Design of an Automated Trituration System for Isolation of Cardiogenic Cells

Department of Biomedical Engineering

A Major Qualifying Project to be submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science
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Abstract

Cardiovascular disease is the number one cause of death in the United States. Approximately 8 million people in the United States suffered from a myocardial infarction last year. This Major Qualifying Project looks to automate the process of isolating cardiogenic cells. These cardiogenic cells provide an opportunity to help regain lost heart function after a myocardial infarction. This was accomplished through the design of an automated trituration system for isolation of cardiogenic cells.

Introduction

Cardiovascular disease is the number one cause of death in the United States today. According to the 2007 American Heart Association Statistical Update, approximately 8 million people in the United States suffered from a myocardial infarction (MI) in one year and this statistic is rising (Committee, 2007). MI occurs when the blood supply to a portion of the heart is interrupted. This interruption is caused by a blockage or buildup of plaque in a coronary artery. The affected area of the heart loses its blood and nutrient supply causing the heart tissue to die. The heart cannot effectively perform its required functions for the body. A method is needed to repair or replace the damaged heart muscle in order for the heart to regain normal function.

It is estimated that over one billion cells die when a major MI occurs, which results is loss of heart function or even heart failure (Murry, 2006). This death of cardiac tissue is detrimental to the function of the heart because cardiac myocytes, the cells that make up the myocardial tissue, have no or limited ability to regenerate after damage from a MI. One treatment method for cardiovascular disease involves the delivery of cardiogenic stem cells to the heart. In order to acquire cardiogenic cells, the process that is currently used produces spheroid bodies, which then generate cells expressing myocardial markers that can be isolated. Once these cells are isolated, they can then be sorted into cardiogenic and non-cardiogenic cells. These viable, cardiogenic cells can then be used to treat cardiovascular damage.

The objective of this Major Qualifying Project is to develop a method for isolation of cardiogenic cells produced through spheroid body formation. The method must allow for maximized cell isolation in a completely sterile environment while

ensuring that the cells retain their cardiogenic potential. The deliverables will include a paper detailing the process, a working prototype of the device and methods used.

Literature Review

Significance

Cardiovascular disease is the number one cause of death in the United States today. According to the 2007 American Heart Association Statistical Update, approximately 8 million people in the United States suffered from a myocardial infarction (MI) in one year and this statistic is rising (Committee, 2007). MI occurs when the blood supply to a portion of the heart is interrupted. This interruption is caused by a blockage of a coronary artery. The affected area of the heart loses its blood supply. One of the most detrimental consequences of this blockage is that oxygen cannot be delivered to this area of the heart, potentially causing this part of the heart tissue to die due to lack of oxygen. The function of the heart is to pump blood throughout the body. Blood circulates oxygen, nutrients, and removes waste.

The heart cannot effectively perform its required function of circulating blood throughout the body when a large part of the heart muscle has died as a result of a MI. A method is needed to repair or replace the damaged heart muscle in order for the heart to regain normal function.

It is currently believed that after a MI, cardiac myocytes are unable to regenerate. In order to help the heart regain its normal function, regeneration or replacement of the damaged area is needed. One method that is being researched is isolating stem cells and delivering them to the affected area for cardiac repair (Stamm, 2003). These cells can be delivered through a variety of methods including tissue scaffolds, injection of cardiac myocytes directly into the heart, or into the blood supply. Ideally these cells will proliferate and replace the damaged tissue.

The Heart

The heart supplies the body with blood, oxygen and nutrients. It is located between the lungs, behind and to the left of the sternum in the chest. The entire organ is surrounded by a two layered sac called the pericardium.

The heart is about the size of a fist, and is comprised of four chambers. The two smaller, upper chambers are called the atria, while the larger, lower chambers are called the ventricles. The left and right sides of the heart are separated by a structure called the septum. The left ventricle is the largest, most muscular chamber because this chamber is responsible for pumping blood from the heart to the entire body (Marieb, 2007). The blood flows in a specific path. The blood enters the heart from the body via the superior and inferior vena cava into the right atrium. The blood then flows through the tricuspid valve into the right ventricle. From the right ventricle the blood is pumped through the pulmonary valve to the lungs via the pulmonary arteries. An exchange of gas occurs at the capillary level in the lungs as CO_2 is exchanged for O_2. The now oxygen rich blood is carried back to the heart via the pulmonary veins and into the left atrium. From the left atrium, the blood flows through the bicuspid valve into the left ventricle (Marieb, 2007) . The left ventricle then pumps the oxygen enriched blood to the body via the aorta. The blood and nutrients are carried through the body via the cardiovascular system which consists of the heart and its network of arteries, arterioles, capillaries, venuoles and veins (Marieb, 2007).

The heart contractions are controlled by electrical impulses. The electrical signal starts at the sinoatrial node (SA node), that is located near the top of the right atrium. The electrical impulse travels from the SA node to the atria via the muscle fibers, made up of

cardiac myocytes, causing the contraction to occur and blood to be pumped. These impulses come at a constant rate unless the body's demands change due to stress, physical needs or hormonal factors (Marieb, 2007). The impulses then travel through the fibers to the atrioventricular node (AV node), which causes the ventricles to contract. The heart beat is actually a two stage process. The first stage occurs when the SA node triggers the atria to contract and force blood to the ventricles. Once the ventricles are full, the AV node fires and the ventricles contract and pump the blood to the lungs and body.

The heart supplies itself with blood and nutrients, as well. The heart has its own vascular network called the coronary arteries. Two arteries stem off of the aorta and break into smaller vasculatures and supply the entire heart with blood and nutrients. The right coronary artery mostly supplies the right side of the heart, and the left coronary artery supplies the left side. When one of these vasculatures narrows or has a plaque buildup, blood flow is lessened. When there is a complete blockage and all oxygen and nutrients are cut off to a section of the heart an acute myocardial infarction (MI) or heart attack can occur (Gray, 2000). The oxygen shortage or ischemia causes part of the heart tissue to die. In fact, it is estimated that over one billion cells die when an MI occurs (Murry, 2006). This death of cardiac tissue is detrimental to the function of the heart because cardiac myocytes, the cells that make up the myocardial tissue, have no or limited ability to regenerate after damage from an MI.

Cardiac Myocyte

Cardiac myocytes are the cells that form cardiac muscle, which is responsible for pumping blood through the heart through involuntary contractions. These cells are

affected by an MI. Cardiac muscle cells are striated muscle cells which differ from smooth muscle cells which line the gastrointestinal tract or skeletal muscle cells which make up the quadricep. The physical attributes of cardiac myocytes are marked by the cross striations that are formed by alternating layers of protein filaments in the sarcomere. The sarcomere is the basic unit of a muscle cross-striated myofibril (Marieb, 2007). The myofibril is the organelle in the cell that contains bundles of actomyosin filaments that are attached at either end of the cell. The main structural proteins of the myocyte are myosin and actin. The actin filaments are thin and the myosin filaments are thicker. The actin protein aids in muscle contractions and myosin produces the contractile force that drives the contractions (Marieb, 2007).

Specialized pacemaker cells at the entrance of the right atrium designated the sinoatrial (SA) node, pace the contractions of the heart. These nodal cells are different from other types of cells in the body due to their myogenic affinity (Gray, 2000). This means that the cell uses self-excitable stimulating contractions to mediate the heart pumping without aid from an electrical impulse coming from the central nervous system. These action potentials are then conducted throughout the rest of the myocardium causing the muscle to contract in a rhythmic synchronic fashion. These cells are stimulated by the autonomic nervous system and will continue pacing each other unless the contraction is disrupted, for example, by a MI. The myocytes are connected together by intercalated discs which allow for propagation of theses action potentials across the cell membranes by the use of different types of junctions. These electrical pulses are created by the flow of ions from myocyte to myocyte called action potentials (Gray, 2000).

Intercalated discs are double membranes separating each myocyte within the cardiac fibers. Within the discs, gap junctions aid in the passing of impulses from cell to cell. These junctions allow for the action potentials to extend through the cardiac myocytes by the passing of ions through these channels (Gray, 2000).

Cardiac myocytes are critical to heart function. These cells make up cardiac muscle and allow the heart to pump blood and nutrients to the entire body. A working heart sustains life.

Stem Cells

In order to repair damage to the heart that results from a MI and heart disease, researchers and scientists are narrowing the focus in on stem cells as a solution. Human mesenchymal stem cells (hMSCs) are multipotent cells and are present in adult bone marrow. These cells can be obtained in large quantities and can be cultured without losing the ability to differentiate into different cell types. They can be frozen for long periods of time, thawed out and still display normal cell behavior. They also have the ability to take up introduced genes (Asahara, 2000). As undifferentiated cells, they have the potential to differentiate into the cells that make up bone, cartilage, fat, tendon, muscle, and marrow. HMSCs are a prime example of cells that can be isolated, expanded in culture, and then characterized both in vitro and in vivo (Pittenger, 1999). If hMSCs can be manipulated into cardiogenic cells and form cardiac myocytes, the possibility of someday curing heart disease could be one step closer in becoming a reality. Hundreds of studies involving stem cells, more specifically mesenchymal stem cells, have been conducted in order to repair damaged heart tissue. Along with these attempts to

manipulate the stem cells into cardiac myocytes, myogenic differentiation and different methods of insertion have been tested as well (Gang, 2004).

The methods used to stimulate myogenic differentiation in hMSCs vary. One method used involves the implantation of hMSCs directly into the heart and observing whether or not the cells differentiate. In a study conducted by Toma and colleagues, bone marrow aspirate was taken from the Iliac Crest of human volunteers (Toma, 2002). The stem cells were isolated, labeled with lacZ and 500,000 to 1 million were injected into the left ventricle of CB17 SCID/*beige* adult mice. The mice were observed for several days. After 4 days, the cells did not express any myogenic markers, and many of the cells did not survive after 1 week's time. However, over time the cells began to resemble cardiomyocytes. The study reveals that the cells displayed evidence of desmin, ß-myosin heavy chain, α-actinin, cardiac troponin T, and phospholamban at levels comparable to those of the host cardiomyocytes, and that sarcomeric organization of the contractile proteins was observed. These are all components of normal cardiac myocytes. The study concludes by suggesting that these cells may be used in the future for cardiomyoplasty (Toma, 2002).

