

A Novel In-House design of a Bioreactor for the Modeling of an *in vitro*
Blood Brain Barrier Model

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Executive Summary:

The blood brain barrier is the protector of the central nervous system and a physical barrier that functions to regulate the substances that can pass in and out of the brain; it is the function and integrity of this system that keeps the homeostasis of the central nervous system. Yet this shield against foreign invaders in the blood also prevents drugs designed for treatment of various ailments of the central nervous system from reaching their target in the brain. Developing drugs that can pass through this barrier, and understanding its function has become an area of increasing interest. Many researchers and companies are turning to *in vitro* models of the blood brain barrier to test a drug's ability to pass through this shield and target areas in the central nervous system. Our lab at Cal Poly focuses on developing blood vessel mimics, and tissue engineered constructs that would allow for *in vitro* modeling and testing of blood vessel physiology. Currently the main focus is on coronary blood vessels for stent testing, but expanding this to other areas of blood vessel research is of interest to our lab, and we believe that we can contribute to the blood brain barrier field by using our lab's experience with other tissue engineering constructs. The focus of this project is to design and create aspects of a novel bioreactor that is reusable, can be built in-house, and facilitates better access to the scaffolding. The primary areas of focus in this project were to 1) design and manufacture a reusable novel bioreactor in-house, 2) create a mandrel to collect electrospun fibers to be used as scaffolding, and 3) to evaluate these scaffolds and compare to the literature's characteristics of proper scaffolds for blood brain barrier models. All of these goals were met; we now have a bioreactor prototype that has been manufactured and is currently going through further refinement, modification and testing to optimize the design, there is now a series of new mandrels that are ready to be used to electrospin scaffolds to be used in the new bioreactor, and the scaffolds have been shown to possess some of the characteristics that were outlined in the literature for blood brain barrier models. The completion of all of these aims has allowed for the advancement and progress towards the ultimate goal of creating an *in vitro* model of the blood brain barrier to study its physiologic mechanisms and study drug diffusion. The project will allow for research and advancement in the area of developing pharmacological therapies and strategies for treating disorders and ailments of the central nervous system.

Introduction:

The blood brain barrier plays a crucial role in protecting the brain and regulating its homeostatic environment. This is very important to maintaining the functional performance of the brain. The structure and nature of the blood brain barrier make it difficult for pharmacologic treatments to enter the central nervous system from the blood in order to treat various diseases [1]. This highlights the importance of creating a model of the blood brain barrier that would allow further study of the characteristics of the system, and gain increased knowledge into creating products that can cross this barrier and treat brain related conditions and diseases [2].

Anatomy of the Blood Brain Barrier

The blood brain barrier refers to the capillaries that supply the brain with nutrients and remove waste. It also functions to control the passage of various solutes into the region of the brain, making it a means of defense for the brain against foreign invaders such as bacteria. These capillaries are comprised of endothelial cells which make up the inner layer of all blood circulating vessels. What differentiates this capillary system from others is the selective nature of the endothelial cells, which is characterized by the tight-junctions that exist between the cells; however, these cells also show less of an ability to allow things to pass pinocytotically. The tight junctions prevent solutes from passing in between cells that comprise the vessel wall, and allow for the control of the homeostasis of the brain, and its functionality [3,4].

The vessel lumen is circumnavigated by a single endothelial cell, rather than numerous cells lined end to end as would characterize larger vessels. Attached to the outer wall of the endothelial cells are pericytes, and beyond this are astrocytes [3]. There is not much information in literature on the exact function of pericytes and more of the research has been on astrocytes. Astrocytes are glial cells which have various functions inside the central nervous system. They have out stretched 'feet' which extend out from their main body, and attach to the endothelial cells of the blood brain barrier [1]. They also provide structural support to the nervous system, control the flow of neurotransmitters and ions in order to maintain function of the nervous system. It has also been recently shown that astrocytes help to improve the functional characteristics of the blood brain barrier *in vitro* [5,6]. For the purposes of this project, we have been using bovine aortic endothelial cells (BAEC's), and for C6 glial (astrocyte) cells. These cell types have been shown to be used in previous research on the blood brain barrier and will suit the purposes of this project [1].

Current Blood Brain Barrier Models:

Current testing of pharmacological products ability to cross the blood brain barrier is primarily done in *in vitro* bioreactor systems. The use of *in vivo* systems has occurred; however, most systems used are *in vitro* systems. The use of *in vitro* bioreactors allows for critical testing of drugs and the exclusion of drugs that would be selected against by the blood brain barrier in *in vivo* systems [2,7]. It is believed that the main conditions that must be met in order to recapitulate the phenotype of the blood brain barrier is to 1) have endothelial cells exposed to shear stress within the lumen, and 2) be exposed to differentiation factors that are believed to come from astrocytes [2]. Today, there are a few different *in vitro* systems for modeling the blood brain barrier, and many of these systems are early in their design and leave much to be desired.

Some groups have tried to create *in vitro* models in flat well plates by growing endothelial cells on one side of a porous membrane, with astrocytes grown on the other side [8,9]. Other groups have tried growing endothelial cells with differentiation factor media to induce the endothelial cells to express tight junctions. The media that has been treated with differentiation factor media helps to resolve the issue of using cell types from different species, which can cause complications [10].

The typical bioreactor for the purpose of modeling the blood brain barrier is composed of many hollow fibers onto which the endothelial cells are grown. The CellMax© system which we had previously been using was this type of bioreactor. Media flow is run through these hollow fibers to simulate blood through a capillary tube, and more naturally recreate the *in vivo* environment by creating luminal shear stress. They also include the use of co-cultured astrocytes in order to provide the differentiation factors, or media treated with the differentiation factors. Pharmacologic agents can then be placed into the intra-luminary media, and measurements can be made to determine the amount that passes through the blood brain barrier constructs and into the extra-capillary space. This type of a bioreactor system has been shown to more accurately mimic the blood brain barrier phenotype [11].

Bryan Brandon's Project:

This project was initially started by a fellow student Bryan Brandon. Bryan's focus in this project was in three areas, 1) characterizing the CellMax© system, 2) working to develop protocols for handling the cell types and 3) selecting the media to culture the cells in [12]. The hope was that laying out the protocols for using this system overall would lay the groundwork for future work to be done by improving various parts of the experiment. He later involved Ryan Woodhouse and me to work on various aspects of the project. Bryan focused on using a commercial system from the company

CellMax© and developing a protocol for it while Ryan focused on creating protocols using cell tracker stains and tracking cell types in co cultures [12]. The commercial system can be seen in Figure 1. My own area of focus was on creating a bioreactor so that we did not have to rely on the commercial products which had various faults that we believed could be overcome to allow easier completion of experiments. After Bryan and Ryan both graduated, new students were added to the team. TJ Eames was brought on to assist with culturing the cells and Amin Mirzaaghaeian worked with me on the bioreactor design.



Figure 1: The above image shows the commercial CellMax© DUO system with the CellMax© hollow fiber bioreactor cartridge. The bioreactor can be seen sitting on the bioreactor stage.

The blood brain barrier model protocol that Bryan developed used a co-culture of bovine aortic endothelial cells and C6 glioma cells. Previous work [1, 15, 11, 16, 17 for C6's and 2, 18, 15 for BAEC's] provided by other papers has shown that these cell types can be used to properly replicate the blood brain barrier. In Bryan's project he was able to develop a protocol for passing and culturing the cells [12].

The commercial system that Bryan worked with was the CellMax© DUO system with the CellMax© hollow fiber bioreactor cartridge. In his protocol he worked to evaluate and characterize the pump system in order to determine the correct flow rates that should be used with the CellMax© hollow fiber bioreactor cartridge in order to create the proper shear stress forces on the cells within the lumens. In the course of his work he was able to determine the pump setting that should be used, as well as the correct pin size to use in order to create the proper shear stresses on the inside of the lumen's [12].

The final test that Bryan investigated was the use of media during co-culture of the cell types. The multitude of tests that he performed concluded that a 50:50 mixture of the C6 and BAEC's media resulted in the best cell growth for both cell types. These media combinations were shown to give the cells the best proliferative rate and signs of normal cell vitality. The C6's grew well in the BAEC's media

alone, however it was unknown if culturing the C6's in the BAEC's media could affect the gene expression of the C6's in the long run. Also the C6 media is less expensive than the BAEC's media, and in order to reduce our costs for this experiment, a solution of half C6 media and half BAEC's media was chosen to be the best choice [12].

Bioreactor Design

The Commercial Bioreactor System

The commercial bioreactor that Bryan used, shown previously in Figure 1, is characterized by a plastic tubular housing that encapsulates the hollow fiber scaffolding that the cells are cultured on. There are 60 polypropylene scaffolds that have been precoated with ProNectin™ F. The fibers had an inner diameter of 480 μ m, a wall thickness of 150 μ m, and an average pore size of .2 μ m. The idea of this type of bioreactor is to grow the cells in a 3-D dynamic environment to more accurately recapitulate the *in vivo* system. The cells can be grown within the scaffolds or between the exteriors of the scaffolds, and the scaffolds support gas exchange between the extra-tubular space and luminal space as seen in Figure 2.

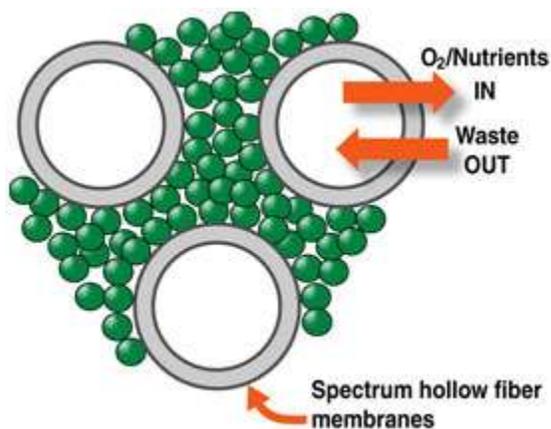


Figure 2: A cross sectional view of the hollow fibers. The BAEC's cells will be grown on the inner surface of the scaffolds, while the C6 glioma cells will be grown on the exterior of the scaffolds. The scaffold tubing allows for gas and nutrient exchange between the inner and outer spaces of the tubing.

