnology progress* (): 102-108. Print.

[4] Circe's biomedical's hepatassist 2000 system. (2001). Retrieved from http://biomed.brown.edu/Courses/BI108/BI108_2002_Groups/liver/webpage/Circepg.htm

[5] Dayton, P, and Z Israili. "Human alpha-1-glycoprotein and its interactions with drugs."*Drug Metabolism Review* 33 (): 161-235. Print.

[6] Hoekstra, Ruurdtje, et al. "Phase 1 and Phase 2 Drug Metabolism and Bile Acid Production of HepaRG Cells in a Bioartificial Liver in Absence of Dimethyl Sulfoxide." *Drug Metabolism and Disposition* 41.3 (2013): 562-567.

[7] Macdonald, Jeffrey. "A Novel Multi-Coaxial Hollow Fiber Bioreactor for Adherent Cell Types. Part 1: Hydrodynamic Studies." *Biotechnology and Bioengineering* 77 (): n. pag. Print.

[8] Matsumura, K. Nn, et al. "Hybrid bioartificial liver in hepatic failure: preliminary clinical report." *Surgery* 101.1 (1987): 99-103.

[9] Palsson, B. European Patent Office, (1993). Methods, compositions and devices for maintaining and growing human stem and/or hematopoietic cells (EP 0 629 236 B1)

[10] Sauer, I., & Gerlach, J. (2002). Modular extracorporeal liver support. Artificial Organs, 26(8), 703-6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12139497

[11] Seagle, C, et al. (2009). High-throughput nuclear magnetic resonance metabolic footprinting for tissue engineering. Tissue Engineering, 14(2).

[12] Sharma, Ruchi, et al. "Three-dimensional culture of human embryonic stem cell derived hepatic endoderm and its role in bioartificial liver construction." *BioMed Research International* 2010 (2010).

[13] Yu, H, et al. (2000). Hepatocyte encapsulation for enhanced cellular functions. Tissue Engineering, 6(5).