In a study entitled, *Autologous bone-marrow stem-cell transplantation for myocardial regeneration,* tests were completed using the direct injection technique in six human patients (Stamm, 2003). All of these patients had sustained a myocardial infarction and undergone coronary artery bypass grafting (CABG). The surgeons injected up to 1.5×10^6 autologous AC133+ bone-marrow cells into the infarct border zone in each patient. Three to nine months after the surgery, all the patients were alive and doing well. Global left-ventricular function had enhanced in four of the patients, and

infarct tissue perfusion had improved dramatically in five of the patients. The study concludes by suggesting that "implantation of AC133+ stem cells to the heart is safe and might induce angiogenesis, thus improving perfusion of the infarcted myocardium (Stamm, 2003)."

The overall goal in the implantation and experimentation of mesenchymal stem cells is to manipulate them in such a way that they will express cardiogenic markers. The cells can then be implanted into the heart in various ways in order to repair damaged heart tissue. In order for this step to be possible, a method for isolation of the cells after differentiation is needed.

Cardiogenic Cells

Cardiogenic cells are cells that express certain cardiogenic markers. These cardiogenic markers include the following; sarcomeric α-actinin, α-myosin heavy chain, cardiotin, and atrial natriuretic peptide (ANP). Cardiogenic markers are expressed in cardiac cells, such as cardiac myocytes, as well as cardiogenic cells. These markers show that the cells are either cardiac cells or that they are beginning to differentiate into cardiac cells. One possible use of cardiogenic cells is for transplantation into damaged heart tissue. It has been show that cardiac stems cells exist in normal heart tissue. This gives hope that the use of cardiogenic cells to replace damaged tissue may be an effective method to regenerate normal heart function (Beltrami, 2003).

Isolation Methods

Currently there are a variety of ways to isolate cells. The variety of isolation techniques varies depending on what types of cells are being isolated and how the cells are going to be used. In a variety of articles researched, trypsin is used to separate the growing cells from the culture plate. This is the first step in the process of isolating the cardiogenic cells from the non- cardiogenic cells. Once the stem cells have been trypsinized they can undergo further preparation for storage and use at a later date (Techawattanawisal, 2007).

When the hMSCs are grown according to our specific lab procedures they form a spheroid. This spheroid is a densely packed clump of cells. Cardiogenic cells are believed to come off of the spheroid and move outward (Potapova, submitted). These free cells need to be separated from the spheroid. It is also desirable to be able to break up the spheroid itself so that these cells can be tested for cardiogenic markers. This separation process can be executed by pipetting the spheroids repeatedly. Techawattanawisal, et al. suggests pipetting cell clumps repeatedly approximately 150 times to obtain single cell suspension (Techawattanawisal, 2007). The pipetting or trituration of cells causes the cells to experience shear stress and break apart from each other. This added shear stress can induce cell membrane rupture leading to cell death. This is why it is important to pipette the cells for the empirically determined amount of time. Once the cells are isolated from one another they can then be separated individually based on certain characteristics.

Individual cells can be assayed to see if they express certain cardiogenic markers. One method to determine which cells express certain characteristics is flow cytometry.

Flow cytometry is a process where single cells suspended in a fluid pass through a beam of light and scatter the light in different patterns. Fluorescent chemicals attached to the cells may be excited into emitting light at a lower frequency than the light source. This allows the user to determine how many cells express certain characteristics. Fluorescence-activated cell sorting (FACS) is a method that uses flow cytometry to determine which cells display certain characteristics and then sort these cells out from the rest of the cells that do not express these characteristics. Cells are separated according to different factors including cell surface antigens, enzymatic activity, proteins, and membrane potential. Specific antibodies can be used to bind to antigens on target cells, making them easy to separate (Fu, 1999).

This method has been proven to be effective in isolation of adipose tissue-derived stem cells (ASCs) (Yamamoto, 2007). This technique has worked for stem cells derived from adipose tissue, it therefore warrants investigation to determine if this technique would also work for hMSCs. Flow cytometry may turn out to be an effective method to sort cardiogenic cells from non-cardiogenic cells.

Some cardiac markers that these hMSCs expressed included the following as stated in an article by Gaudette's Laboratory.

> The cells spreading from the spheroids expressed cardiac specific proteins, including sarcomeric actinin, cardiotin, and atrial natriuretic peptide, and the cell cycle marker cyclin D1. They also express a calcium current similar in amplitude to that of ventricular myocytes (Potapova, submitted).

This means that the cardiogenic cells express certain specific traits such as sarcomeric actinin, cardiotin, and atrial natriuretic peptide. These unique markers could be used in FACS to separate the cells displaying these cardiogenic traits versus cells not exhibiting

cardiogenic traits. This would allow the cells to be separated and the cardiogenic cells to be used in treatment of post myocardially infarcted hearts.

Conclusion

The heart is a complex organ that is needed in order to sustain human life. When damaged, the chance of survival decreases significantly. With an advanced knowledge of the heart, stem cells and isolation methods, scientists are in search of a method to repair damaged heart tissue. The initial steps in this search require a method for isolation of the cardiogenic cells in vitro after differentiation.

Project Approach

Hypothesis

The isolation of cells from a spheroid body containing hMSCs will yield viable, cardiogenic cells.

Assumptions

A major assumption of this project is that the spheroid bodies contain cardiogenic cells that are able to be isolated and maintain their viability.

The other assumptions of our project are the number of cells that will be viable and cardiogenic after the process. The first is that 50% of the original amount of cells will have cardogenic potential, the next is that 50% or greater single cells isolated from spheroid bodies, the next is that 80% or greater pure population of sorted cardiogenic cells and finally that there will be 80% or greater cell viability of the sorted cardiogenic cells. The final assumption of this project is that starting with approximately 12,500 hMSCs in the spheroid body will theoretically yield at least 2000 viable, cardiogenic single cells.

Goal

Our team goal is to develop a device that will effectively isolate cardiogenic cells formed from a spheroid body.

Design

Initial Client Statement

The initial client statement is to develop a method to effectively isolate single cardiogenic cells from a spheroid body. One treatment method for cardiovascular disease involves the delivery of cardiogenic stem cells to the heart. The process that is currently used to grow these cardiogenic cells leaves them in a spheroid body that needs to be broken up into single cells. Once the cells are isolated they can then be sorted into cardiogenic and non-cardiogenic cells and then can be delivered to the heart to potentially treat cardiovascular disease in the future.

Revised Problem Statement

The objective of this Major Qualifying Project is to develop a method for isolation of cardiogenic cells produced through spheroid body formation. The method must allow for maximized cell isolation in a completely sterile environment while allowing the cells to retain their cardiogenic potential. The design must follow the Good Manufacturing Process and scalability is desired. The deliverables will include a paper detailing the process, a working prototype of the device and methods used.

Objectives

Defining Objectives

One of the main objectives of this design project was to isolate cardiogenic cells from a spheroid body. The design of this device/method is based on separating cells in the most effective manner possible. The cells must first be freed from the plate that they are cultured on with trypsin. After this the spheroid body must be broken up into single cells. This is accomplished by pipetting the spheroid body.

After the spheroid body cells are isolated into single cells, the cardiogenic cells must be sorted from the non-cardiogenic cells. Once the cardiogenic cells are separated from the other cells they can be used to treat a post MI heart. These cardiogenic cells can be delivered to the infarcted area of the heart and regenerate cardiac muscle.

The cells being cultured grow within a spheroid body. As they grow, some of these cells separate from the spheroid body and move outward. Some of these cells have already been proven to express cardiogenic markers (Rosen, 2007). The cells within the spheroid body itself have not been studied to see if they express cardiogenic markers. For the purposed of this project, it has been assumed that these cells will also display cardiogenic markers.

A reliable device will work when it is operated properly. It is important that each time the device is operated it completes the operation and there are no setbacks. This will allow the user to maximize his/her time and maximize the usage of the device. The device will be considered reliable if it has few moving parts and operates every time the operator wants.

A durable device is essential. If the device is always breaking, then the usefulness of the device decreases. A durable device will not have any unexpected breakages if the

maintenance plan is followed. This allows the user to operate the device whenever it is necessary without any unforeseen problems. The device is considered durable if it has few moving parts, is constructed soundly by using proper connecting parts, and will remain unaffected by an occasional bump.

Repeatable results are important to any scientific device. It is required that a device produce repeatable results so that the data has statistical significance. If the device continuously generates different outputs from the same inputs then it is of no real scientific use. The device will be considered to produce repeatable results if it has an automated process which has few moving parts that could possible fail.

The device should be efficient. Cost and time need to be taken into consideration. It takes a significant amount of time to grow the hMSCs to the stage where they begin to display cardiogenic markers. The process is also expensive. For these reasons it is most desirable that the process be efficient. This means that the device will deliver an output of 80%+ cardiogenic cells from the total number of cardiogenic cells put in.

It is important that the device is easy to use.   If every time a user, especially a new user, operates the device they are confused and have many unclear options to choose from the device will not be considered easy to use.   It is important that the device is simple to operate so that the correct process can be carried out every time. By carrying out the same process, then the same results will be obtained. This is important so that the data collected can have statistical significance. The device will be considered easy to use if it has only a few obvious choices for operation and is automated.

The device should be automated throughout the whole process.   By having the device be automated, there is less variability from user to user. This allows a variety of

different people to use the device and obtain the same results. An automated device also makes it so that the user does not have to go through intense training. The user is also less likely to do something incorrectly that would lead to the device breaking or a variation in the results. An automated device will also save time because a person does not have to carry out all of the processes manually.

The device should be easy to clean and sterilize. If the device is easy to clean then the cleaning process will take less time and the user is more likely to clean the device regularly. The device will be considered easy to clean if all parts are disposable, can be autoclaved, or can be wiped down with disinfectant easily.

It is desirable that the device complete its functions in a short amount of time. If this happens, then the rest of the process can continue without a hold up at the isolation/ separation step. The device will be considered timely if it completes its functions in a few hours.

The device must meet safety requirements. The device must meet these safety requirements so that it can be brought to market. It must also meet safety requirements so that it does not injure the user or anyone who is near it. If the device injures the operator or other support staff that come in contact with it, it will not be accepted by the client. It is also unethical to create a device that the designer knows is not safe and can harm the user. The device will be considered to meet safety requirements if all the moving parts are enclosed, there are no sharp edges, and it meets International Organization for Standardization (ISO) requirements. However, the device will be used in a laboratory setting for research purposes only and, therefore, is not considered a medical device by the Food and Drug Administration (FDA).