Limitations of the Commercial System

After the work that Bryan did with the CellMax© systems, we realized that the CellMax© DUO bioreactor design left much to be desired. The first problem that we came across with the commercial bioreactor was that some of the scaffolds within the bioreactor system were broken before our initial set up of the system. This meant that when we would try to seed our cells on the interior of the scaffold

tubing that the BAEC's would be released into the extra-tubular space, and either die or grow on the outside of the scaffolding. In a system where we are trying to recreate the cell layering that is normal in native tissue, the loss or misplacement of cells is detrimental to the model.

The next thing that we noticed with the commercial CellMax© bioreactors, that actually was a large flaw in our eyes, was the inability to reuse the system. The housing of the bioreactor had no way of accessing the scaffolding within, removing these or replacing these. The only ports into the bioreactor system were the inlet and outlet tubing ports meant to facilitate fluid flow, or media sampling. This meant that once we had completed an experiment and wanted to analyze the scaffolding system to determine the morphology of the cells and their characteristics, we had no way of getting to the cells. Ideally we would be able to take the scaffolds out of the system, embed them in paraffin or some type of holding medium and stain them for cell markers. Another technique to analyze them would be to fix the cells onto the scaffolding and stain the cells to look for cell attachment on the luminal wall of the scaffold. However, since the bioreactor doesn't allow us to easily remove the scaffolding from the bioreactor, performing these analyses becomes more difficult. In order to get to the scaffolding and perform analysis, we had to cut the outer housing of the bioreactor. To do this we used a band saw with the help of Mr. Martin Koch and Mr. Dave Laiho. Using the band saw we cut through the housing and the scaffolding at each end of the bioreactor housing, an image of the bioreactor after being cut can be seen in Figure 3. Cutting through the housing compromises the bioreactor housing, and to perform more experiments, more bioreactors need to be purchased. Yet at \$469 per bioreactor, our funding for this project would quickly be depleted.

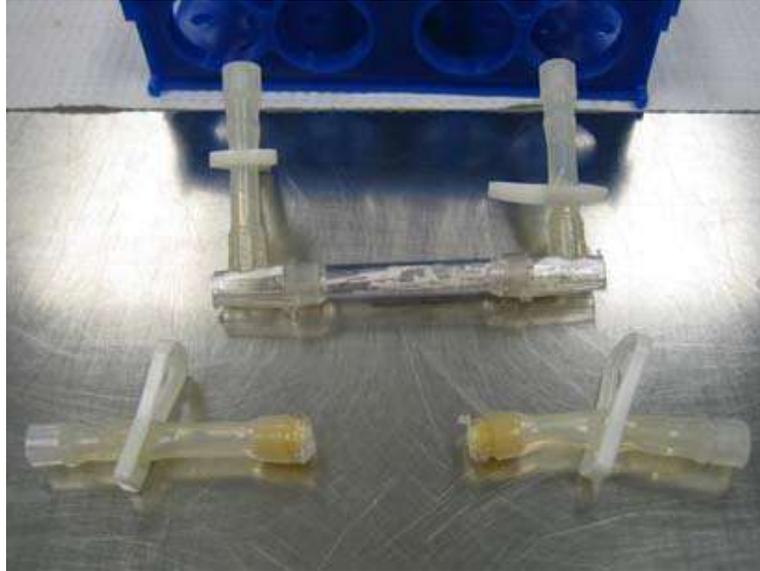


Figure 3: The above image shows the hollow fiber bioreactor after being cut with a band saw. The inlet and outlet ports to the luminal space were cut to allow removal of the scaffolding.

Another issue that arises with the lack of access to the scaffolds is the inability to perform time trial experiments. We would like to be able to check our system by removing singular scaffolds as experiments progress to check on the development of our blood brain barrier model. It would be impossible to do this with the current bioreactor systems since removing a scaffold would require cutting the bioreactor, thus compromising the system.

While the CellMax© bioreactor system allowed us to set the foundation for this project and gain basic understandings of the components and requirements for creating a blood brain barrier model, there were various shortcomings with the design. The flaws that we saw in the system were that they would sometimes have scaffolds broken before the inoculation of cells into the system, they were not reusable and were difficult to deconstruct for analysis, and would not allow for time-trial analysis. To remedy this, we decided to undertake the goal of creating our own bioreactor that we could build in-house, was reusable, and allowed access to the scaffolds without destroying the bioreactor.

Design Criteria for New Bioreactor

Our plan for a new bioreactor was to identify and resolve some of the issues with the commercial bioreactor system. Our main concern with the commercial bioreactor was that it was not reusable. To resolve this issue, we hoped to create a bioreactor that allowed for easy access to the scaffolds within the housing, while still maintaining its integrity and not compromising sterility. This would also mean that

the components that the bioreactor was made of needed to be able to be sterilized. By allowing access to the scaffolding we addressed not only the issue of reusability, but it would allow us to perform time trial studies as well, as we could now select scaffolds from the bioreactor, remove them, then close the system and continue running the experiment. The issue with scaffolds being broken before the experiments began was partly an issue of there being so many scaffolds within the bioreactor, so our design looks to reduce the number of scaffolds so we can be sure of their integrity before performing an experiment. Taking these factors into account, we can list our design criteria for the bioreactor as: 1) to be able to reuse the bioreactor, 2) have easy access to the scaffolding, and 3) to be able to create the bioreactor in house.

Bioreactor Designs

In order to manufacture our own bioreactor and address the flaws of the commercial system, we came up with various potential designs to choose from. These ranged from making slight modifications to current in-house bioreactor systems we use, to completely manufacturing a new bioreactor. Among the various designs, there were 3 main designs that we considered for creating our own bioreactor, all that we envisioned would allow for better access to the scaffolding while still being reusable.

The first design that I had considered involved modifying the in-house bioreactors that we currently use for the blood vessel mimics (BVM) experiments. The current bioreactor used for the blood vessel mimics uses a Lock & Lock® Tupperware container as the bioreactor housing. These containers are able to be autoclaved, and the Lock & Lock® system of securing the lid seals the container to prevent any contamination from occurring during experiments. With the addition of various barbed and luer lock fittings, inlet and outlet ports have been created in the container wall to allow for the placement of a polymer construct onto which endothelial cells can be cultured for blood vessel mimic experiments. My design would keep the housing system the same, but use a different interface within the bioreactor to hold the scaffolds. The current system uses barbed fittings that ePTFE (expanded polytetrafluoroethylene) scaffolds are slid over and then secured using suture to tighten the scaffold onto the barb. The new design that I had created used a type of diffuser cap that fit onto luer lock fittings extending from the wall. The larger end of the diffuser caps would have been open and allowed for polymer scaffolds to be glued into place using medical adhesive. Similar designs had been used to secure the housing in other bioreactor designs for this type of model [14]. This design would readily allow access to the scaffolds through removing the lid of the container. Also the materials used in the container are able to repeatedly be sterilized, so that the same bioreactor could be used over and over. A problem with this system is that with the scaffoldings simply placed in the hollow ends of the diffuser caps and glued in, it would be hard to remove the scaffolding for time trial experiments. It would also be hard to repeatedly get the same

orientation of scaffolds. Also the diffuser caps would most likely need to be manufactured in-house as there was limited availability of commercially available diffuser caps that would fit our needs for this design. An initial sketch of this design can be seen in Figure 4.

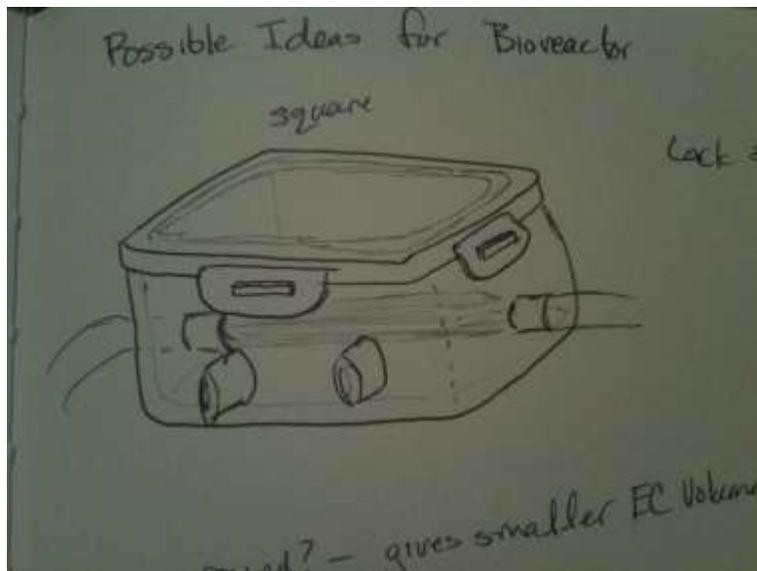


Figure 4: Bioreactor design that incorporates use of Lock & Lock® housing, with cartridge of scaffolds that integrates into the system.

The second design that we considered was more of a traditional bioreactor design. This design would have used a polycarbonate type of polymer for the bioreactor housing. The bottom of the bioreactor would be made of optical glass. This would allow for imaging of the scaffolds while they are in the bioreactor system and the experiment was still ongoing. The glass would be adhered using silicon adhesive. The top of the bioreactor could be made of acrylic or some type of clear polymer and would secure to the rest of the housing body through screws. Inside the housing of this design would be an inlet and outlet port which has a similar interface to the previous design. The ports would be large enough to allow for scaffolding to be layered within the ports and medical grade adhesive to be around the scaffolding to hold it in place and seal the extra-tubular space from the luminal space. This design, because of its interface with the scaffolding has the same flaws as the previous design. The ability to achieve a similar orientation of the scaffolding with each experiment would be hard, as well as the removal of individual scaffolding for time trial experiments. Also, optical glass is an expensive material, and for our initial prototype may be more than we can afford at the current time, however, in future iterations of this design after modifications, this may be a plausible addition to the design. The use of screws to attach the clear polymer to the rest of the housing would also make opening the bioreactor somewhat tedious. A sketch of the design can be seen in Figure 6.

