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Preparing Cell Seeded Microthreads for the Clinic

Biomedical Engineering Department

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by

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Approved:

Prof. Glenn Gaudette, Major Advisor

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1. Introduction

Cell therapy is a technology that is pivotal to many new medical procedures that are incredibly beneficial. Many researchers have had great results using cell therapies to heal patients suffering in a number of ways. More specifically, human mesenchymal stem cells (hMSCs) have the potential to be turned into living tissue that can replace dead or damaged cells. These engineered cells are advantageous because they do not run the risk of being rejected by the patient. Research is currently being conducted, at Worcester Polytechnic Institute, on hMSCs to repair dead heart tissue that results from a heart attack. About 295,000 people year in the US suffer a myocardial infarction (out of hospital cardiac arrest) and a high percent must undergo invasive heart surgery to repair the damaged tissue. After a heart attack, part of the heart tissue dies. This dead tissue in the heart is very different from healthy living tissue. Dead heart tissue can be stiff and does not expand or contract very easily.

The research being done at WPI uses fibrin microthreads as a delivery vehicle for hMSCs. The current delivery device and bioreactor for these microthreads, a small piece of medical tubing with plastic clamps, is rudimentary in design. With continued success, this therapeutic cardiovascular procedure will soon move to clinical trials. There is currently no optimal way to prepare, ship, and store cells to be used in these types of procedures. Sterility, viability and safety are among a few of the major problems that must be addressed. Another aspect is the ease of use for the doctors who will be delivering the cells. The system currently being applied was designed simply with lab technicians in mind and does not take into account these crucial factors. Transporting the cells from the lab to the clinic, and their storage on site, must also be taken into consideration.

If a device and system could be developed to account for these issues, than it would allow for the progression of many cell therapies and other techniques that are currently being studied. The goal of this project will be to design an efficient and safe system to get the cell seeded microthreads to the patient. An ideal method of storage and administration of the cell seeded microthreads will be developed.

2. Literature Review

2.1.The Heart

The heart is a myogenic muscular organ that is found in all animals with a circulatory system[i]. It is responsible for operating in conjunction with the circulatory system to pump blood and oxygen throughout the entire body. It is located in the chest cavity just behind the breastbone and in between the lungs, and it is divided into four main chambers (the left and right atria and the left and right ventricles). The atria receive the blood and the ventricles pump the blood from the heart to different organs in the body[ii]. The heart is a vital organ that can be damaged by a variety of factors which lead to serious health problems such as myocardial infarction[iii].

2.2 Myocardial Infarction

Myocardial infarction, or a heart attack, generally occurs due to the interruption of blood supply to part of the heart. Damage occurs if blood flow is not restored to the organ within 20 to 40 minutes of the blockage[iv]. The result of blood interruption causes irreversible death of the heart muscle. A heart attack is usually due to the narrowing or blockage of a coronary artery. These blockages usually arise from a buildup of plaque or a rupture in the wall of the artery. Approximately one million Americans suffer a heart attack each year, and four hundred thousand of them die as a result of their heart attack[v]. Most heart attacks are minor and people ignore them because they confuse the chest pains with other problems.

Death is more likely to take place when symptoms are ignored and a more fatal attack occurs. When it comes to medical treatment, the aim is to unblock the affected artery and restore blood flow to the area of the heart in danger as soon as possible. Today there are many medicines that are used to reduce the chance of heart attack. Anti-platelet medicines, for example, are used to prevent the tendency of platelets to clot and cause blockages[vi]. In severe cases defibrillators are used and open heart surgery is common. Transplants can be used if the heart is beyond saving, but there is a limited supply of donors. This is why there is so much funding going into research for new methods to treat heart failure.

2.3 Mesenchymal Stem Cells

Stem cells are very unique cells that can undergo differentiation into a variety of specialized cell types. They can also maintain the ability to proliferate while maintaining the undifferentiated state. The discovery of human mesenchymal stem cells (hMSCs) has been a suggested pathway to bypass ethical hardships confronted by embryonic stem (ES) cell research[vii]. Furthermore, while ES cells can theoretically differentiate into all of the cells in the body, HMSCs with their multipotent characteristics can be isolated from adult bone marrow and induced *in vitro* and *in vivo* to differentiate into an array of cells that constitute bone, cartilage, fat, muscle, and other kinds of

connective tissue such as tendon[viii]. Bone marrow is considered the top candidate for isolation of mesenchymal stem cells because they exhibit a high congregation of MSCs while still remaining fairly accessible. HMSCs can be obtained in large quantities, cultured, and frozen for preservation without losing their capacity to form a variety of cell types, including cardiomyocytes. Credited to these versatile qualities, HMSCs are the best candidate for cell delivery for cardiac tissue among numerous adult stem cells[ix].

The goal in the implantation and experimentation of hMSCs is to manipulate them in such a way that they can express cardiogenic markers. However, the techniques used to stimulate myogenic differentiation in hMSCs vary. Experimental data produced by Strauer and colleagues in their study titled, *Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans,* suggest that bone marrow-derived cells may contribute to the healing of myocardial infarction (MI)[x]. The method used in their study was to treat 10 patients by intracoronary transplantation of autologous, mononuclear bone marrow cells (BMCs) via balloon catheter in addition to standard therapy in another 10 patients after MI. In the following 3 months, the infarct region had decreased significantly within the cell therapy group and was also significantly smaller compared with the standard therapy group. While infarction wall movement velocity increased significantly only in the cell therapy group, further cardiac examinations that were performed for the cell therapy group showed significant improvement in stroke volume index, left ventricular end-systolic volume and contractility, and myocardial perfusion of the infarct region. These results suggest that selective intracoronary transplantation of autologous, mononuclear BMCs is safe and seems to be effective under clinical conditions[xi].