The outcome of this project is to separate as many cardiogenic cells as possible. To achieve this, the cells must be kept alive. This is done by maintaining a sterile environment for the cells. If the cells die because they are exposed to a non-sterile environment, then the cell separation was meaningless since the sample can no longer be utilized. A non-sterile environment would contaminate the cells and make them of no use. A sterile environment can either be in a cell culture hood, or in a fully enclosed sterile environment outside of the culture hood.

The device must keep cells alive throughout the entire separation and isolation process. If the cells die at any point during the process, they are of no use. The cells must be kept at a relatively constant pH, temperature, pressure, and be in a medium that supports them. Excessive force must not be applied to the cells when trying to separate them, or at any point in the process.

Pairwise Comparison Charts

Pairwise Comparison charts, as shown in Tables 1-7, were used to help rank objectives early in the design process. These charts compare the different design objectives in a paired fashion (Dym, 2004).

Table 1: Pairwise Comparison Chart of Main Objectives

MAIN OBJECTIVES	Isolates Cells Effectively	Sort cardiogenic cells from non-cardiogenic cells	Keep separation between free cells and spheroid contained cells	Reliable	User Friendly	Safe	Total
Isolates Cells Effectively	x	1	1	1	1	1	6
Sort cardiogenic cells from non-cardiogenic cells	0	x	1	1	1	1	5
Keep separation between free cells and spheroid contained cells	0	0	x	0	1	1	3
Reliable	0	0	1	x	1	1	4
User Friendly	0	0	0	0	x	0	1
Safe	0	0	0	0	1	x	2

Isolates Cells Effectively =   6   6/21= .286

Sort cardiogenic cells
from non-cardiogenic cells =   5   5/21= .238

Keep separation between free
cells and spheroid contained cells=   3   3/21= .143

Reliable=   4   4/21= .190

User Friendly=   1   1/21= .048

Safe=   +2   2/21= .095

                                                       21

        Table 1 compares the importance of isolating cells effectively, sorting cardiogenic

cells from non-cardiogenic cells, keeping separation between free cells and spheroid

contained cells, reliability, user friendliness, and safety compared with one another. As

shown, isolating cells effectively was found to be the most important objective.

Table 2: Pairwise Comparison Chart of Sub-Objectives of isolates cells effectively

sub objectives of isolates cells effectively	From spheroid	From free cells	Total
From spheroid	X	0.5	1.5
From free cells	0.5	x	1.5

From spheroid=   1.5   1.5/3= .500

From free cells=   +1.5   1.5/3= .500

                      3

Table 2 compares the importance of the sub-objectives of isolating cells effectively. The sub-objectives are isolating cells effectively from the spheroid and from the free cells. As shown, when ranked against one another, the two were found to be equally important.

Table 3: Pairwise Comparison Chart of Sub-Objectives of reliable

sub objectives of reliable	durable	repeatable	efficient	Total
durable	x	0	0	1
repeatable	1	x	1	3
efficient	1	0	x	2

Durable=   1   1/6= .167

Repeatable=   3   3/6= .500

Efficient=   +2   2/6= .333

                  6

Table 3 compares the importance of the sub-objectives of being reliable. The sub-objectives are durable, repeatable and efficient. As shown, when ranked against one another, repeatable was found to be the most important.

Table 4: Pairwise Comparison Chart of Sub-Objectives of efficient

sub objectives of efficient	isolates cells in hours	high yield return	Total
isolates cells in hours	x	0	1
high yield return	1	x	2

isolates cells in hours=   1   1/3= .333

high yield return=   +2   2/3= .667
                                 3

Table 4 compares the importance of the sub-objectives of being efficient. The sub-objectives are isolates cells in hours, and has a high yield return. As shown, when ranked against one another, high yield return was found to be the most important.

Table 5: Pairwise Comparison Chart of Sub-Objectives of user friendly

sub objectives of User Friendly	easy to use	automated	easy to clean	timely	Total
easy to use	x	0.5	0	1	2.5
automated	0.5	x	1	1	3.5
easy to clean	1	0	x	1	3
timely	0	0	0	x	1

easy to use=   2.5   2.5/10= .250

automated=   3.5   3.5/1-=.350

easy to clean=   3   3/10= .300

timely=   +1   1/10= .100
                    10

Table 5 compares the importance of the sub-objectives of being user friendly. The sub-objectives are easy to use, automated, easy to clean, and timely. As shown, when ranked against one another, automation was found to be the most important.

Table 6: Pairwise Comparison Chart of Sub-Objectives of safe

sub objective for safe	safe for user	safe for cells	Total
safe for user	x	0	1
safe for cells	1	x	2

safe for user=   1   1/3= .333

safe for cells=   +2   2/3= .667
                    3

Table 6 compares the importance of the sub-objectives of being safe. The sub-objectives are safe for the user, and safe for the cells. As shown, when ranked against one another, safe for the cells was found to be the most important.

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Table 7: Pairwise Comparison Chart of Sub-Objectives of safe for cells

sub objective for safe for cells	sterile	keeps cells alive	Total
sterile	x	1	2
keeps cells alive	0	x	1

Sterile=	2		2/3= .667
keeps cells alive=	+1 3		1/3= .333

Table 7 compares the importance of the sub-objectives of being safe for the cells. The sub-objectives are sterile, and keeps cells alive. As shown, when ranked against one another, being sterile was found to be the most important.

Objectives Tree

An objectives tree was used to help better understand the client statement. It is a hierarchal list that branches out into a tree-like structure. Each main objective has a set of sub-objectives that helps clarify and give more detailed descriptions of the objectives above them in the tree. The main goal is located at the top of the tree and the objectives and sub-objectives then branched out below the main goal. The numbers assigned to each objective and sub-objective was given in accordance with the weighting they were given in the pairwise comparison charts in the previous section of this report. Figure 1 is the weighted objectives tree for this project. The objectives tree gives a more visual way to see the importance of each objective in the final design.
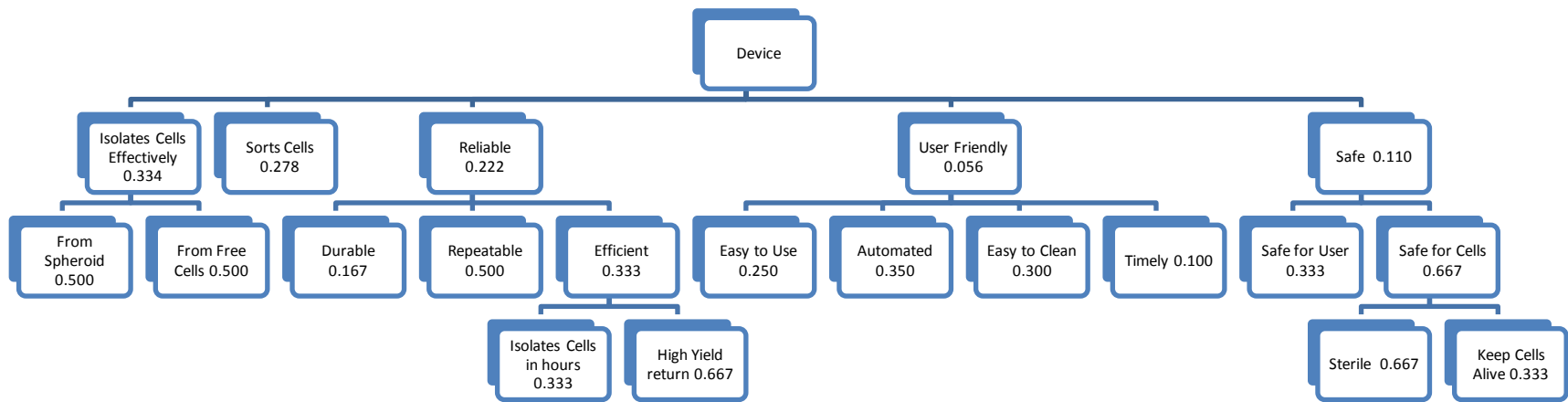
Figure 1: Weighted Objectives Tree

Design Constraints

The constraints of a design are the limitations or restrictions that are placed on the project. The constraints for this MQP are time, cost, total isolation time of cells, sterility and resources availability. The project must be completed by April 5, 2008 because this is one week before project presentation day and all aspects of the project must be finalized in order to create a complete and organized project presentation. The total cost of the project should not exceed $468, as each student in the group was allotted $156 each by the Worcester Polytechnic Institute's Biomedical Engineering Department. This will put limitations on the types of materials that are able to be used in the working prototype, but will force the group to be able to generate innovative ideas regarding alternate methods and materials used in the prototype. The next constraint is the total amount of time it will take to isolate the cells. The design must be able to isolate the cells in minutes or hours, rather than days or weeks. By limiting the design with this constraint, it will allow the user to isolate cells in bulk in a time-efficient manner. Next, the design must be able to isolate the cells in a sterile, non-toxic environment as not to damage, kill or compromise the viability of the cells in any manner. This can be achieved by either having the design fit into a sterile lab hood, or by having the entire system enclosed in a sterile box. Finally, the limitations on the resources the group will have access to while completing this project will constrict the design. The limited amount of time to grow hMSCs to test the design with, as well as the solutions that they will grow in and the solutions that are used to isolate the cells will limit the amount of testing that the group is able to do to create a proven working prototype. Also, access to

the major equipment in the lab, including incubators to culture the cells in, availability of a sterile lab hood if need be, and also the use of other equipment like a flow cytometer if needed. These constraints will limit the project but it will also force innovative thinking, creative ideas and produce an original design prototype that will be able to achieve all the objectives within the scope of this project.

Good Manufacturing Process

Good Manufacturing Practice (GMP) is a regulation for medical devices that is enforced by the U.S. Food and Drug Administration (FDA), more specifically the Center for Devices and Radiological Health (CDRH) (21 CFR Parts 808, 812, and 820 Medical Devices; Current Good Manufacturing Practice (CGMP); Final Rule., 1996). The most up to date GMP practices are referred to as the current Good Manufacturing Practice (cGMP). All manufactures of medical devices that are sold commercially in the United States must follow these practices. The reasoning behind this is so that the American people receive medical devices that do what they are designed to do, and do not cause unwarranted injuries.