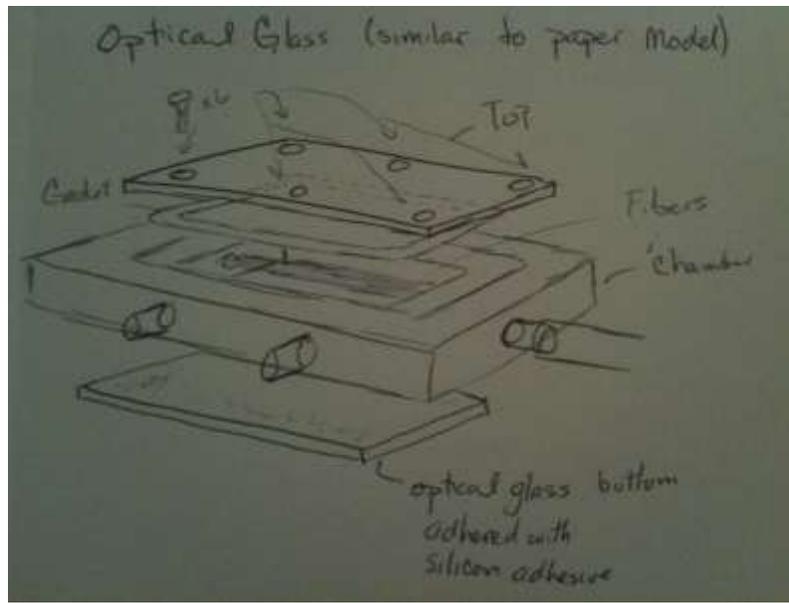


Figure 5: Bioreactor design using polycarbonate housing, optical glass bottom and acrylic top to allow for imaging of scaffold constructs.

The last design that we considered drew from some designs that I had come up with previously, but largely came from a design created by Amin. The design called for a tubular housing compartment that would have a window cut into the housing tube. Within the tube would be two caps at each end that would have inlet and outlet ports on each exterior end respectively. Between these two caps would be where the scaffolding would be. The scaffolding in this design was held in place by a cartridge design that would screw into the end caps at either end. The cartridge design had individual holes for each scaffold tube to attach too thus allowing for adequate spacing to be maintained between scaffolds for easy removal of the scaffolds. The disadvantage of this design is that it would require much more manufacturing on our part than the other designs. With the other designs, more of the components can be commercially bought. With this design however, the end caps would require custom machining, as would the housing tube. Yet with this design, we felt that it best identified and remedied the flaws of the commercial CellMax© system. It allowed easy access to the scaffolding through the window port, allowed time trial experiments, and could be made of materials that would be able to be autoclaved.

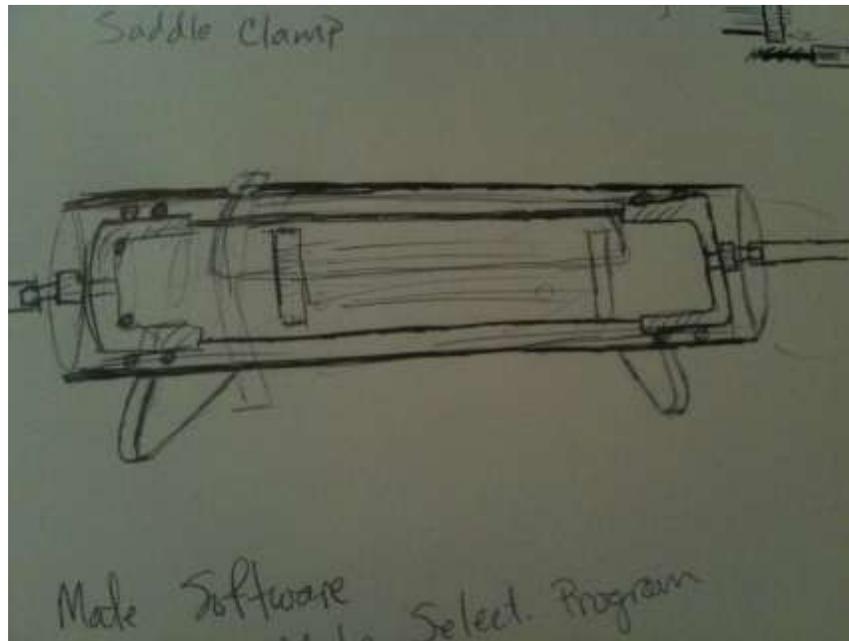


Figure 6: Sketch of the final design of the bioreactor. The image shows the acrylic tubing on the outside, with the end caps at both ends and end caps to support the scaffolding.

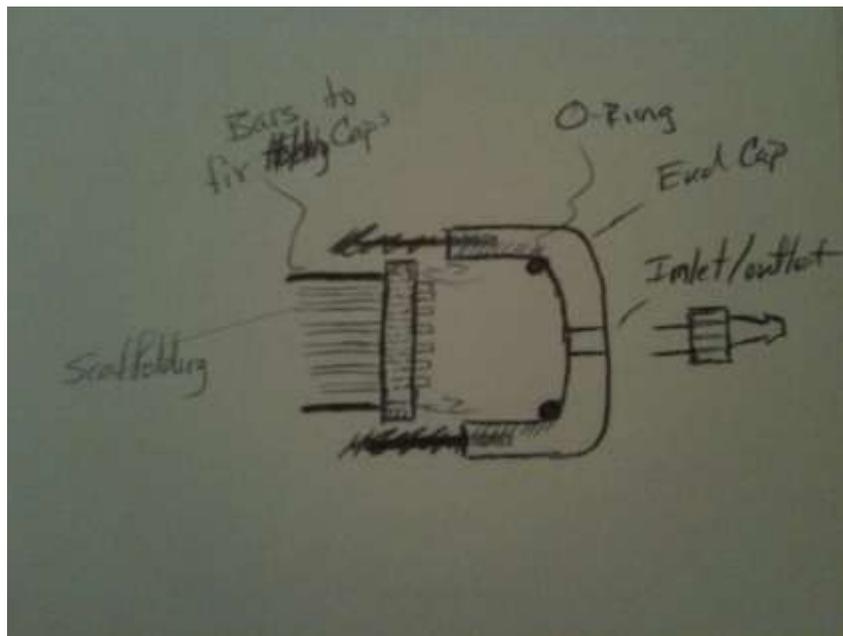


Figure 7: Image showing the end cap, and scaffold cap that support the scaffolding for the final bioreactor design.

New Bioreactor

To do this project, I, as well as another student Amin Mirzaaghaeian, designed a novel hollow fiber bioreactor system. After consideration of various designs, we decided to focus on a design that

utilized a tubular housing, and a cartridge of hollow fibers that could be slid into the tube, with proper sealing at both ends.

Our main concern in designing this new bioreactor system was the fact that the CellMax© systems were not reusable. This was a large challenge to the goal of the overall Blood Brain barrier project since for each experiment we would have to purchase more of the bioreactor systems at \$469.00 per bioreactor [12]. For drug companies wishing to test their pharmacologic products this cost may seem reasonable, but for an academic lab and student experiments, this was beyond our budget. The design of the CellMax© bioreactors meant that after an experiment was complete, the bioreactor housing had to be destroyed to get to the hollow fibers. There was no means of take the housing apart so that it could be reconstructed as the ends of the chamber were sealed as one unit. In order to get access to the hollow fibers the housing chambers were cut open using a band saw in the departments machine shop with the help of Lab Technicians Mr. Martin Koch, and Mr. Dave Laiho.

We wanted our new bioreactor to facilitate access to the hollow fibers without compromising the bioreactor itself. Rather than cutting the housing apart, we decided to cut a window view port into the side of the bioreactor housing. This would allow for removal of the window inside a sterile laminar flood hood so that the hollow fibers could be sectioned out for analysis. In selecting the material for the tubing of the bioreactor, we wanted something that was clear to allow visualization of the scaffolding, but also something that had strength and wasn't too brittle. Based on these desires, we selected to use cast acrylic from McMaster-Carr. While acrylic cannot be autoclaved for sterilization, we were able to have the material gassed using ethylene oxide. The acrylic used for the housing was a clear cast acrylic tube, 3" outer diameter, 2.5" inner diameter from McMaster-Carr. We chose this size of tube because it would allow for maneuverability within the housing when removing scaffolding. The window port had a silicon rubber gasket (McMaster-Carr P/N 5787T63) around its edge which allowed it to properly seal against the outside of the acrylic housing. The window was then held on using hose clamps with thumb screws that fit around the acrylic housing and acrylic window. Once the hollow fiber of interest was removed and the free ends sutured shut, the window could be placed back into position, and the experiment could be allowed to continue running. Figure 8 shows the housing with the stainless steel end caps in place, and gives a good view of the window view port.



Figure 8: Bioreactor prototype. In the image can be seen the hose clamps with the screws that tighten the housing around the stainless steel parts. The viewport window can be seen cut into the acrylic housing including the silicon gasket around the edge.

To allow for the reusability of the bioreactor meant that the materials used for the bioreactor should be able to be sterilized. For the housing of the bioreactor we decided to use a clear cast acrylic tube. The cast acrylic gives a good optical clarity and is also more impact resistant than other clear plastic materials. An issue that arises by using this material is that it cannot be autoclaved, which is how a majority of the sterilizing is performed in our lab. The high temperatures in the autoclave are beyond the operating range of the acrylic, and results in the acrylic deforming and degrading over time. Instead of using an autoclave, we decided to try using the Ethylene Oxide sterilizing method and see if the housing would withstand the process, which it did, as well as any silicon gaskets on the housing. For the cartridge and end caps that would slide into the acrylic housing we used 316 stainless steel. These can be autoclaved, and made sterile for repeated use in the bioreactor.

The manufacturing and machining of these materials can all be done in house at the machine shops at Cal Poly. The 316 stainless steel parts were machined out of house for our first prototype. This was done because there was limited time to get training on the CNC machines in order to machine some of the features on the steel, however for future production of other bioreactors, the training to operate these machines can be completed and the design can also be refined to use some other machining techniques and materials. The stainless steel parts can be seen in Figure 9. The acrylic parts can also be machined in the shops at Cal Poly. The acrylic housing was cut using a band saw, while the window that was cut into the housing was cut using an end mill.