In a similar study titled, *autologous bone-marrow stem-cell transplantation for myocardial regeneration,* tests were concluded using the direct injection technique in six human patients. All of the patients had experienced MI and undergone coronary artery bypass grafting (CABG). Surgeons injected up to 1.5×10^6 autologous AC133+ bone-marrow cells into the infarct border zone in each patient. In the following 3-9 months after the surgery, global left-ventricular function had improved in four of the patients, and infarct tissue perfusion had significantly improved in five of the patients. These results suggest implantation of AC133+ stem cells to the heart is safe and might induce angiogenesis, therefore improving perfusion of the infarcted myocardium[xii].

2.4 Culturing hMSCs

The hMSCs are first isolated a healthy donor. The MSCs from the marrow sample can then be cultured and grown in a Petri dish containing human mesenchymal stem cell media with basil media and fetal bovine serum in order to acquire the cells needed for coculturing with human cardiomyocyte. Under culture conditions, when there is direct contact of cardiomyocytes and hMSCs, the hMSCs begin to express the cardiac specific proteins myosin heavy chain, β-actin, and Troponin-T. During coculturing human cardiomyocytes can be labeled with a fluorescent indicator cell sorting and then mixed with the hMSCs in a smooth muscle medium and seeded at the desired cell density. Cells should be cultured in an incubator at 37°C for duration of 48 hours. Once 48 hours has passed the cells can washed with buffered saline solution to remove excess fluorescent label. Trypsin can be added to detach the cells from the surface of the Petri dish[iv].

2.5 Microthread Seeding Procedure

The microthreads that are inserted into the heart must have a certain number of cells attached in order for them to be effective. There are many things that the cells will need in order to survive and adhere to the microthreads including, temperature, fresh media and a strong ECM (extracellular matrix). hMSCs are placed in to the bioreactor. The purpose of the bioreactor is to act as a stable environment for the cells to live and naturally adhere to the microthread rather than the surrounding walls.

The exact procedure of seeding the microthreads involves many steps and different chemicals to feed cells, and hydrate the threads. The first step in the process is the removal of cells from a flask through the use of Trypsin. hMSCs are anchorage dependent and the Trypsin is an enzyme that will release the cells from the flask. Once returned to a stable environment the hMSCs will reanchor. Next the cells are spun in a centrifuge and the excess Trypsin is removed. The cells are then placed into a media that contains a serum containing proteins that will stop the actions of the Trypsin. Before the cells are placed in the bioreactor with the threads, the threads are hydrated in order to increase cell adhesion. Since the cells are super concentrated they require a large volume of media in the bioreactor in order to survive. Inefficient levels of media in the bioreactor can lead to high levels of waste which could be harmful to the cells and result in dead cells remaining adhered to the threads. The bioreactors are placed into a plastic vial which attaches to a rotator and the entire assembly is placed in an incubator. Currently the cells are incubated for approximately 24 hours before they are ready to be used for implementation.

Different approaches to this process could be taken to perfect the cell seeding system. Larger volumes of media could be used while maintaining the same percentage of cells to possible yield a higher adhesion amount. There is also the possibility of having communal bioreactors in which multiple threads are seeded at once. Also the amount of time that the time they are incubated for has yet to be thoroughly researched.

2.6 Tissue Engineering

WPI's microthreads are currently pending a US patent. They are created from collagen and fibrin using a thread model of in-vitro ACL scaffold regeneration. They are created to have similar mechanical and structural properties as collagen threads; they are made up of a structural protein that is found in the provisional matrix during wound healing. The focus of microthreads is to incorporate them into the process of complex ligament tissue engineering.

Tissue engineering is a field of biomedical engineering which focuses on biomaterials and cells; also is known as regenerative medicine. Most tissue engineering involves biological functions, liquids and materials. Some specific tissue engineering categories are bone, cartilage, and blood vessels. When focusing on one aspect scientists must look at the structural and mechanical properties that coincide with the specific aspect. When looking into each aspect one must research the tissue's properties

and work on improving their functions and growth. Using the properties researched and realizing the need for this specific field, much advancement has been made.

One category of tissue engineering is called bone tissue engineering and works with the structure of the bone and properties it holds. Examining these properties they are then used to create bone supplements/substitutions. Many of the key factors that go along with bone tissue engineering are harvested cells, recombinant signaling molecules and 3D matrices. Using a scaffold to attach the harvested cells and improving the growth of the bone, one must make sure the cells will multiply and transform to where they contain bone-like properties.

Since bone tissue engineering technologies have been emerging; more and more techniques have been discovered to work and not work. Some current methods of bone replacement are autografts, allografts, and metallic replacements. Within those replacements there have been some arising challenges, such as the amount of time it takes the cells to transform and survive on scaffold, design systems, complex scaffolds, and new biomaterials.

Another category for tissue engineering focuses on the cartilage engineering. Cartilage is a tissue that can sometimes degenerate by causes of trauma and disease. However since this is a new focus for tissue engineering the current repair/treatment methods haven't been perfected yet, but does hold a great promise in the future. The basic procedure that has come from cartilage engineering is the use of a biocompatible scaffold that holds the bioactive cells needed to differentiate the other cells. The best approach of cartilage engineering is using scaffolds that are created by natural and synthetic biomaterials.