The GMP regulation set forth in the Quality System (QS) regulations are promulgated under section 520 of the Food, Drug and Cosmetic Act (CDRH, Medical Device Quality Systems Manual: A Small Entity Compliance Guide, 1999). It requires that domestic or foreign manufacturers of medical devices intended for commercial distribution in the United States establish and follow a quality assurance (QA) program (CDRH, Medical Device Quality Systems Manual: A Small Entity Compliance Guide, 1999). The GMP rule is a flexible program. It recognizes that companies and the medical devices that they manufacture can, and do vary greatly. For this reason it is set

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up as a list of rules on how to design and manufacture medical devices in the safest most effective way possible. The rules listed in the cGMP apply only to those medical devices for which they are needed.

If a company is manufacturing only one device that is simple then a good portion of the rules are not going to apply because they are not needed do to the simplicity of the medical device. This medical device can be manufactured safely without all the restrictions which may be needed for a more complicated device or factory which is manufacturing a variety of different devices at the same time. The regulation requires that specifications and design controls be established for each medical device and that the finished devices meet these specifications. This makes sure that each device does what it is supposed to. By doing this, the GMP regulation helps assure that medical devices are safe and effective (CDRH, Human Factors Implications of the New GMP Rule Overall Requirements of the New Quality System Regulation, 1998).

> Design controls are an interrelated set of practices and procedures that are incorporated into the design and development process, i.e., a system of checks and balances. Design controls make systematic assessment of the design an integral part of development. As a result, deficiencies in design input requirements, and discrepancies between the proposed designs and requirements, are made evident and corrected earlier in the development process. Design controls increase the likelihood that the design transferred to production will translate into a device that is appropriate for its intended use (CDRH, Design Control Guidance For Medical Device Manufacturers, 1997).

Here the FDA gives its definition and explanation for the need of design controls.

The GMPs for medical devices were updated when analysis of recalls and adverse reaction reports showed that about half of reported device failures involve traditional GMP problems. Between 1985 and 1989, FDA compiled data through its recall database

that demonstrated that 45 to 50 percent of all device recalls stemmed from poor product design (including problems with software) (CDRH, Human Factors Implications of the New GMP Rule Overall Requirements of the New Quality System Regulation, 1998). For this reason, Congress passed the Safe Medical Devices Act (SMDA) of 1990, which gives FDA authority to require good manufacturing practices necessary to ensure proper device design (CDRH, Human Factors Implications of the New GMP Rule Overall Requirements of the New Quality System Regulation, 1998). This act was the impetus for "Medical Devices; Current Good Manufacturing Practice (cGMP), final rule", which required design controls to be implemented into the design and manufacturing of medical devices.

These revised GMPs for medical devices were incorporated into a quality system regulation.

> The Quality System Regulation includes requirements related to the methods used in, and the facilities and controls used for, designing, manufacturing, packaging, labeling, storing, installing and servicing of medical devices intended for human use. The revised GMP Regulation was released as a final rule on October 7, 1996. The section that deals specifically with design controls is section 820.30 of Title 21, of the Code of Federal Regulations ("21 CFR"). 21 CFR 820.30 requires manufacturers to establish and maintain procedures to control and verify device design to ensure that design requirements are met. More specifically, it requires manufacturers to establish and maintain plans that describe or reference the design and development activities and indicate responsibility for their implementation. It further requires manufacturers to establish and maintain procedures to ensure that design requirements relating to a particular device are appropriate and address the intended use of the device, including the needs of users and patients. It also requires manufacturers to establish and maintain procedures to ensure that design output meets the design input requirements. The regulation is effective June 1, 1997 (CDRH, Human Factors Implications of the New GMP Rule Overall Requirements of the New Quality System Regulation, 1998).

cGMP and Our Device

The device designed in this project falls under the category 862.2750 Pipetting and diluting system for clinical use.

> Subpart C--Clinical Laboratory Instruments
> Sec. 862.2750 Pipetting and diluting system for clinical use.
>
> (a) Identification. A pipetting and diluting system for clinical use is a device intended to provide an accurately measured volume of liquid at a specified temperature for use in certain test procedures. This generic type of device system includes serial, manual, automated, and semi-automated dilutors, pipettors, dispensers, and pipetting stations.
>
> (b) Classification. Class I (general controls). The device is exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to 862.9.
>
> [52 FR 16122, May 1, 1987, as amended at 65 FR 2309, Jan. 14, 2000] (CDRH, [Code of Federal Regulations] [Title 21, Volume 8], 2007)

This device must follow cGMP practices. Only the class I devices with an asterisk (*) are also exempted from the GMP regulation, except for general requirements concerning records (820.180) and complaint files (820.198), as long as the device is not labeled or otherwise represented as sterile (CDRH, Medical Device Exemptions 510(k) and GMP Requirements, 2008). 862.2750 Pipetting and diluting system for clinical use are not exempt from cGMP regulation. This means that if this medical device was manufactured and sold commercially in the United States it would have to follow cGMP regulations.

Functions

The functions of the device must include several characteristics. The device will have several wells in order to speed the process. The device must also isolate cardiogenic

cells into single cardiogenic cells. Then, the cardiogenic cells must be sorted from the non-cardiogenic cells. Through this process, the device must maintain cell viability and ultimately produce single cells. The cells must be isolated from the spheroid body and from the plate. It must provide the operator with a user interface or manual of instructions. The cells must be kept sterile throughout the entire process. The temperature of the media and the O_2 and CO_2 must be kept constant as well. The operator must use gentle handling throughout this process.

Specifications

The device must work with the cell culture media. It must keep the cells at a constant temperature of 37°C ± 1°C. It must maintain a 5% CO_2 concentration. The device must isolate at least 50% of the single cells from spheroid bodies. The device must produce 80% or greater pure population of sorted cardiogenic cells. The device must produce a cell viability of at least 80% for the sorted cardiogenic cells. Starting with approximately 12,500 hMSCs in the spheroid body will theoretically yield at least 2000 live, cardiogenic single cells if these percentages are followed. Increasing the yield at one of these stages will increase the final yield.

Design Alternatives
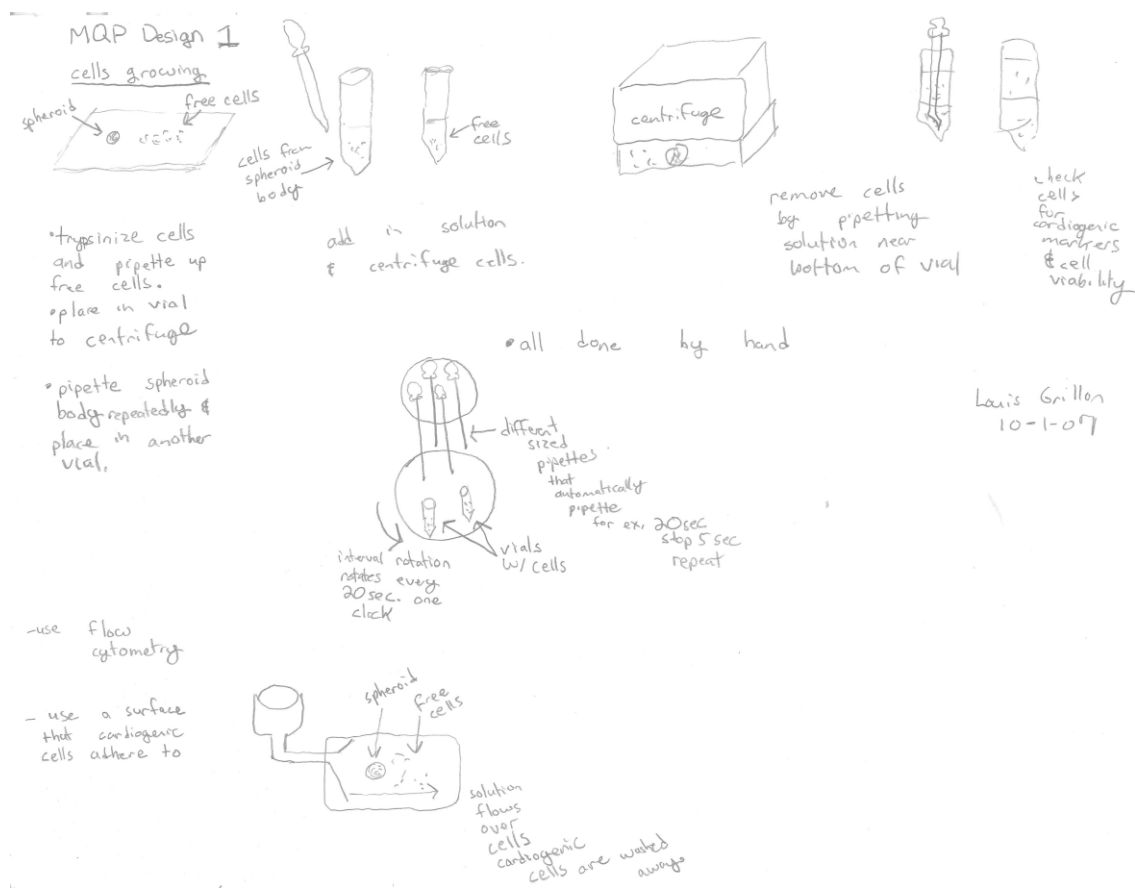
Design Alternative 1

The spheroids are grown until the spheroid body is ready to be harvested, at this stage Design Alternative 1 begins. The cells are trypsinized to remove them from the cell culture container. The spheroid body is aspirated with a pipette and set aside for trituration. The free cells are then washed in media to neutralize the trypsin. The free

cells are pipetted to make sure that they are isolated into single cells. These single free cells are then placed in a centrifuge vial. A similar process is followed for the preparation of the spheroid body cells. These cells are pipetted until they are isolated into single cells. They are then place in a centrifuge vial. After centrifuging the cells at low speeds the vials are removed. The cells at the bottom of the vials are removed by either pipetting them up or by decanting the top solution. Once the cells at the bottom of the vial are isolated they are resuspended into media. These cells can then be tested for cardiogenic markers and tested to see if they are viable. This process is completed manually.

To improve upon this process the trituration of the spheroid cells can be carried out in an automated process. The vials containing the cells can be placed in a rotating shelf. A series of various sized pipettes can then drop down and pipette the cells for a set amount of time, then rise up. The plate containing the vials can then rotate to the next position where a smaller sized pipette would drop down and pipette the cells. This process would continue for some time until the cells were pipette through the smallest pipette and were isolated into single cells. A diagram illustrating this design is shown in Figure 2.