Figure 9: To the left is an image of the stainless steel parts. At the top and bottom are the endcaps. In the middle are the scaffolding caps which will hold the scaffolding. The image on the right is all of the parts to the bioreactor disassembled.

Evaluation of Bioreactor Design

After we received our stainless steel parts from the machinist we realized that the depth for the o-ring groove was cut too deep to fit the standard size o-rings we had selected from Cole-Parmer. We believe that this can either be attributed to an error made in our SolidWorks© designs that we had sent in, or that the machinist made. To remedy this we layered the o-rings that we had originally planned to use in the groove. To test if this solution allowed for the housing chamber to be properly sealed against the stainless steel parts, we put the system together, then filled the chamber with water and left the bioreactor to sit for 24 hours. After the 24 hours we found that there was water that had leaked out and deemed that we needed to follow this up with an alternative solution. We thought that perhaps using o-rings with different durometer may provide a tighter seal against the bioreactor housing. We tested o-rings of varying durometers but decided upon o-rings with a shore A:70 durometer. This durometer wasn't too soft to the point that it would tear when the cartridge was placed inside the housing, but also wasn't too hard so as to prevent the o-ring from forming a proper seal. Yet the o-rings still didn't prevent the system from leaking. To resolve this problem Amin developed the idea of cutting the bioreactor longitudinally along its axis. We had previously discussed making the acrylic tube two pieces that were hinged together

and could be closed around the steel parts but thought this too difficult to seal. So, to compromise between the hinged tube idea and our initial prototype, we made one cut longitudinally, thus leaving the tube as one piece. Into this cut we placed some of the same silicon gasket material used to seal the window against the housing. Hose clamps with screws were used to tighten the acrylic housing tube around the stainless steel parts. Using this method of cutting the housing in addition with the hose clamps with screws and the layering of the original silicon rubber o-rings, we were finally able to seal the housing and prevent water from leaking from the housing after 24 hours. We also performed this test over a period of 72 hours, and there was still no leaking that occurred. A benefit of creating this cut down the longitudinal axis was that assembling the bioreactor became easier. Before, sliding the stainless steel end caps into the acrylic housing was somewhat difficult due to the amount of friction created by the tight fit between the o-rings and the acrylic housing. The current modification to the housing, inserting the stainless steel parts is much easier. The assembled bioreactor can be seen in Figure 10.

To further evaluate the bioreactor we hooked the system up to the CellMax© DUO pump system to be sure it could handle the pressure from the pump. From Bryan's work with the system, we knew to select the smallest pin size for the pump in order to get the proper physiologic flow and shear stress. We then hooked the inlet and outlet ports of the bioreactor to the pump accordingly and turned the system on using water as the medium. Since we hadn't developed scaffolds yet for our bioreactor at this point, we used two 18 gauge stainless steel hypodermic tubes which we had left over from our mandrel design for electrospinning which will be detailed later. The other holes in the scaffold caps were plugged using medical adhesive that was used to secure the scaffolding. The space outside of the tubes in the bioreactor housing was left empty and void of water. We had already proved that this area could hold water, and had no leaks, this test with the pump was to see if there was leaking between the scaffold caps and end caps of the device. With no water inside the extra-tubular space, if any water started to accumulate there, we would know there was leaking occurring. We then turned the pump on at the lowest setting which was the setting that Bryan had determined gave the proper shear and flow rates. After allowing the system to run for a few hours, we came back and found that there was no water that had leaked into the extra-capillary space, indicating that there was a proper seal between the scaffold caps and the end caps of the bioreactor. To be sure that the seal would hold, we turned the pump up to a higher setting and found that it was still able to hold water.

Once we had confirmed that the bioreactor housing was properly sealed and would not leak and compromise the sterility of any experiments being performed, we felt that the next step would be to attempt using cells in the system. If we could confirm that our system allows for cell adhesion and shares

similar properties as those *in vivo*, then we would be able to begin experiments to attempt to recreate the blood brain barrier. However before we could put cells in, we needed to obtain a material to use for scaffolding.



Figure 10: The above image is the fully assembled bioreactor.

Scaffolding:

Once the design of the bioreactor and the housing was complete, and it no longer leaked; we needed to determine what to use for the scaffolds that would be held within the bioreactor. The scaffolds that were used in the commercial bioreactor were polypropylene tubes coated in ProNectin™ F which acts to help cells adhere to the scaffolding. Inside the commercial bioreactor system there were 60 of these tubes. Each fiber has an inner diameter of 480 μm , wall thickness of 150 μm , an average pore size of .2 μm , an inner surface area of 100 cm^2 and an extra-capillary space volume of 1.5mL. The choice of polypropylene was made since it allows cells to adhere in environments where there will be shear stress and gas diffusion. The hydrophobic membrane allows the diffusion of gasses but not proteins or other molecules, similar to the epithelial tight junctions seen in the blood brain barrier. Our goal was to use a material that was similar to the commercial bioreactor, yet could either be made in house, or be purchased without exceeding our funds.

Scaffolds within Our Bioreactor

Our bioreactor was designed to only hold 8 scaffolds. We believed that eight would give us a good number to sample from to perform time-point experiments, and also allowed excision of scaffolding without the bioreactor chamber becoming too crowded with scaffolding. If more or less scaffolds are desired, other end caps can be made to accommodate various sizes, patterns and numbers. Only using eight scaffolds also allows us to use less scaffolding material per bioreactor and thus save costs on material purchases.

When trying to search for distributors of hollow fibers that met the specifications for our bioreactor, we had trouble finding products that would fit within our budget. From GE Healthcare a sterile ready-to-process hollow fiber cartridge of scaffolds with 10 kD molecular cut off, and .5mm inner diameter size cost approximately \$1,075 [13]. Alone this would have taken up the entire budget for our project. It was also difficult to find producers of polymer tubing that matched the characteristics that we wanted for our bioreactor. We tried contacting

several companies, such as Zeus Inc.©, but either wouldn't always hear back or didn't receive much help in finding the product that we needed.

Electrospinning

At this point we decided to try creating the scaffolding in house. There are various means to create polymer scaffolding such as dipping mandrels into polymer solution and letting them dry, then sliding them off the mandrel. However, this process wouldn't yield the correct material characteristics for what we needed in order to recreate a blood brain barrier model. Our lab does perform electrospinning experiments to create scaffolding for blood vessel mimics experiments. Electrospinning is a process that can make a polymer scaffolding by using electrical charge to draw out long, fine fibers of a liquid polymer. These fibers are collected on a collector that can be of various shapes and sizes. In our case, the collector was a mandrel that spins and forms a tube using poly (lactic-co-glycolic acid) or PLGA. Creating the scaffolding this way provides many benefits.

Benefits of Electrospinning

First off, the electrospinning process of collecting these fibers results in a very stochastic looking pattern of webs and fibers under high resolution. This characteristic is beneficial because it acts to more accurately mimic the natural environment in the body which has various layerings of fibers of collagen and other natural connective tissue materials. The more natural patterning of the material may allow for better adhesion of cells to the scaffold and facilitate a better anchoring of the cells, especially under shear stress forces. The patterning may also allow for a more natural blood brain barrier endothelial phenotype of the cells once they have been cultured on the material. Also this layering of fibers creates many pores in the material, and fine tuning the various aspects of this process can allow control over the characteristics of the pores as well as the fiber sizes that comprise the scaffold.

Another benefit of performing the electrospinning is that our lab has experience using electrospun scaffolds. Purchasing scaffolding material from a commercial dealer would mean we would have to totally evaluate the new material and it's properties in regards to cell adhesion and growth. By using the electrospun scaffolds that we create in house, we can use the previous

experience of our labs work with these scaffolds to more quickly optimize the scaffolds for use in our blood brain barrier system. The PLGA scaffolds have been used in the blood vessel mimic models performed by other students in the lab, and they have shown that endothelial cells will adhere and display proper phenotype on the scaffold. Other students have also been working on performing cryo-sectioning and histological analysis on blood vessel models using this scaffolding material. Also by doing this in house, we would be able to tailor our scaffolds slightly to experiment with what characteristics are essential to creating a blood brain barrier phenotype. With all the research and experiments being performed on the material in our lab currently, much of the groundwork has been laid out to use this material, and doing so will save us time and money in developing our blood brain barrier model.



Figure 11: Image of the electrospinning machine at Cal Poly.

Mandrel for Blood Vessel Mimics

The student who currently has led the experiments and research on electrospinning PLGA scaffolds is Yvette Castillo. She has worked to develop various different experiments to determine what the best settings are in order to create a scaffolding that facilitates the best environment for endothelial cells for blood vessel mimics. Some of the various factors that contribute to the characteristics of the PLGA scaffolding include percentage of polymer in solution, flow rate of the polymer, electric charge, distance to the collector, and rotation of the

mandrel. Most of her current work has been done to create scaffolds for the blood vessel mimics being pursued in the lab. These require larger diameter tubes than what we want for our blood brain barrier model. The collecting mandrels used for the blood vessel mimics are 4mm in diameter, 14cm in length and are made of 303 stainless steel. The mandrels feature a shallow hole drilled into one end to allow attachment of a live center on the machine for the mandrel to spin. The live centering interface is attached to a threaded screw that can be moved in and out to allow for the mandrel to be held tightly in place. The other end of the mandrel has a 1/16" diameter, 1/2" length stainless steel slotted spring pin put through both sides. This pin acts as an interface with the driving component of the electrospinner and allows for the mandrel to be spun in order to allow collection around the rod.



Figure 12: Live centering interface of the electrospinning machine. The pin that acts to center the mandrel is within the cylinder shown in the image.



Figure 13: The interface end that drives the pin end of the mandrel and spins the mandrel.