Over the past few years numerous advances have come out that pertain to cartilage tissue engineering. Such are cell–scaffold composites which are to help with the lack of cell retention rate. In addition the scaffold being used should almost be identical to what the repaired tissue is in order to be successful. A second advancement is the use of mesenchymal progenitor or stem cells for cartilage engineering. Using these cells provide less of a concern when looking at the donor site, lifespan and cell dedifferentiation. With these advancements already scientists are looking to overcome more challenges such as if the tissue has a hostile environment or if this can be combined with gene therapy.

Blood vessel tissue engineering is becoming more and more important in the tissue engineering field. The approach to blood vessel engineering is recellularization, which occurs when one strips the living cells from the donor leaving the extra matrix as a scaffold. Once the scaffold is formed it is seeded with the new cells which are able to form back into the original tissue because of how the scaffold is structured. Recellularization is becoming more and more frequent in laboratories, and even in bone tissue engineering. One advancement scientist had discovered is using adult stem cells to created functional blood vessels that can later replace synthetic grafts which are usually require in vascular bypass surgeries. They had determined that one can build the blood vessel using the donor's tissue and an animal's adult stem cells, using these blood vessel the complications of the surgery would be reduced.

Blood vessels have also been created another way which is to harvest skin cells and remove them leaving just the scaffold and used endothelial cells of the patients because they had discovered that the fibroblasts can clog the blood vessel. These endothelial cells are able to hold a smooth blood

flow in the interior of the vessel created. Today blood vessel engineering is proven to be used in kidney dialysis and to have been effective and successful.

Tissue engineering has clearly become an advancing study in the biomedical field. A great contribution to the reason it has been advancing so rapidly, is the need to transplant organs, and tissue growth/replacement. This specific type of engineering incorporates biologists, chemical engineers, material scientists, surgeons, and other clinical researchers, which is important because each role plays a key part in coming up with different tissue engineering procedures. The greatest advancement is the focus of skin tissue and the ability of skin grafts and using the patient's own skin cells to repair the skin of patients with skin disorders or burn victims. However over the next few years there will be many obstacles that scientists must overcome such as the lifespan of the cells, how long it takes the tissue re-growth to become effective, how many times one must go through surgery, and if there is an immunity rejection when using a donor's cells into a patient's body.

3. Project Strategy

This project was completed in two stages, one being completed in December 2010 and the second in May 2011. The two stages were necessary because I would be graduating in December and my three group members would continue my research. My project would focus on improving the current design of the bioreactor, which had many issues. The rest of the team would continue my research and examine transportation methods and storage. Dr. Gaudette was an asset throughout the entire project and he played the roles of both client and advisor.

3.1 Initial Client Statement

The initial client statement was as follows:

Design a system to deliver cell seeded microthreads to the clinic. The system should consider storage on site; preparation prior to delivery and shelf life.

3.2 Clarification of Initial Client Statement

The initial client statement was a vague description of the problem that needed to be solved and it needed both clarification and specification. Extensive background research on relative topics, such as, stem cell research, cell culture and storage, cryopreservation, and standard operating procedures was done in order to gain a better understanding of the factors involved. Informal interviews were done with Dr. Gaudette as well as students who had been working directly with the original bioreactor were held to find limitations and dislikes. Some of the major concerns with this design involved an exposed needle that presented safety issues, as well as the two plastic clamps which made the device awkward to work with (see Figure 1). Up to this point, no research had looked into the longevity of the cells if kept in this bioreactor. After conducting this background research and having a more elaborate understanding of the device, we formulated a revised client statement:

Design a system of delivering cell seeded microthreads from the laboratory to the operating room of a clinic. This system should focus specifically on the time between arrival at the clinic and the time of insertion of cells into patient. This design must consider preparation prior to delivery to the patient and also provide a minimum shelf life of one week and storage on site. The final design must maximize cell viability while still guaranteeing safety to all the users involved.