Figure 2: Design Alternative 1

Design Alternative 2

Once the spheroid bodies have been plated, and sit for several days, the media containing cells that surrounds the spheroid body will be carefully sucked up by a pipette and placed into separate wells underneath and automated trituration system and on top of a vibrating plate. Six pipettes move up and down in the system above the wells on the plate and triturate the cells in each well by pumping the fluid in and out of the well. The vibrations from the plate underneath help to isolate the cells further. After several

minutes, everything will be pipetted of each of the wells manually and placed into separate vials. The vials will be centrifuged at a certain speed for a given amount of time. The heavier (cardiogenic) cells should sink to the bottom of the vial when centrifugation is complete. These cells will then be marked and examined under a microscope for cardiogenic markers and cell viability. A diagram illustrating this design is shown in Figure 3.
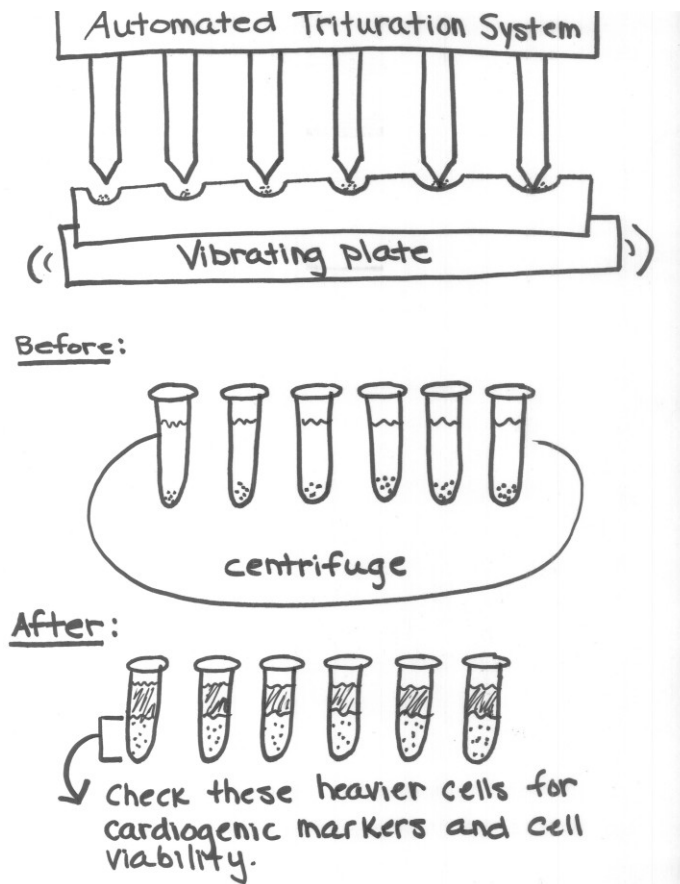

Figure 3: Design Alternative 2

Design Alternative 3

In this design alternative the process of isolating the spheroid cells is automated. But, before entering the device, the free cells are trypsinized and pipette from the spheroid plate, leaving the spheroid behind. Next, these free cells are put in a vial with

cell media to be later sorted. Next the plate of now just spheroids is placed on the conveyer belt at the entrance of the device. The plate then slides through the entrance where it enters a completely sterile environment. At the top of the box there is a removable pipette shelf that is replaced with new sterile pipettes every use. The pipettes are then lowered into the box directly on top of the spheroids. The spheroids are then triturated for a predetermined amount of time, until desired isolation is met. Trypsin is then added to the isolated cells to release them from the plate. The pipette shelf is then removed with the pipettes containing the now isolated cells. These cells are then placed in a separate vial that contains cell media to deactivate the trypsin. These two vials are then placed in a centrifuge in order to sort them into cardiogenic and non-cardiogenic cells. Finally, the cardiogenic cells, which are heavier, are sucked from the bottom of each vial a tested for cell viability and cardiogenic markers. The live, healthy, cardiogenic cells are then used.   A diagram illustrating this design is shown in Figure 4.
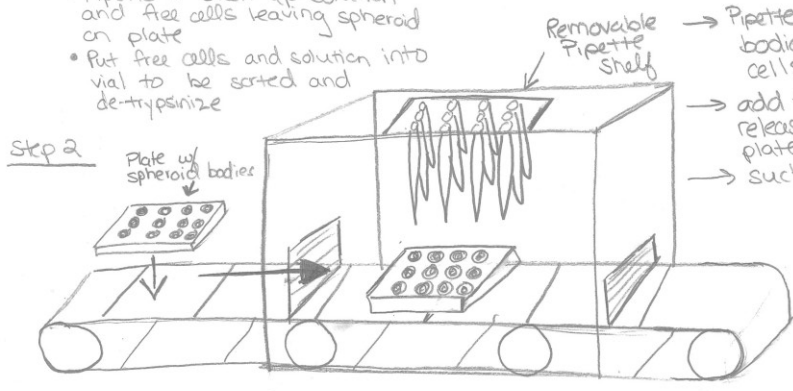
The handwritten figure contains the following text:

MQP Design Alternative 3
Amber Dangelo

Step 1
Before entering device:
• Trypsinize "free cells"
• Pipette or suck up solution and free cells leaving spheroid on plate
• Put free cells and solution into vial to be sorted and de-trypsinize

Step 2
Plate w/ spheroid bodies

Removable Pipette Shelf

Step 3

→ Pipette spheroid bodies to isolate cells
→ add trypsin to release cells from plate
→ suck cells up

Spheroid   Free Cells
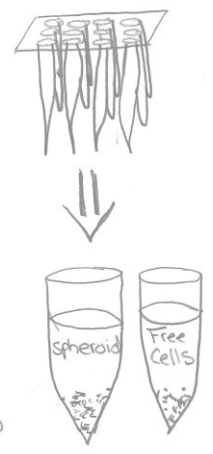
→ Centrifuge to sort cells
→ both vials

Figure 4: Design Alternative 3

Design Alternative 4

The hMSCs will be plated and after several days, placed into an automated trituration system. Once triturated, the cells will be placed into a separate holding container. Some sheet of material soaked in a substance that attracts cardiac cells will be dipped into the media holding the hMSCs. The cells will stick to the sheet. The sheet will

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then be placed in a media containing trypsin to release the cells from the sheet. These cells will then be marked and examined under a microscope for cardiogenic markers and cell viability. A diagram illustrating this design is shown on the top portion of Figure 5.

Design Alternative 5

The hMSCs will be plated and after several days, placed into an automated trituration system. Once triturated, the cells will be placed into a separate holding container. A device sending out electrical impulses will repel the cardiogenic cells toward the opposite end of the holding container, the cells will fall onto a sloped edge and drop down into a vial of media. These cells will then be marked and examined under a microscope for cardiogenic markers and cell viability. A diagram illustrating this design is shown on the bottom of Figure 5.
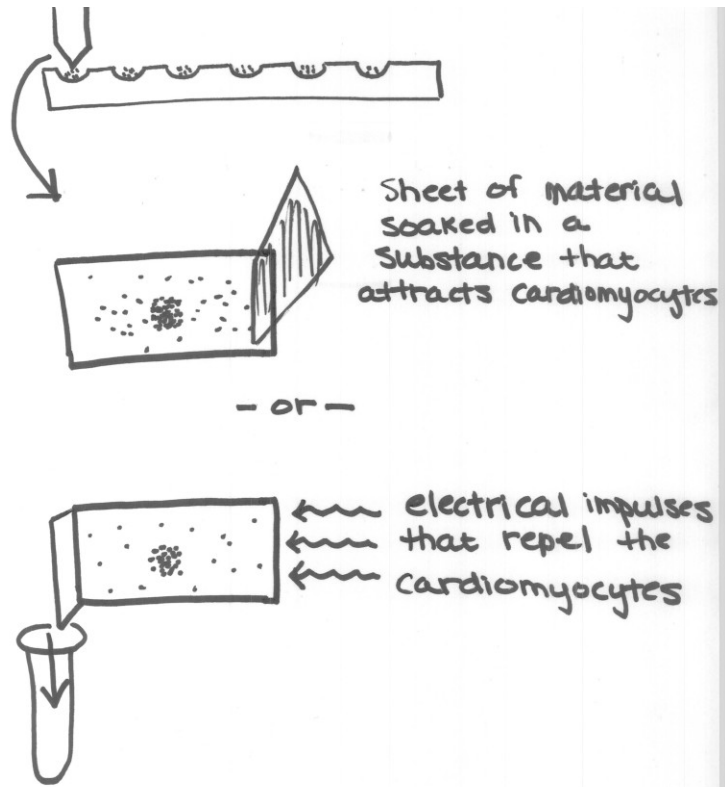
Figure 5: Design Alternatives 4 and 5

Design Matrix

Constraints & Objectives	Weight %	Design 1	Design 2	Design 3	Design 4	Design 5
C: Time		☑	☑	☑	☑	☑
C: Budget		☑	☑	☑	☑	
C: Sterility		☑	☑	☑	☑	☑
C: Resources		☑	☑	☑		
O: Isolates Cells Effective	33	33	33	33	16.5	16.5
O: Sorts Cells	28	28	28	28	14	14
O: Reliable	22	15	20	20	8	8
O:User Friendly	6	0	4	4	3	6
O: Safe	11	11	11	11	11	11
Total	100	87	96	96	52.5	55.5

☑ = meets constraint

Figure 6: Design Matrix

Design Matrix Constraints

Time

The constraint of time was met by all five designs. The design should meet its purpose in less time than it would take the user to complete the process manually.

Budget

Money is one of the constraints for this project. This project has a limited budget and all of the designs had to fall within this amount to be considered. All the design alternatives except design alternatives 4 and 5 were determined to have a projected cost that falls within the budget.

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Sterility and Resources

The constraint of sterility was also met by all of the designs. All of the designs can either be fit inside a sterile hood, or are themselves contained in a sterile box. The resources for the designs were only met by designs 1, 2 and 3. These 3 designs follow the same basic procedure and the materials and resources will all be available. Design 4 did not meet the criteria for resources because the substance used for attracting the cardiac cells may not be available to the team. Design 5 also did not meet the resources criteria because a magnetic separator is not available for the team at this time. If it does become available, then the resource criteria will be met.