Designing our Own Mandrels

In order for us to electrospin scaffolds for use in a blood brain barrier model, we need mandrels that are a smaller diameter. A point of contention was what the exact size of the smaller mandrels should be in order to get the best results in our model. Most all models of the blood brain barrier experiments in the literature use 1mm or smaller diameter hollow fibers. One source we found that listed desirable characteristics for scaffolding in a bioreactor similar to ours was in U.S. Patent 6667172 by Damir Janigro and Mark S. McAllister [14]. From their assessment, to get a proper phenotype for a blood brain barrier model, the scaffolding should have a molecular weight cut off of anywhere from 1kD to 1000kD, and a pore size from $.01\mu\text{m}$ to $5\mu\text{m}$ (or they say ideally from $.01-.64\mu\text{m}$). They also say that the inner diameters for the hollow fibers should be between $20\mu\text{m}$ and $1000\mu\text{m}$, larger than this can limit the diffusion, and smaller makes the scaffolding difficult to create and to seed cells onto. They state that the wall thickness should be approximately $2\mu\text{m}$ to $200\mu\text{m}$. These are some of the requirements that we want to have our scaffold meet. We decided that an inner diameter of 1mm would be the best for our design. Smaller diameters would make the handling of the scaffolds difficult and increase the risk of damaging the scaffolding. We created holes in our scaffold caps that allow for scaffolds with an outside diameter of 2mm, but plan on the outside diameter of the scaffolds to be approximately 1.5mm, meaning the wall thickness would be approximately $250\mu\text{m}$. One

characteristic that wasn't in the literature but that we were aiming for was to have a fiber size of approximately $2\mu\text{m}$ to facilitate proper cell growth and adhesion.

Mandrel Design Considerations

In order to create this we needed to create our own mandrel collector since the current ones are too large. The mandrel needed to fit in the machine's interface points but still allow for easy removal of the scaffold from the mandrel once the process was complete. In order to do this, many different mandrel designs were devised.

One design we considered was to just modifying the current mandrels by machining the center of the mandrel down from a 4mm diameter to 1mm diameter. The idea was that by doing this, it would not require the purchase of more stainless steel stock, and would only require the use of a lathe. However, machining the mandrel down to such a small diameter would prove difficult on the lathe. Also, if we only machined the middle, then the barbell shape of the mandrel would prevent the scaffolding from being able to slide off the mandrel.

The second design considered was similar to the first, but addressed the issue of removing the scaffold. It would require the 4mm mandrels to again be machined down, but instead of just machining down the middle portion of the mandrel, we would machine down about $\frac{3}{4}$ of the mandrel's length. This would provide a means of removing the scaffold by sliding it off the end of the mandrel. Yet this doesn't identify the issue of machining the steel down to such a small diameter. It is unlikely that you would be able to machine the steel down to this diameter without damage the mandrel. Also it would be difficult to get a good finish on the mandrel because the material would likely flex when it got down to a small diameter. This would make removing the scaffold from the mandrel harder as there would be increased friction, and the fibers would likely be torn and sheared as they were caught on the rough surface of the mandrel. Also if the mandrel was machined down to 1mm, it would be hard to have a deep enough dimple to align in with the live center so that the mandrel stays centered while spinning.

At one point we considered trying to use just a 1 mm diameter stainless steel rod, similar to the original mandrels used, but with the entire rod at this diameter. This would allow us to remove the scaffolding without having to worry about a poor finish on the surface of the metal

that might damage the inner surface of the scaffold. There would also be minimal machining that needed to be done on the mandrel. This would mean that students would not need to gain training on the lathe, and time machining would be minimized. An issue with this design; however, is the need to have interfaces with the electrospinning machine. To try to put a 1/16" pin through the 1mm rod would mean trying to drill a hole into the 1mm rod. This would prove to be extremely difficult, and would risk damaging the rod if the drill slipped on the small surface of the rod. The interface with the electrospinning machine at the other end of the rod where it interfaces with a live center would also prove difficult to incorporate since drilling onto the end of the small diameter rod would prove difficult.

The last design that was developed looked at modifying the idea of using a 1mm rod. Rather than trying to place the 1/16" pin through the 1 mm rod, we decided it would be best to attach something to the 1mm rod that had a larger diameter, and would thus allow the pin to spin the rod. While looking for something that could be used to attach to the rod, Mr. Laiho and I thought of the idea of using collets similar to ones that are on the end of drills and allow for different drill bits to be used on the drills.

Manufacturing the Mandrel

After searching on McMaster Carr, Mr. Laiho found several pin vises that would work and ordered some that had various collet sizes in order to determine which would best hold the 1mm rod. The ones that were ordered were 5/16" in diameter, and 3 3/4" long with pin vise sizes ranging from 0-.125" (P/N 8455A12). These were fairly cheap and cost \$12.84 each. These pin vises were double-ended, meaning that there was a pin vise at each end. The pin vise was then cut in the middle so that there were now two separate pin vises. The collets that were in the vises could hold various sized object depending on which end was facing outward. While we only needed one size to hold our 1mm rod, this gives our lab the options of using various small diameter rods to act as a collector for the electrospinning machine, and thus many different experiments can be performed to determine what size scaffolding works best for various types of models. For the end of the pin vises that had been cut to separate the double ended pin vise into two separate pin vises, a 1/16" hole was drilled through the diameter of the pin vise, and the 1/16" pin was placed through it to allow for the vise to be spun, an image of this can be seen in

Figure 5. Now that the attachment for the rod was finished, we needed to find 1mm stainless steel rod.



Figure 14: The top image shows the pin vise that was modified to fit into the electrospinning machine interface. The bottom image shows the pin that was put through the diameter of the pin vise to allow for the spinning drive to spin the mandrel.

The original hope for the rod was to find a solid 1mm 303 stainless steel rod. At this diameter however, there were not any internet searches that turned up products; instead, all searches regarded the use of 1mm wire instead. Wire would not work for our purpose as it would bend and flex and could distort the fibers, as well as not being able to fit the interfaces of the electrospinning machine. We considered stiff wires such as piano wire as it may have proved more durable; however, it could also have bending issues. I then looked into using hypodermic spinal needles, searching various vendors; however, showed that these needles were very expensive. At Atlantic Medical Supply Inc. the price for a box of ten 18 gauge spinal needles that were 6" long each was \$98.00. Instead, I found 3' stock hypodermic tubing available on McMaster Carr that was listed at \$12.75 and which we ended up purchasing. The hypodermic tube could easily be held by the pin vises, and since it was tubing and thus hollow, the hole provided a means for interfacing with the live centering hole. The 19 gauge hypodermic tubing

was precision miniature 304 stainless steel, welded and drawn with an outer diameter of 1.0668mm. 304 stainless steel has essentially the same material properties as the 303 stainless steel that was used with the original electrospinning mandrels, yet is slightly more difficult to machine. Yet with the size of the material we are using, and the limited amount of machining we would be doing on this tubing, this would not present itself as an issue. With the attachment we had created, we were not sure what length of rod would be best to fit in to the electrospinning machine. Having the stock tube would allow us to cut various lengths and then determine which fit best into the machine, and being tubing and not solid rod, had added rigidity that would prevent it from easily being bent. Once we had the stock hypodermic tubing, we decided to cut the tubing at lengths of 5 ½” and 5” and add the pin vise attachment to each. Then to determine which length would best fit into the electrospinning machine, we tried to fit each into the interfaces of the machine and found that the 5” rod worked the best. An image of the finished mandrel can be seen in Figure 15.

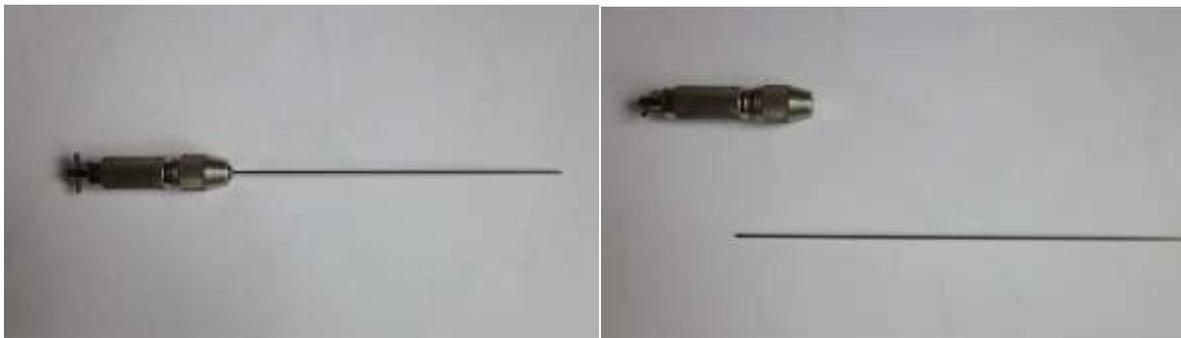


Figure 15: The image to the left shows the mandrel in complete assembly. The stainless steel hypodermic tubing fits into a collet which the pin vise tightens around. The image on the right shows the pin vise and hypodermic tubing separately.

Testing the Mandrel

The next step was to run the electrospinning machine with the newly made mandrel in place. One concern of ours with this project was how the mandrel would handle being spun at high speeds. The pin vise, while small, had considerable weight to it since it was made of steel. Our fear was that if the mandrel was off center in the machine, it could harm the drive of the electrospinning machine, or perhaps even fly off the machine and injure the students performing the experiment. Because of the safety concerns, Yvette Castillo and I tested the mandrels in the presence of Mr. Laiho. The electrospinner was in a fume hood, with polymer box encasing it,

further preventing any injury from occurring if the mandrel did spin off. Once the mandrel was in place the translation setting of the mandrel was set to 3, and the rotation speed was set to 5 on the machine. There were no issues with the mandrel coming out of the machine interfaces during spinning. The mandrel did however seem to show some slight bending when spun in the machine, although it is very slight, and we don't believe it is significant enough to cause issues with collecting the scaffolding polymer.