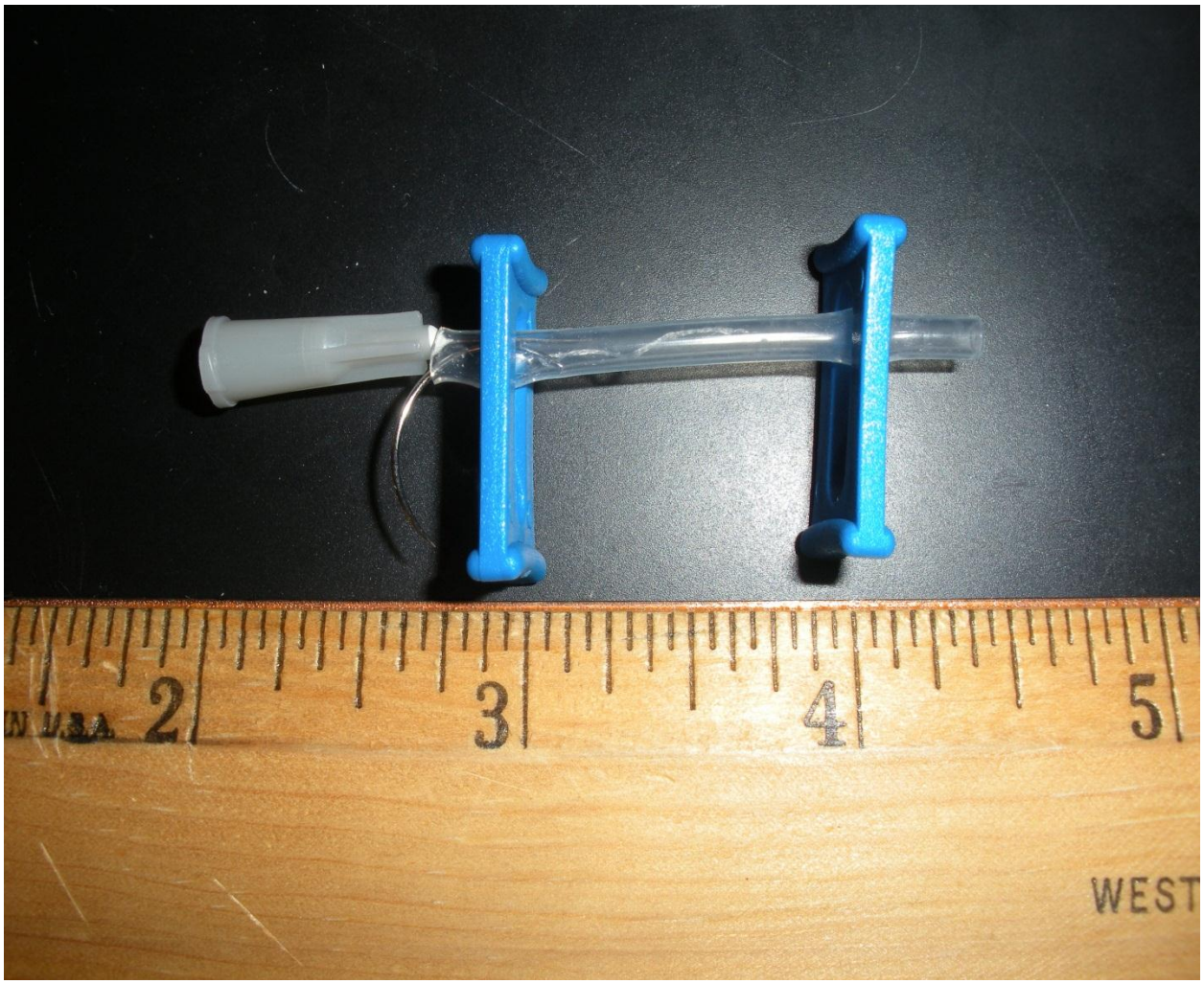

Figure 1

4. Alternative Designs

4.1 Objectives, Functions & Specifications

From our research, and speaking with our client and potential users, we began to form list of objectives pertinent to our project:

- Maintain cell viability
- Safe
- Device must be small
- Short application/preparation time
- Maximum shelf life
- Environmentally Friendly
- Simply Design/User Friendly

After these objectives were then confirmed by our project advisor and client, Professor Gaudette, we began to develop specifications. To better understand each of the aspects involved with this project, the team evaluated all of the objectives, functions and constraints. To evaluate the importance of each of the objectives that were created, a pairwise comparison was conducted. This simple chart form of comparing ideas is a good way to rank objectives. Each member of the group completed a pairwise comparison and the results were merged and averaged to create an overall list of ranking. The top three ranking objectives were safety, cell viability and sterility and the lowest ranking objective was the ability to hold >1 microthread.

Safety was ranked the most important objective involved in our project because without the wellbeing of the user and cells, our device would not be feasible. The original bioreactor design contained an exposed needle that created a serious safety concern for any one coming in contact with it. There is a lot of handling required with the bioreactor when it is being prepared for seeding and prior to surgery when the sutures are removed for implantation. If a technician or surgeon was punctured with this needle it would not only be a potential biohazard, but that particular suture would have to be disposed as well. Speaking with a student, Andrew Kazanovich, he noted that it was irritating and difficult at times to work around the exposed needle. The safety of the cells was another important aspect of safety that we worked on. The cells would to be kept from being shaken, exposed to extreme temperatures, UV light and other unwanted biological agents. The team began working solutions to this problem and created a list of ways that this safety condition could be improved. This list of safety concerns was broken into two categories, user and cell safety. A list of ways to promote safety in both situations was created:

- Safety
    - Cell Safety
        - air tight packaging
        - durable packaging
        - bio-agents
        - temperature control
        - UV protective cover

        - minimize time out of incubator
        - O_2 and $CO_{2\ regulation}$
    - User Safety
        - No exposure to cells
        - No exposed needles
            - magnet
            - hard cap/container
            - well or depression
            - "cork"
            - Sleeve (with an antiseptic

In order to maintain efficacy of the procedure, the cell viability would also have to be accounted for. The hMSCs need to be stored in a container that offered them protection from contamination from the environment. Our client wanted cells to be viable for up to a week, so the device would need to have the proper attributes to allow this. If the cells could not adhere to the microthread in the device or remain alive the device would be considered a failure. To protect the cells the device would also have to durable and not be affected by human contact. Another function desired was the devices ability to hold more than a single microthread at one time. It was desired that the device would contain multiple threads and maintain viability. The options for a container that could hold multiple threads were either to have individual bioreactors or one large communal bioreactor.

To maintain the life of the cells, the device needed to be sterilizable. We considered many methods of sterilization such as using an autoclave, ethylene oxide and cold sterilization. If the device could not be sterilized, it would not be accepted under the guidelines of the FDA for a medical device[xiii]. The sterilization is also necessary to stop and airborne pathogens or dirt that could hinder the cells.