Design Matrix Objectives

Isolates Cells Effectively

The five designs received different rankings in their ability to isolate cells effectively. The first three designs received an equal score of 33/33 since each design would work through trituration, which is a proven effective method of isolation of other types of cells. The other two designs would require use of methods that are uncommon. There are many factors that would make designs four and five inefficient. Design four could be inefficient since the material may also absorb some of the non-cardiogenic cells, not all of the cells, or drop some of the cells when pulls from the media. Design five could be inefficient since the magnetic waves could push non-cardiogenic cells off the plate if those cells are stuck to the cardiogenic cells. It might also not be powerful enough to repel the cardiogenic cells. Therefore, designs four and five received a lower score of 16.5/33.

Reliable

The different design alternatives were considered to be reliable if they were automated, had few parts, and would require little user interaction to complete the isolation and separation process. The design alternative was considered unreliable if the device required a large amount of user interaction, had many moving parts, and require constant maintenance. Based on this criteria design 1 received a score of 15/22. This design alternative required a large amount of human interaction and was not automated. Design alternatives 2 and 3 received scores of 22/22 because they are both automated and require little user interaction. Design alternatives 4 and 5 received 8/22 these methods require user interaction and may not always separate the cells as wanted.

User friendly

User-friendly is defined as a device or method that requires little user interaction and which is automated. Design alternative received a 0/6 for this because it is all done by hand and not automated. Design alternatives 2, 3, and 5 received a 6/6 because they are automated and easy for the user to operate. Design alternative 4 received a 3/6 for this objective because it is mostly automated, but requires some user interaction.

Safe

In the design matrix the final objective to reach for each design was "safe." This means that the design would be safe for the user to operate and safe for the cell, meaning that the design would be able to maintain cell viability and cardiogenic traits. All 5 designs completely met this objective; therefore they all received an 11/11. The reasoning behind this decision is that the designs show no potential hazards for the user or the cells.
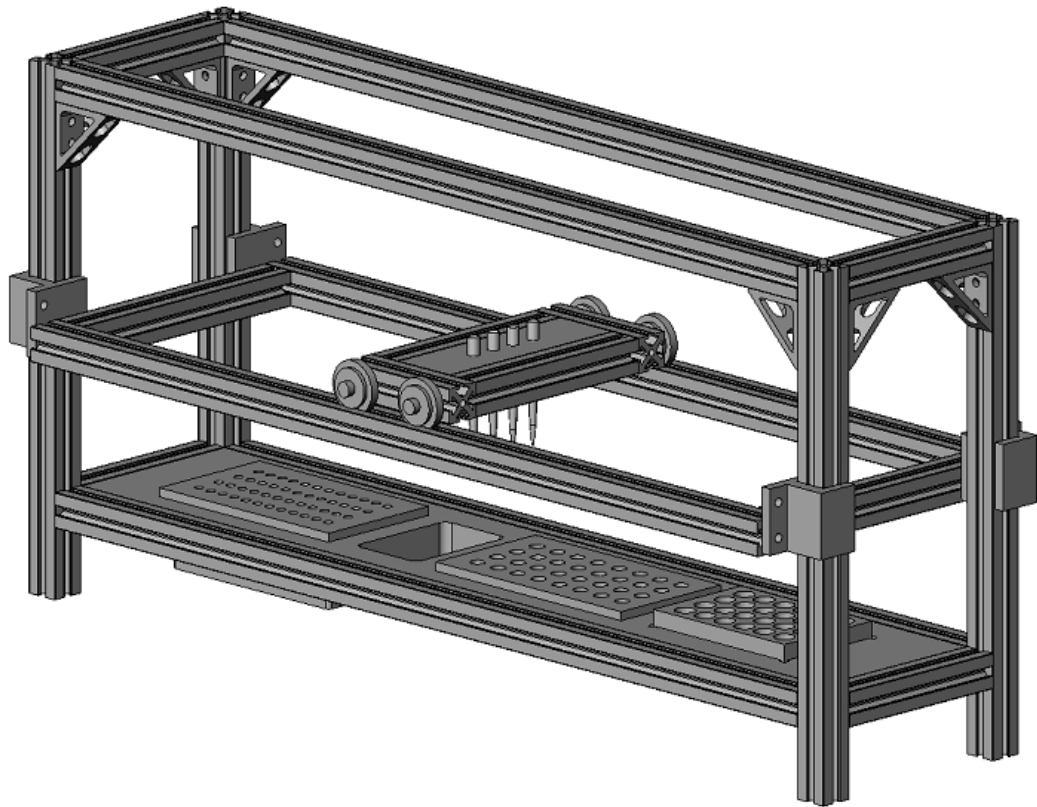
Final Design

The final design that we chose is a combination of design alternatives two and three. The objective of this design is to automatically separate spheroid bodies or other clumps of cells into single cells. This fully automated design is described as follows.

The overall shape of the design is a rectangular box frame. The base frame has two sub frames. The first sub frame is on the bottom of the main frame and holds both the 24 well plate with the spheroid bodies and a test tube rack. This bottom sub frame moves horizontally. The horizontal motion of the sub frame is guided by two guide tracks, one on each side of the sub frame, parallel to the direction of motion. The bottom sub frame is moved by a pulley system attached to a motor which is controlled through LinControl Software© provide by Lin Engineering (Santa Clara, CA).

The second sub frame is located near the top of the base frame and holds the pipette cart, motor and thread screw that controls the movement in the x direction. This sub frame will be referred to as the pipette frame. This sub frame moves up and down on four guide tracks. The pipette sub frame is powered by a motor attached to a thread

screw system which is controlled through LinControl Software© provide by Lin

Engineering (Santa Clara, CA). This device allows the user to load the necessary

components into the machine and press the start button. After this, the device takes care

of everything else. The device can take solution from the test tubes and but it into the 24

well plate containing the spheroid bodies and cells. It can also pipette the cell solution to

separate the cells into single cells. This allows the device to automatically triturate the

cells and separate them into single cells.

Figure 7: CAD Drawing of Preliminary Design Frame
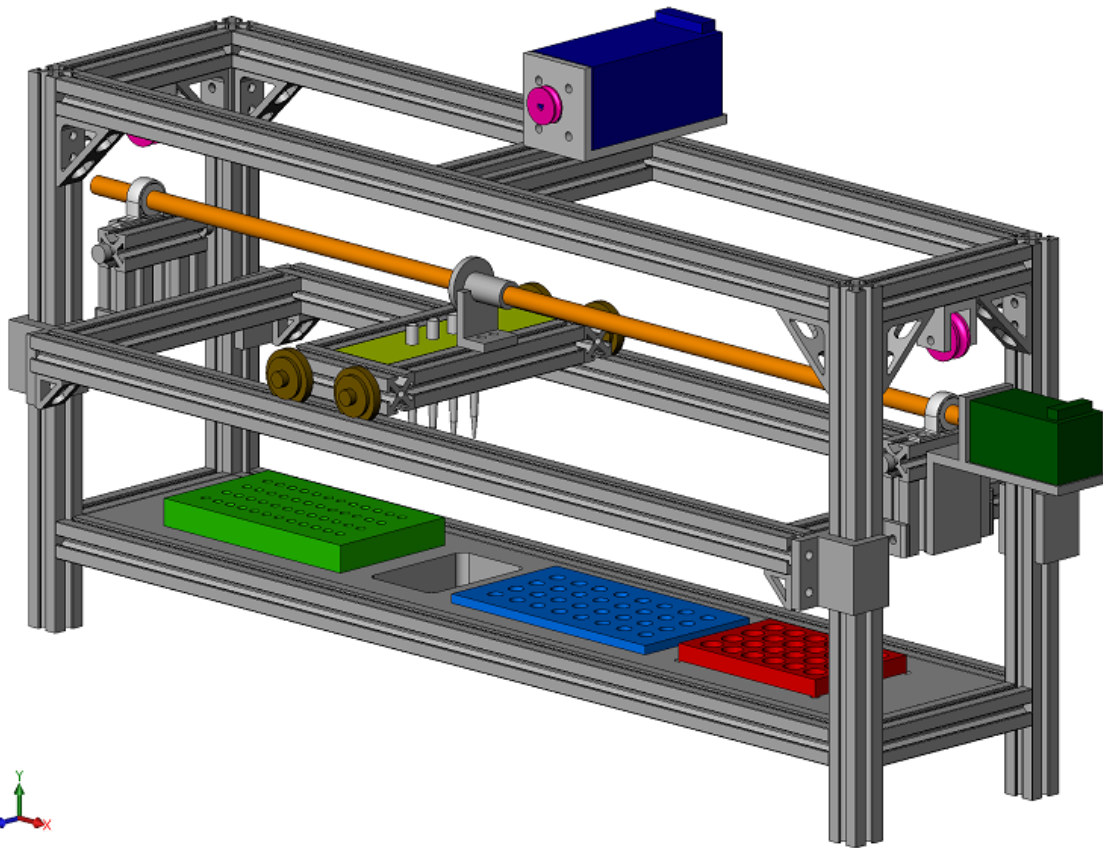
Figure 8: CAD drawing of final design

Figure 8 above is a CAD drawing of the final design that incorporates the thread screw, stepper motors and pulley system used to automate the device. The base plate and all its inserts can be seen as well.
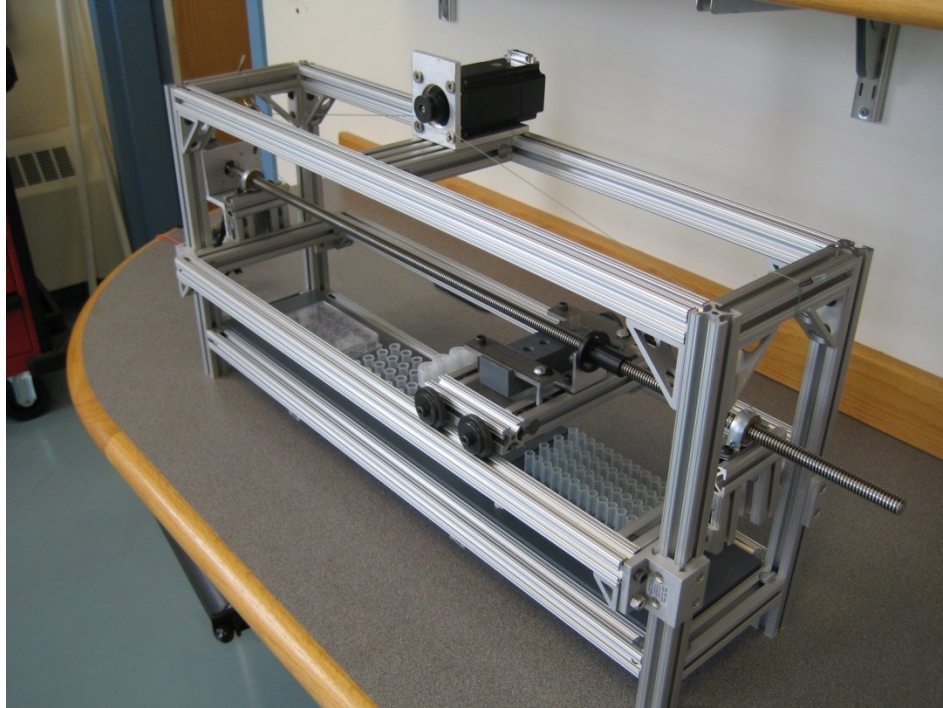
Figure 9: Right isometric photograph of the device