Once we knew that the mandrel would be able to interface correctly with the machine we wanted to try creating some scaffolds. The new 1 mm mandrel was given to Yvette to spin. Since we weren't sure how the scaffolds would turn out, similar settings that were used for the normal blood vessel mimic scaffolds were used to create the blood brain barrier scaffolds. The settings used to create our scaffolds was a distance of 8 inches from polymer syringe to collector, a flow rate of 6.0mL/hr, a solution of 15% PLGA, and a voltage of 18kV. Initially a volume of 3mL of solution was spun onto the mandrel however as can be seen in the next section this created very thick wall thicknesses and for the subsequent spin a smaller volume of solution was used. When we received the spun mandrels from Yvette, we discovered that the pin vise was covered along with the length of the mandrel. In order to get the scaffolding off from the mandrel we used a scalpel blade to cut around the circumference of the rod up next to the pin vise, and then slid the scaffolding off from the rod.

Evaluating Scaffolds

Scanning Electron Microscope

Once the scaffolds were created using the electrospinning machine we needed to evaluate some of the characteristics of the scaffolding. Preferably we wanted scaffolding that met some of the characteristics outlined in previous literature. The main things that we want to characterize with our scaffolds are the fiber sizes, the wall thickness, and the porosity of the material. To do this we require some means of viewing the scaffolds at very high magnifications. In order to do this we used a scanning electron microscope (SEM). A scanning electron microscope works by sending a high energy beam of electrons at the object being imaged. The beam is then moved across the object in a raster pattern, and the resulting electrons and x-rays are collected and processed to produce a highly detailed image of the surface of the object. SEM's range in size and the magnification level that they can produce. On campus Dr. Lily Laiho has two desktop SEM's that are for our department to use and have previously been used to evaluate electrospun scaffolds. Yvette ran the mandrel once using the same settings she uses for the blood vessel mimic scaffolds. When we got the scaffoldings from her we cut the scaffolding and slid it off the mandrel as previously mentioned. Then we imaged the scaffolding at various magnifications in order to get an idea of the characteristics of the scaffold.

ImageJ Evaluation of Wall Thickness of the 1st Scaffold

To image the scaffolds we cut various sections off of them. We made a cross section cut to allow us to evaluate the wall thickness of the scaffold, as well as the fiber size. We also made some cuts longitudinally down the length of short sections of the scaffolding in order to give ideas of the fiber diameters and variations in fiber size within the scaffolding. With the first spin that Yvette created, we found that the wall thickness of the scaffolding was larger than we had desired. Previous literature had stated that a wall thickness of about 200 μm was appropriate for blood brain barrier models, and our goal for these scaffolds was approximately 200-300 μm . A macro SEM cross sectional image of the first scaffold created can be seen in Figure 16. Using the measure feature in the ImageJ software, I was able to measure the wall thickness of the scaffolding by setting the scale to match the scales provided at the bottom of each image. The images I used to determine the thickness of the walls can be seen in Figure 17 and Figure 18. To

determine this thickness I would measure from the inside of the luminal wall to the exterior wall. Within each image I would measure in the middle of the cross section, as well as the top and bottom halves of the image. In some images measuring to the exterior wall became difficult as some of the fibers appeared to be frayed and were coming off from the bulk of the scaffold wall so that a definite edge was hard to determine. After collecting wall thickness measurements on both images I averaged the values to get an average wall thickness of 1042 μm . This was much thicker than the 200 μm range that we had hoped for, however we felt that an easy solution to this would be to use less volume of polymer solution on the next spin, so instead of using 3mL, approximately 1mL was used. While the wall thickness on this first scaffold was not what we had hoped for we still wanted to evaluate the fiber size to get a rough approximation of how this scaffold compared to the literature.

Measuring Fiber Size of the 1st Scaffold

For measuring the fiber size we used images that were taken of the lumen of a longitudinal cross section of the scaffolding tube. The same method used to measure the wall thickness of the scaffolding was used to measure the fiber sizes. This data was collected from Figures 19 and 20. The images were split into 6 square areas, with one measurement of a fiber taken from each area, so 6 measurements were taken from each image for a total of 12 measurements. These measurements were then averaged together to get an average fiber size of 5.39 μm with a standard deviation of 2.56 μm . This indicates that there was a varying degree of fiber sizes in the scaffold. Some of the fibers appeared fairly large, which would not be ideal for our endothelial cells to adhere to, but the exact parameters that control fiber size are still being determined by students performing experiments with the electrospinning machine, and hopefully will be optimized in the future to provide the best results. This fiber diameter should still work well enough for our initial tests.

Measuring the Wall Thickness of the 2nd Scaffold

The second scaffold was spun using approximately 1mL of polymer solution in the hope of decreasing the thickness of the scaffolding wall. The rest of the parameters were kept the same. Once the scaffold was created, it was then evaluated using the SEM just as the initial scaffold was. Measurements of the wall thickness and fiber size were taken in similar manner to

get the same number of measurements. Figures 21, 22 and 23 were the images used for the evaluation of the wall thickness of the second scaffold. After again inputting this data into excel and the average wall thickness was found to be 296.43 μm with a standard deviation of 60.97 μm . This wall thickness is much closer to our desired wall thickness (between 300-200 μm) and I believe that once we have gained more experience using this mandrel, and effects of the different electrospinning parameters are better known, we will be able to more accurately produce more scaffolds with this wall thickness.

Measuring Fiber Size of 2nd Scaffold

For the fiber size measurements Figures 24 and 25 were used. The fiber size measurement method again followed the same ImageJ protocol that was used with the first scaffold. After collecting this data, the average fiber size was found to be 5.31 μm with a standard deviation of 2.13 μm . This is very similar to the results that were found with the first scaffold created, and should facilitate our need for now, however smaller fibers would be desired if possible. The success in creating a scaffold with an ideal wall thickness is a large step for our project, and will allow us to soon start running experimental tests with cells to determine their true viability in regards to creating a blood brain barrier model.

Pore Size

One parameter that is important to our project, yet would be difficult to measure through image analysis is the pore size of the scaffolding. The SEM can only view the surface of the material, so knowing what pore sizes were deep in the material would be difficult. Even to attempt to use image analysis of the surface images collected would be difficult since the material is made of many fibers, and determining of boundaries of pores could be problematic and inconsistent. Instead of using a direct image analysis method I propose that determining the pore sizes of the materials would best be done through physical experimental procedures. This would include using fluorescent microspheres of a known diameter and flowing them under pressure into the lumen of the scaffold at a known concentration. Then the concentration of the microspheres that passed through the walls of the scaffold could be measured in any media that went through the scaffold walls. This would give an idea of average pore size in the scaffold. Besides using fluorescent microspheres, any molecule of a known size and concentration could

be used to determine the pore size, however this could take many iterations of the experiment to get a proper idea of the true relative pore size.

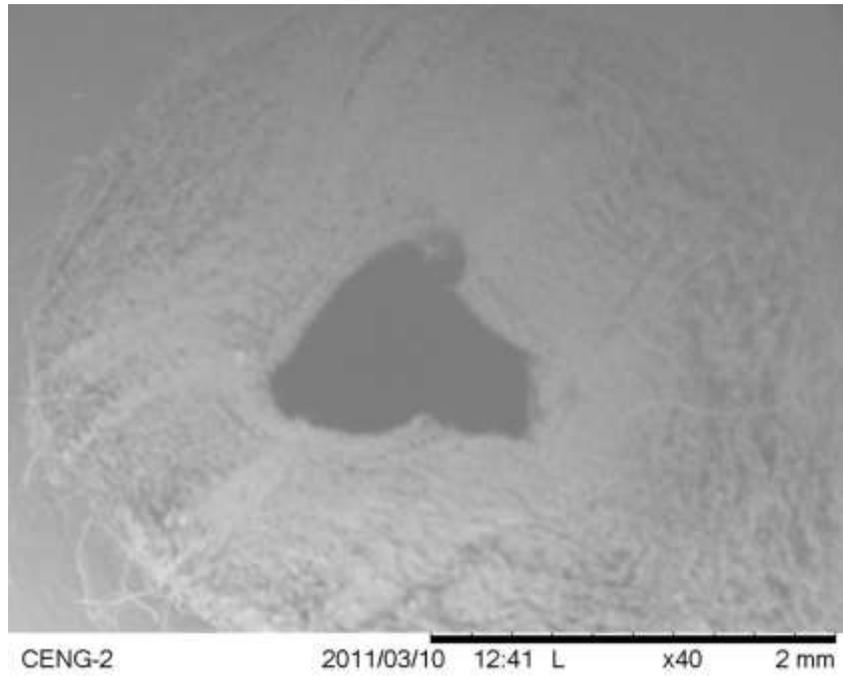


Figure 16: x40 SEM view of the 1st Scaffolding

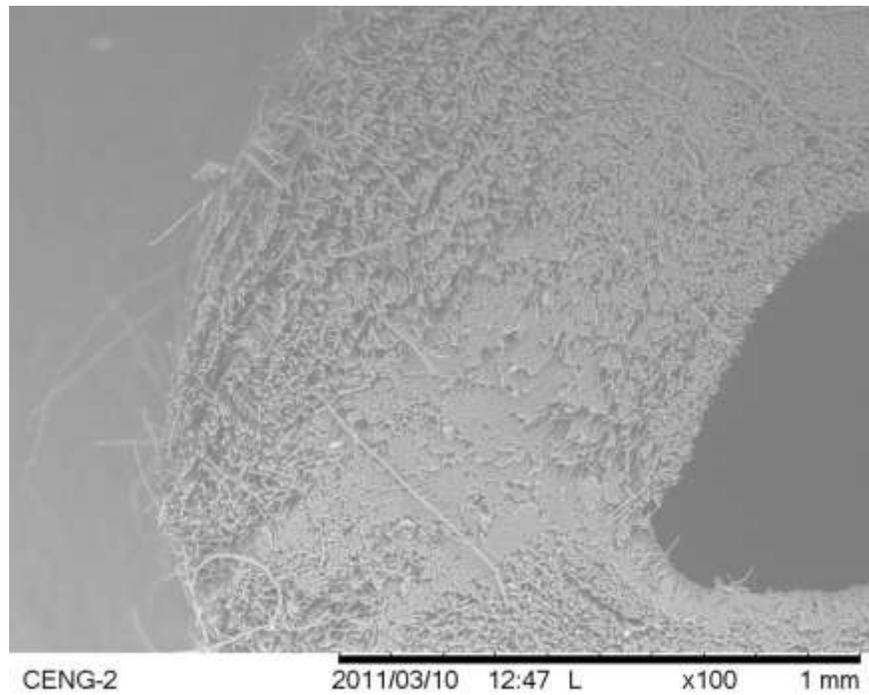


Figure 17: Image of left side of scaffolding for assessment of wall thickness.