Although we were presented with minimal constraints, they were all vital for successfully completing this project. The main constraint we were confronted with was the time limit that we were given. The first section of this project needed to be completed within seven months. Since I would be graduating it was imperative that the project was completed by the previously stated deadline. Another constraint involved with our project was the monetary assistance that we were given, which was $156 per group member. Finally the size of the device was an important constraint that needed to be considered. Our device would need to be big enough to contain the microthreads and small enough to fit in a standard incubator. A small device size was also important to minimize the overall cost involved.

4.2 Conceptual Designs

After compiling a detailed list of functions and constraints our team began to create basic designs. Brainstorming sessions were held to develop possible designs for our device. For each specification we had we created a design that would facilitate it. Here are some of the conceptual designs that we created:

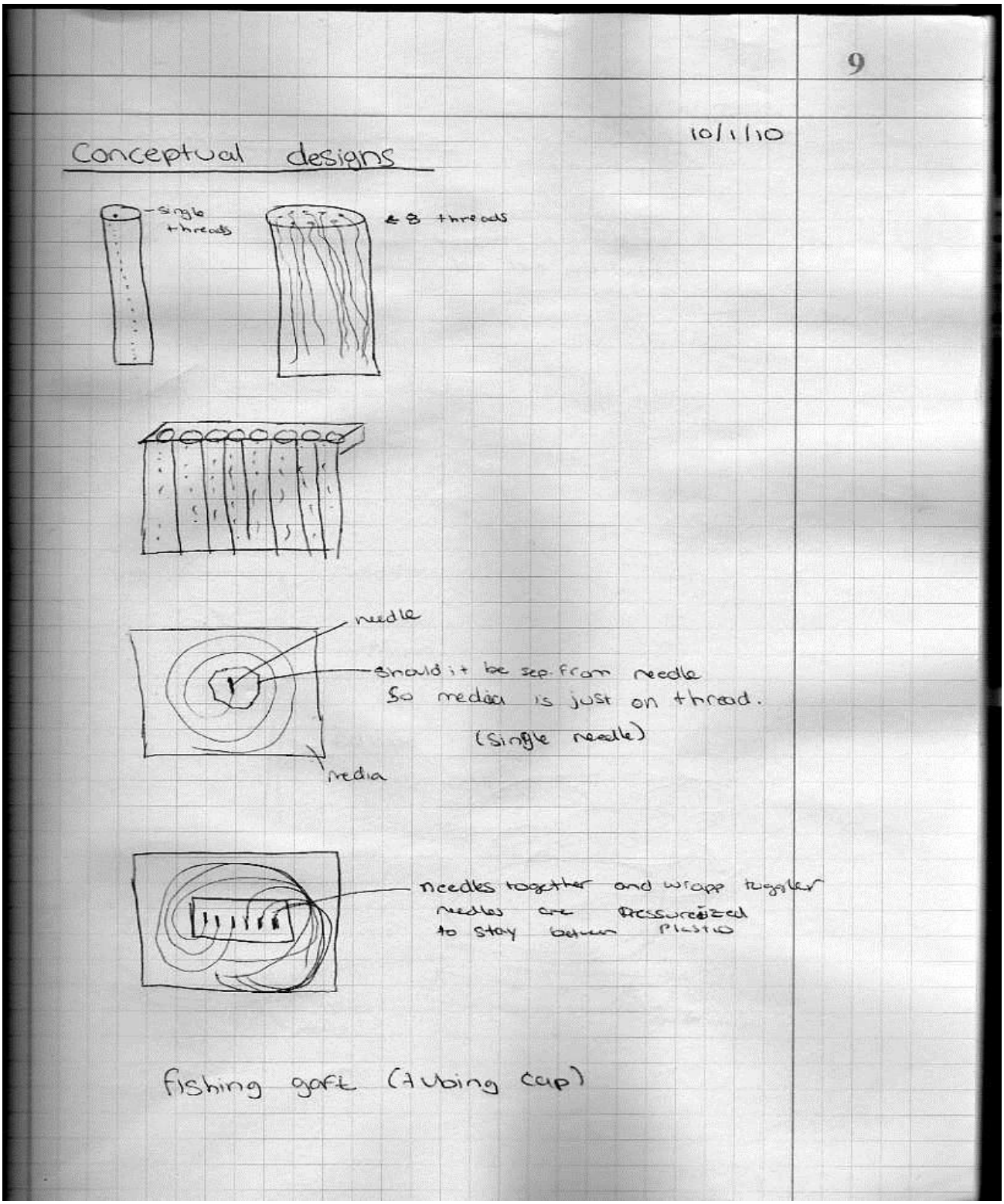

Figure 2

[1) A circular 8 well bioreactor with cap.2) A rectangular 8 well bioreactor.3) A square single thread bioreactor.4) A square multiple thread bioreactor.]