Figure 9 above shows a right isometric view of the finished device and all its
components. The pulley system is located on the top which is run by a stepper motor.
This lifts and lowers the track. The cart is attached to the thread screw run by a motor.
This moves the cart left and right for the transfer of materials.
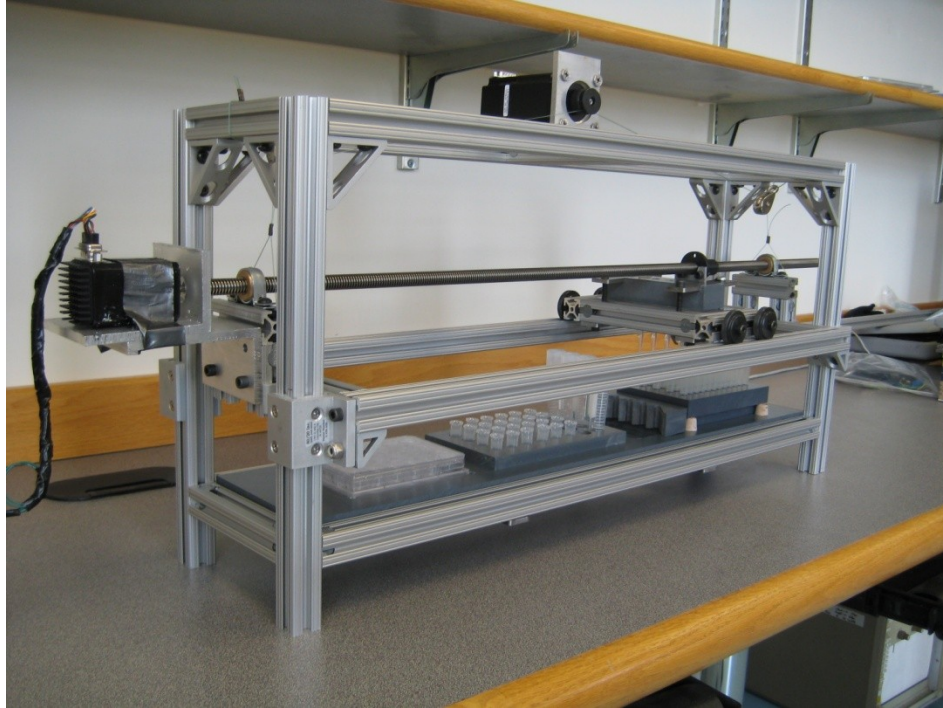
Figure 10: Left Isometric view of device

Figure 10 above shows a left isometric view of the finished device and all its components. The motor on the left powers the thread screw, as shown. The bottom unit can be seen in this photograph, which houses the 24 well plate, the removable insert containing eppendorf tubes, the pipette tip remover and the pipette holder.
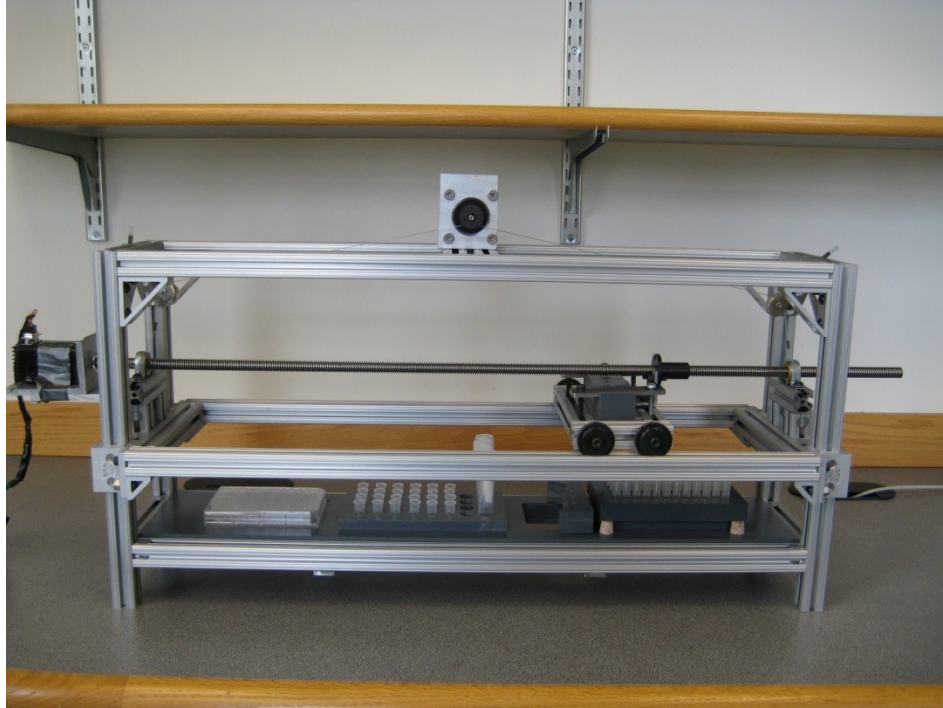
Figure 11: Front view of device

Figure 11 above is a front view of the device and all its components.
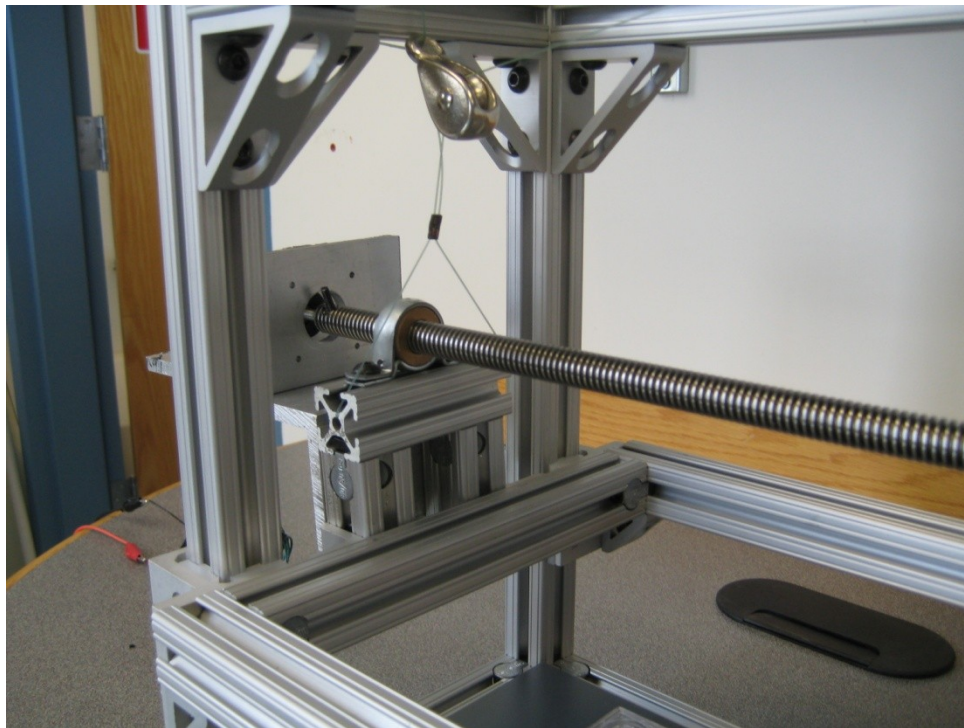
Figure 12: Photograph of Pulley System and thread screw

Figure 12 above shows how the pulley system is connected. 40 lb test fishing line was used within the pulley system since it is strong enough to withstand the weight of the track. The line is fed through the hole on the aluminum that houses the mounted bearing. The line is then crimped to be held in place. The crimp was used rather than a knot since it would hold more effectively.
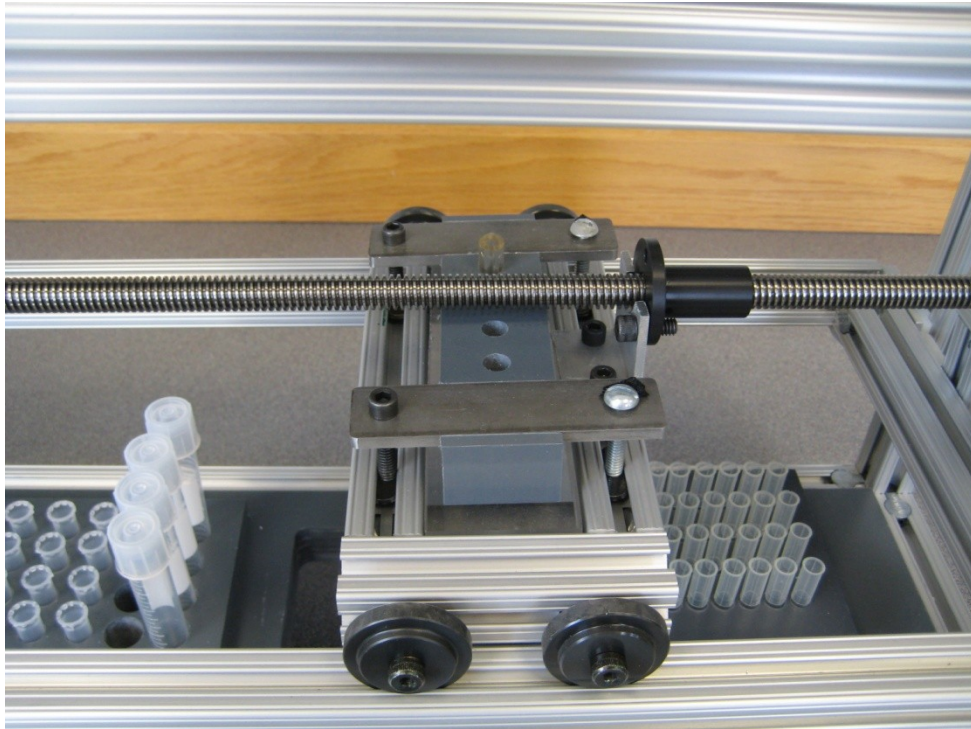
Figure 13: Photograph of Pipette cart