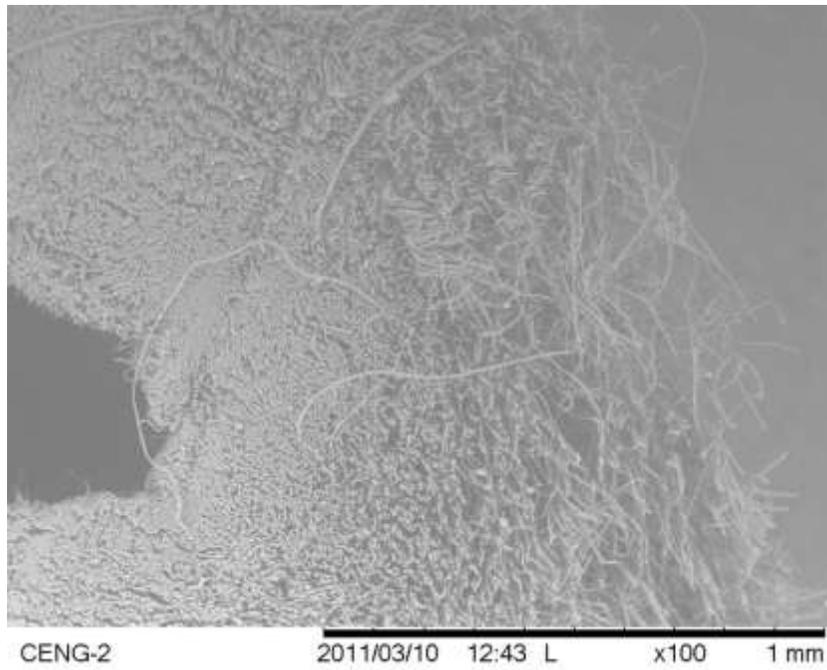


Figure 18: Image of right side of scaffold for wall thickness measurement.

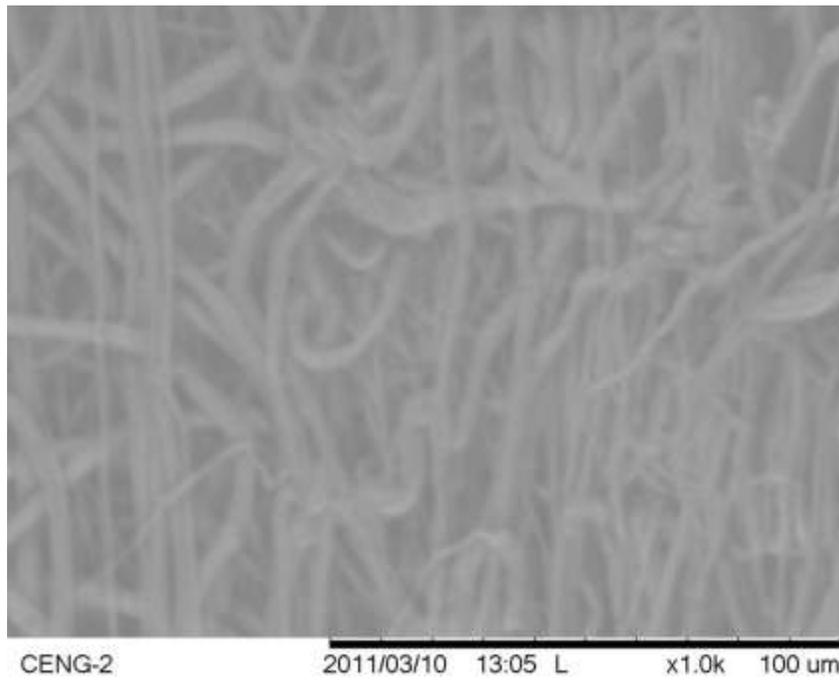


Figure 19: SEM image of inner lumen of first scaffold for fiber size measurement.

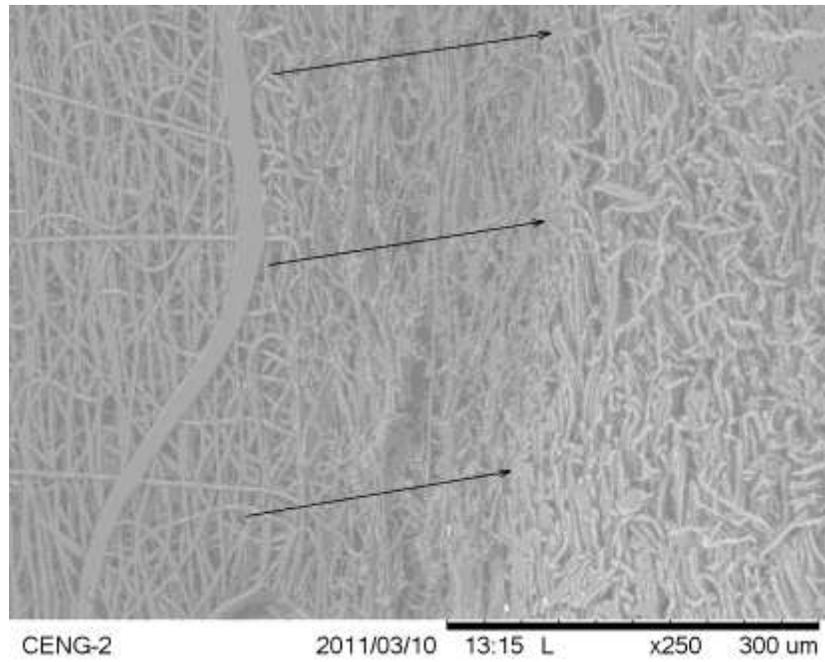


Figure 20: Second SEM image of inner lumen of first scaffold for fiber size measurement. Black arrows indicate the wall of the scaffold, with the left side being the lumen.

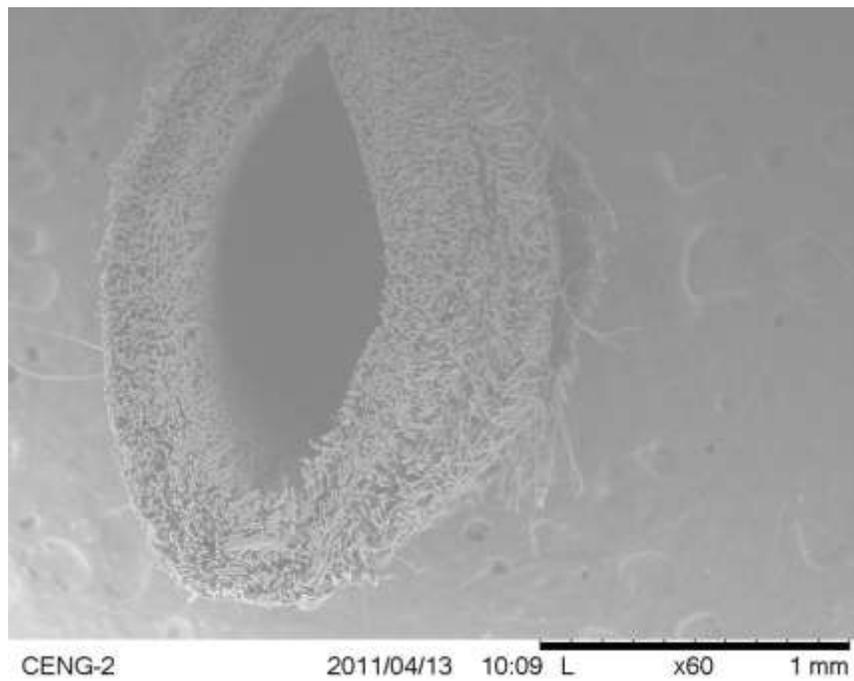


Figure 21: x60 SEM macro view of the cross section of the second scaffold.

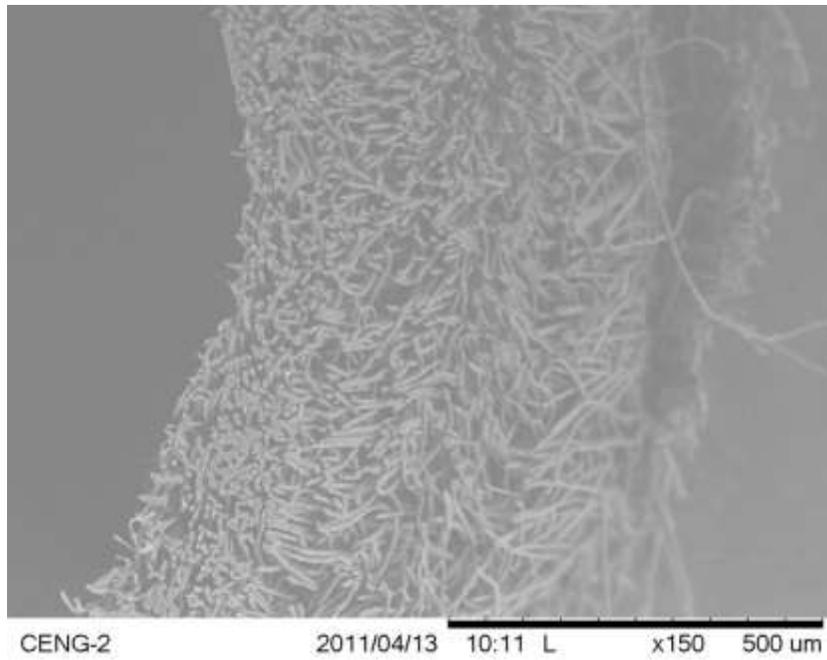


Figure 22: Image of right wall of the second scaffolding for wall thickness measurement.