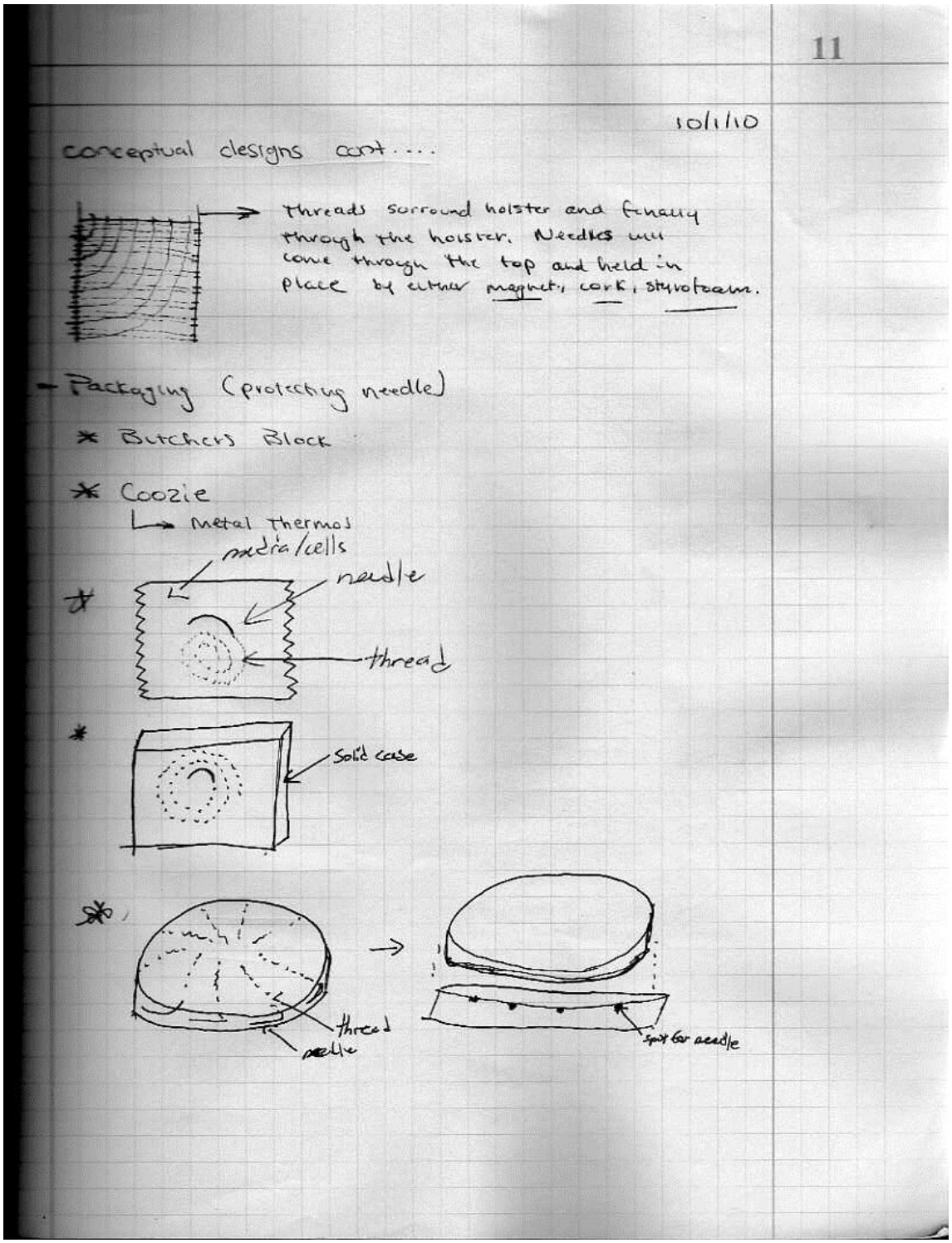

Figure 3

[1) Disposable, individually packaged microthread, could use as many desired at one time. 2) Reusable, individually packaged microthread. 3) Petri dish style, 8 microthread, communal bioreactor.]

A series of designs were considered including both communal and individual bioreactors. After evaluating our conceptual designs it was decided that the final design would multiple tubes to contain a total of 8 microthreads. To achieve our size constraint, a cylindrical design would be utilized. A tapered end would be applied to the cylinder allowing it to be inserted into a standard rotisserie. The rotisserie machine is a piece of equipment that it used to aid in the process of seeding the cells the microthreads. The spinning of this device allows for constant movement of the cells and media inside the bioreactor, not allowing it to settle. This cylindrical design resembled the same shape as the previous bioreactor that was being used. Finally this design was chosen in order to maximize the ease of use for both the lab technicians and the surgeons.

4.3 Final Design

After choosing the different aspects from many conceptual designs, we formulated a preliminary design that would achieve the maximum number of functions. In order to create a prototype of this preliminary design, it needed to be drawn in using CAD software. Using Solidworks, the design of the prototype was created so that it could be processed by the Rapid Prototyping machine. The Rapid Prototyping machine has the ability to print 3D objects, layer by layer, out of plastic. A prototype would enable the team to examine the compatibility of the device with the microthreads and necessary equipment. Pictures of the drawings can be seen here:

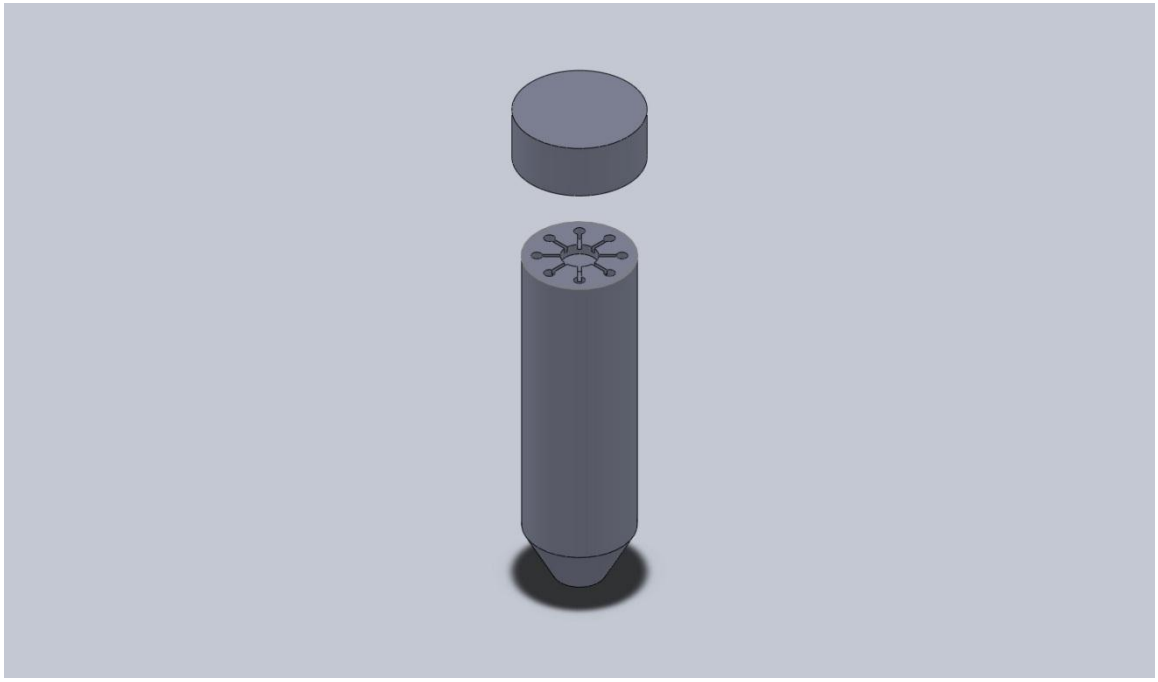

Figure 4

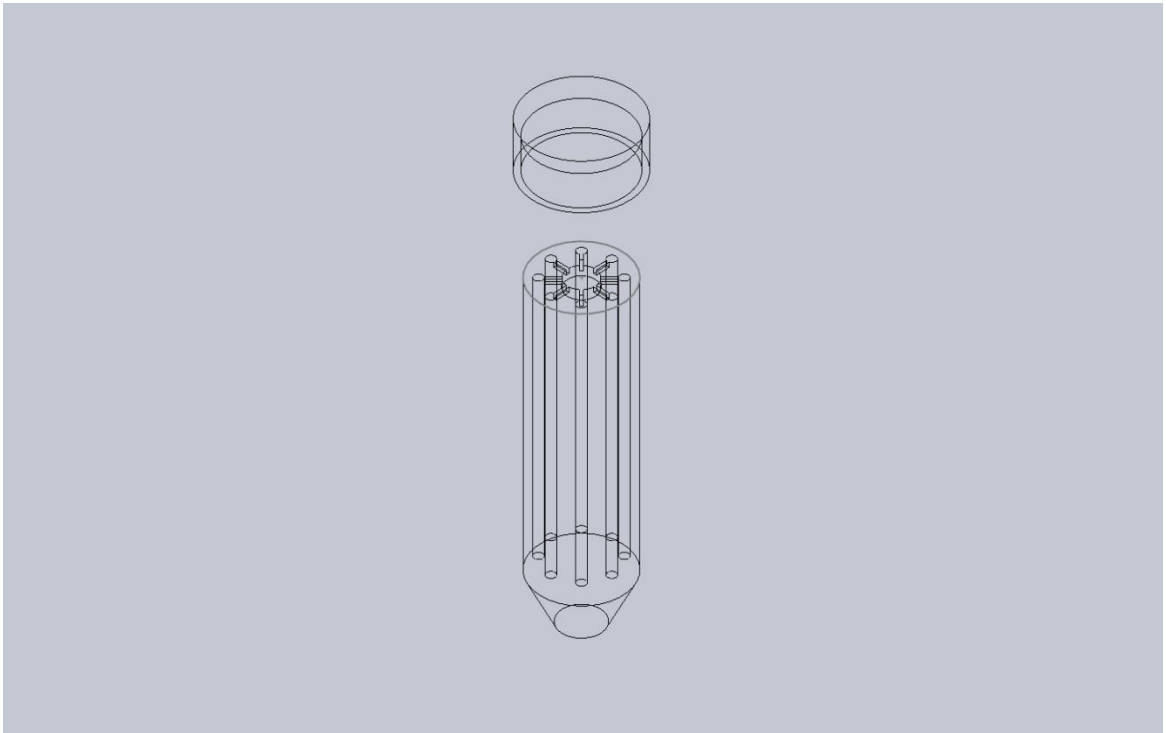

Figure 5

5. Design Verification

Using the prototype as model, our group did preliminary testing on the final design that we had created. We examined the devices ability to hold eight sutures at a time. The size of the device was also examined to confirm that it would fit it necessary equipment such as a standard incubator and the rotisserie device. Ethylene oxide was a viable method of sterilization for this device. This design is not only durable but satisfies all of the functions required for maintaining cell viability. The needles rest in the grooves on the top of the device, preventing possible puncture by one of the users. The prototype standing alone and containing microthreads can be seen below.

Figure 6

Further design verification will be conducted by the remaining group members in the following months. A working prototype will be made to test the ability of this device to successfully seed microthreads with cells. The longevity of the cells in the bioreactor will also be determined by examining the amount of time that this device can remain on the shelf while keeping the cells alive.

6. Discussion

Previously the device being used as a bioreactor was complicated and needed a lot of developments. Our group met the objectives of designing a system for expediting and improving the process of preparing cell seeded microthreads for the clinic. The progression of this process is still ongoing however; we have already made significant improvements to this medical procedure. The remainder of this section will confirm that each of the objectives was met.