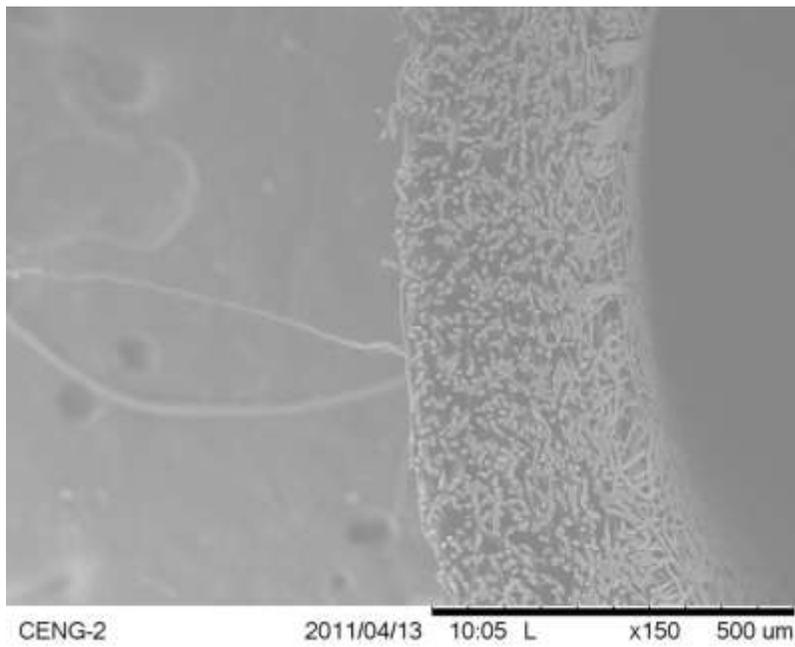


Figure 23: SEM image of left wall of second scaffold for wall thickness measurement.

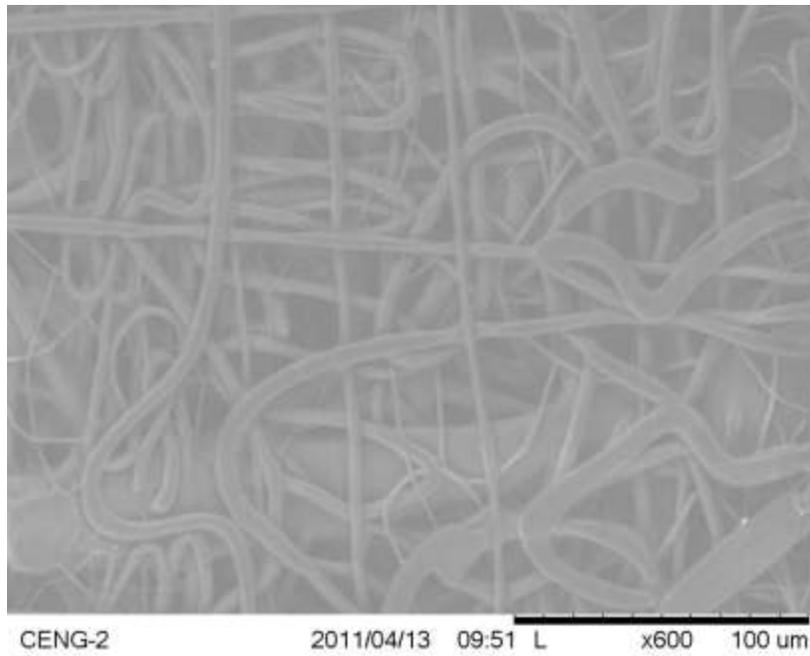


Figure 24: SEM image of the lumen wall of the second scaffold for analysis of fiber size.

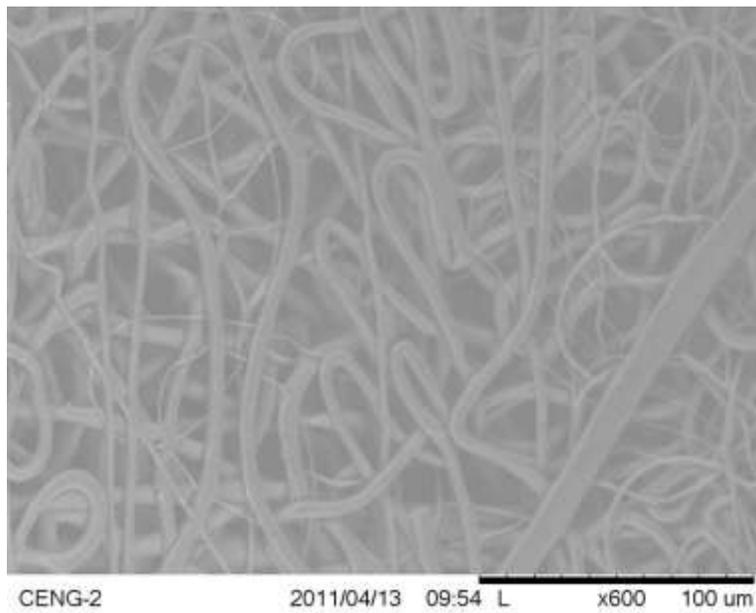


Figure 25: Second SEM image of lumen wall of second scaffold for fiber size analysis.

Discussion and Conclusion:

The overall focus of this project has been to develop a model of the blood brain barrier *in vitro* that would allow our lab to contribute to the field of tissue engineering, as well as drug delivery to the central nervous system. This project was initially started by former biomedical engineering student Bryan Brandon, and has since been broken into various aspects and divided amongst several students to facilitate advancement of the project. Myself and now Amin Mirzaaghaeian have been tasked with working on the design and creation of an in house bioreactor system to house our experiments and attempts to recapitulate the characteristics of an *in vivo* blood brain barrier. T.J. Eames has been working to better develop the means of biologically testing for markers of a true blood brain barrier model, characterized by different surface proteins and tight junctions between the endothelial cells.

I have described in this paper what my role has been in the development of the bioreactor for the *in vitro* model. My main contributions have been in the design and manufacture of the bioreactor, the design of the mandrel for creating electrospun scaffolding for our model to be cultured on, and the evaluation of these scaffolds. The previous system used to initially develop a protocol for culturing bovine aortic endothelial cells, and C6 glioma cells, was the CellMax© hollow fiber bioreactor cartridge. While this system allowed us to initially become familiar with the process and steps to create a blood brain barrier model, we felt there were many shortcomings in its design that didn't warrant purchasing a new cartridge for each trial, and that we could improve upon.

The first goal of my portion of this project was the development of a re-usable bioreactor that we could manufacture in house. We were able to successfully design and manufacture a bioreactor that is able to be sterilized for reuse, that allows for easy access to the scaffold constructs, and allows for time trial experiments to be performed using one bioreactor system. All these elements of our bioreactor are improvements over the single use CellMax© DUO system which requires the housing to be destroyed to get to the scaffolds. The parts that our bioreactor is composed of are all able to be manufactured in the Cal Poly machine shops and can be made by students who have received the proper training. While our bioreactor has improved upon the commercial system, it wasn't without its own issues. We had issues with sealing our bioreactor housing. Yet throughout the manufacturing and design process we were able to troubleshoot the various problems that we had and create a bioreactor that is liquid tight, and can be tailored to various experimental design formats.

Once we had completed the design for our bioreactor, we needed scaffolding to act as the construct for our blood brain barrier model. After trying to investigate commercial polymer tubes to

purchase and failing to find a viable option, we decided that we could create our own in house PLGA electrospun scaffolds. We already had an electrospinning machine, and with the work being done with the electrospinner by students, we can soon tailor the scaffold to the right material parameters that we desire. The electrospinner generates a scaffold environment that can be made to properly simulate the environment that the native cells in the body would experience. In order to use the electrospinning machine for our model and experiment, we would need to create a new mandrel to act as a collector to get the proper dimensions for the construct. Doing so required more consideration than we previously thought since we had to incorporate the mandrel into the current interfaces on the machine, which were tailored for larger mandrel sizes. However, as presented in this paper, we were able to successfully create a mandrel that interfaces properly with the machine, while still allowing proper collection of the electrospun polymer.

The final portion of the project was to evaluate the electrospun scaffolds that were created on our mandrels. The evaluation method used image analysis to determine the fiber size, and wall thickness of the scaffolding. This was carried out using the ImageJ software, and upon refinement was able to get scaffolding with the proper wall thickness and close to the correct fiber size. With further work being performed by students to characterize what the different parameters of the electrospinning process do in regards to the material characteristics of the final product, I believe that we will soon be able to create scaffolds that can be tailor made to fit out model specifications.

This project is continuing on the groundwork laid out by Bryan Brandon and his work to develop various protocols for creating a blood brain barrier model *in vitro*. Through his work we were able to recognize the need for a novel bioreactor. The commercial system had many shortcomings which we felt we had the ability to address and improve upon. By creating these bioreactors in house we will effectively reduce the cost of the system, make the system more user friendly, and improve the convenience of the system to facilitate better experiments.

While we have made progress with this portion of the project, there is still room for more work to be done. While we were able to create a few electrospun scaffolds and evaluate them using SEM, cells still need to be seeded onto the scaffolds to ensure that they will adhere properly. Once we are sure that cells will adhere to the scaffolds, we can run the entire system with cells and evaluate the effectiveness of the system in simulating the blood brain barrier environment and its ability to culture the BAEC's and C6 glioma cells.

Future work can also be performed on the design of the bioreactor. The bioreactor that we have created is a prototype and still has many features that can be optimized. With our initial design hopes, we had planned to manufacture the parts through rapid prototyping; however we couldn't find the correct equipment on campus to create our parts. Yet with further research, we believe materials better suited for this project and cheaper could be found.

Overall, I believe that this project is a significant step forward in our pursuit to create a blood brain barrier model. We have created a new design for an in house bioreactor, and have seen the project through to an initial prototype. Our prototype design has been shown to be liquid tight, is reusable, allows easier access to the scaffolding constructs, and allows for time trial experiments. The importance of the ability to perform time trial experiments will prove important in further development of our system with a culturing of the C6 glioma cells and bovine aortic endothelial cells to show the proper model development. The hope is that with further refinement and experiments, we will be able to use our model to test the effectiveness of drugs to cross the blood brain barrier and target various pathologies and diseases that affect the central nervous system. The early nature of this project provides many aspects that can be the focus of future senior projects and master's theses.

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