User safety was improved by creating an area that will contain the needle and protect the users. With the needle safety placed into the "holster" that was created, it is difficult for an accidental puncture to occur when handled properly (Figure 7). The previous design used a flexible piece of plastic tubing that could damage the cells on the thread if squeezed. The new bioreactor is made out a strong plastic that can be constantly handled without damaging the cells or threads. Instead of holding a single microthread, this new design can hold eight at one time, which reduces the amount of time needed for not only seeding but also application. Since multiple sutures are needed for a single surgery this also reduces the number of materials involved in the process. All of the objectives of this project were completed on time and done within the stated budget. This design allows for an easier and more efficient system of preparing cell seeded microthreads for the clinic.

6.1 Manufacturability, Ethics and Influences

The price of the prototype that I had made was very inexpensive costing only about $15.00. This device was not only cheap but easy to manufacture because it can created within 12 hours. This bioreactor device design could be machined out of any material. All of the holes and physical aspects of the device can be machined using only a small number of machines including a drill and a reciprocating saw. A cheap and easy to produce device would create a low cost for not only laboratories but also for the patients receiving treatment. This device could be made out of any number of materials and does not require any hazardous or scarce materials.

This device combined with microthreads inside it will offer a beneficial treatment for patients who have suffered a heart attack. This device will have a very positive societal impact because it will make the lives of all the users significantly easier. The simplicity of this device allows for a decreased preparation time by lowering the number of required materials. The communal design of this bioreactor, among other factors, will lower the application time which means a decrease in medical bills as well as a shorter recovery time.

This product does pose any political concerns because it will help people live better lives. In terms of ethics, this medical device only presents effects as it will be in the technicians, doctors and patients best interest to use it. The device itself can only help people and will assist in creating a good and satisfying life. The communal bioreactor created for this project will house 8 microthreads that will be designed to regenerate dead tissue which lead to an increase in personal heath for many people.

Figure 7

7. Final Design & Validation

The task given to this project tem was to create a bioreactor that would have better characteristics than the one previously in use. After conducting thorough background research and speaking with various users we developed our conceptual designs. The problems involved with the previous design were relatively obvious which allowed us to work quickly toward a final design that would solve these problems. Once we had moved on to our final design that allowed for the accommodation of 8 microthreads it was an easy decision to have a rapid prototype made. This was a cheap and easy way to have a physical model of our test that could examined and used to conduct preliminary tests. The full project breakdown was done by creating a Gantt chart that dictated the dates that each section of work would be completed (Figure. 8).

To test the appropriate size of the device to ensure that it would fir in the rotator device. It was found that the device could fit in the rotator but not easily as it was a little short. The device could easily hold the 8 sutures at any one time and they could be easily inserted and removed for seeding and application. The direct comparisons to the previous design result in less materials and equipment needed. Due to the cap involved with the device, there would no longer be an exposed needle which means that it would now be much safer. Finally this enclosed device would keep the cells protected and the needles sterile.

Number	Task	Resource	Start	End	Duration	% Complete
1	Intro		8/4/2010	8/25/2010	16	50.0
2	Literature Review		8/4/2010	8/25/2010	16	25.0
3	Project Strategy		8/4/2010	9/10/2010	27	
4	Alternative Designs		8/4/2010	10/1/2010	42	
5	Design Verification		8/4/2010	11/5/2010	66	
6	Discussion		8/4/2010	11/23/2010	77	
7	Final Design Validation		8/4/2010	11/23/2010	77	
8	Conclusions & Recommendations		8/4/2010	12/9/2010	88	
9	Identify Problem or Need		8/4/2010	12/9/2010	88	100.0
9.1	Define Project Objectives		8/4/2010	8/25/2010	16	
9.2	Define Project Functions		8/4/2010	8/25/2010	16	
9.3	Define Project Specifications		8/4/2010	8/25/2010	16	
9.4	Develop Conceptual Design		8/4/2010	9/10/2010	27	
9.5	Evaluate Conceptual Design		8/4/2010	9/17/2010	32	
9.6	Develop Preliminary Design		8/4/2010	9/17/2010	32	
9.7	Evaluate Preliminary Design		8/4/2010	9/24/2010	37	
9.8	Design the Device		8/4/2010	10/1/2010	42	
9.9	Build the Device(s)		8/4/2010	10/14/2010	50	
9.10	Test the Device(s)		8/4/2010	11/12/2010	70	
9.11	Choose Final Design		8/4/2010	11/23/2010	77	
9.12	Document Final Design		8/4/2010	12/9/2010	88	
9.13	Identify Areas Needing Improvement		8/4/2010	12/9/2010	88	
9.14	Suggest Future Design Solutions		8/4/2010	12/9/2010	88	

Figure 8

8. Conclusions & Recommendations

The first stage of this project has been successfully completed and the next stage is ready to commence. So far this group has developed a model of a working device that meets all of the functions and constraints that were desired. Overall the bioreactor, cell seeding process and the surgical application have been improved through the creation of this device. This device improves the overall process of seeding microthreads and will also lead to developments in the future.

In the continued research conducted by Dr. Glenn Gaudette and researchers, we have considered possible improvements that could be made to perfect this design. First, a cushion should be inserted into the center of the device that allows for the needles to stuck in place. This would hold the sutures in place during seeding which would potentially allow for better cell adhesion. It would also improve safety by preventing the needles from being exposed unexpectedly during use. Second, the aeration of the device could be improved because oxygen is necessary for the continued life of the cells. Without any circulation of air into the device, the cells would die. Finally, the device would ideally be able to remain on the shelf for an extended period of time to allow for a quicker application by removing the time involved with delivery. One potential solution to this would be to freeze the microthreads, while they are in the bioreactor.

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