Figure 13 above is a photograph of the moving pipette cart. This pipette cart contains 4 pipettes that connect to a syringe pump that activates the triturating mechanism of the device. The pipette cart moves both left and right to transport materials from one location in the device to another.
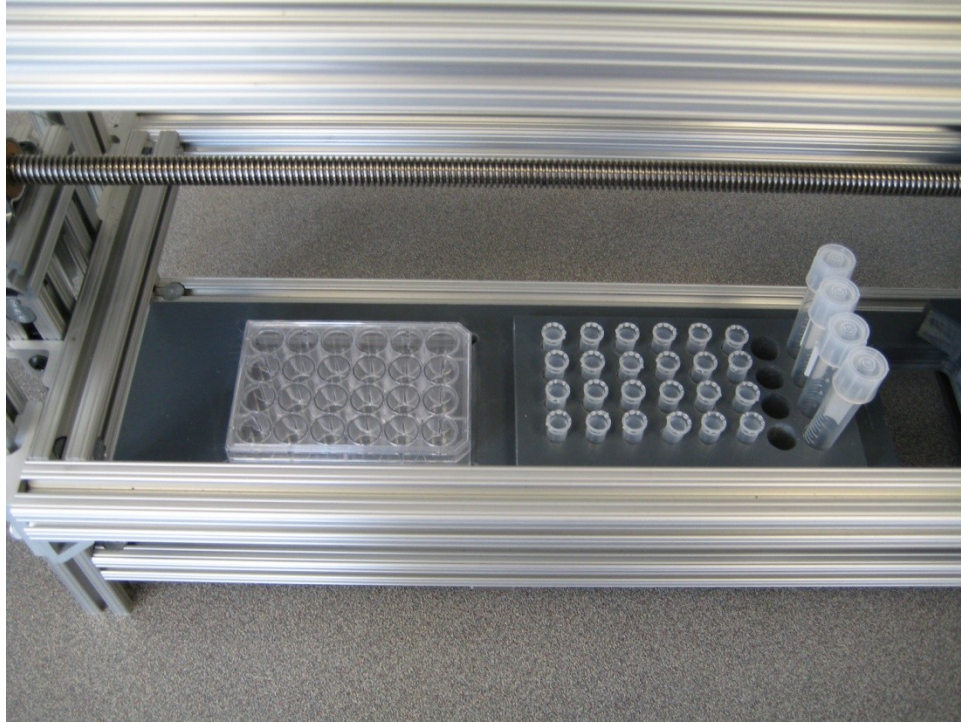
Figure 14: Photograph of removable inserts

Figure 14 above is a photograph of the removable inserts within the base of the device. On the left is the 24 well plate, followed by the eppendorf tube holders, the pipette tip remover and then the pipettes (not pictured).
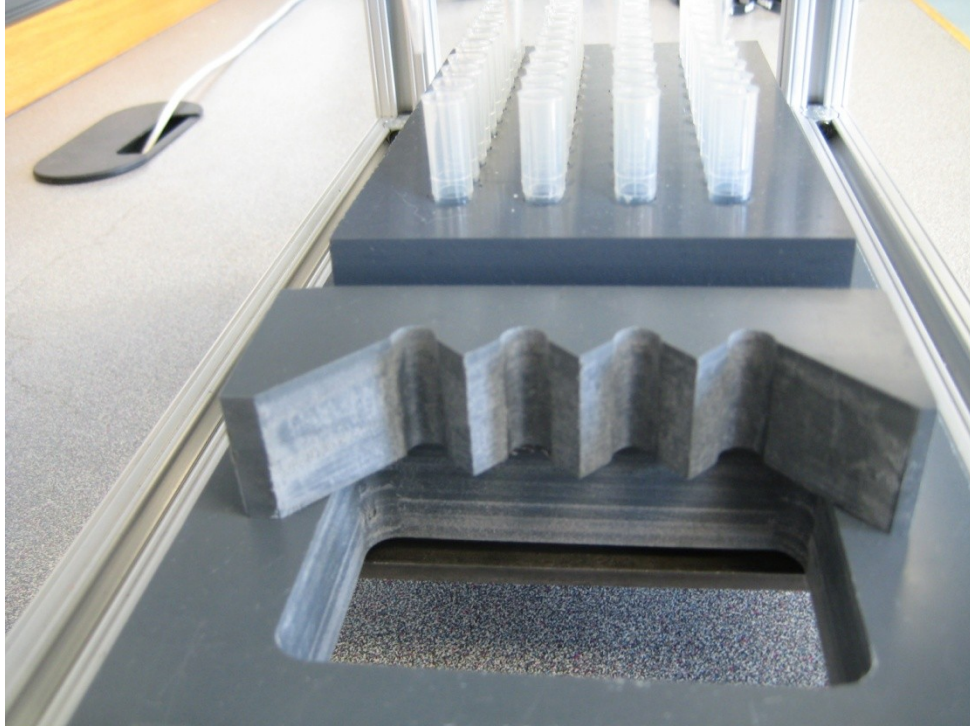
Figure 15: Pipette removal

Figure 15 above shows the pipette tip remover which gently removes the used pipettes tips from the pipette cart once the tips are used. The rectangular hole will house a small trash bag that will collect the biohazardous tips when the process is complete. The sterile pipette tips can be seen following the pipette tip remover.
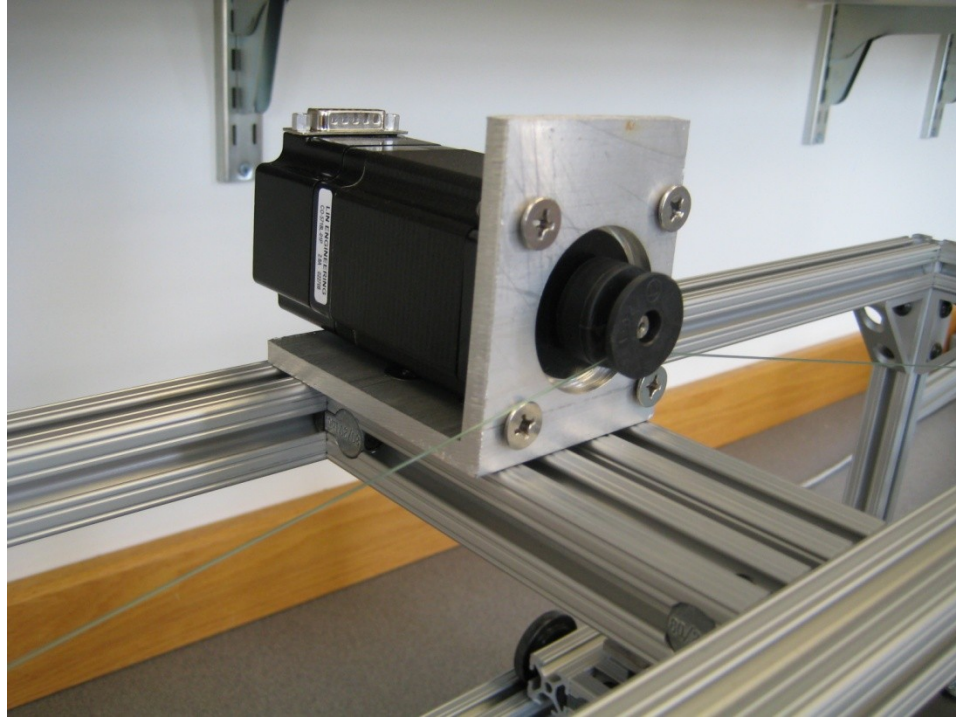
Figure 16: Photograph of top stepper motor

Figure 16 above shows the top stepper motor connected to the upper part of the device. There is a small pulley connected to the motor containing several feet of 40 lb test fishing line.

Motors

One of the basic parts of our design is its ability to automatically isolate cells into single cells. To do this effectively the device must be able to move both vertically and horizontally in a controlled fashion. To automatically move the sub frames you need a source of movement. For this we chose stepper motors. Stepper motors were chose to move the sub frames within the device both vertically and horizontally because they can be easily controlled and are reasonably priced. Once the type of motor to use was chosen, it was required to determine the specification for the movement that the device would undergo. This allowed the acquisition of the proper motors. To determine the specifications of the motors, the weight that each individual motor would have to move was calculated in the Table 8: Weight of Vertical Assembly and Table 9: Weight of Horizontal Assembly below.

Table 8: Weight of Vertical Assembly

Part number	Description	Quantity (units or ft)	Weight (lbs.)	sub total
1010X97"	Aluminum T-Slotted Framing System 1010	7.7	0.51	3.92
6715	10 S Single Flange Linear Bearing	4	0.21	0.82
2281	10 S Roller Wheel	4	0.07	0.28
3090	10 S 10-32 Double Anchor T-Nut T Short Assembly	12	0.06	0.74
	motor weight (estimated)	1	5.00	5.00
	plastic top pipette holder (estimated)	1	2.00	2.00
	screw assembly weight (estimated)	1	3.00	3.00
	Grand total			15.77

Table 9: Weight of Horizontal Assembly

Part number	Description	Quantity (units or ft)	Weight (lbs.)	sub total
1010X97"	Aluminum T-Slotted Framing System 1010	1.72	0.51	0.88
6715	10 S Single Flange Linear Bearing	4	0.21	0.82
2281	10 S Roller Wheel	4	0.07	0.28
	motor weight?	1	5.00	5.00
	plastic top pipette holder	1	2.00	2.00
	screw assembly weight ?	1	3.00	3.00
	Grand total			11.98

Once the weight that the motors would have to move was determined the torque can be calculated based upon the motion transfer mechanism. The general specifications of the different motors can be found in Table 10: Motor and Lead Screw Specifications below. The axial load is calculated by assuming that there is about a 0.1 coefficient of friction between the horizontal sub frame's wheels and the bar they run along. This would give an axial load of 1.2 lbs.

Table 10: Motor and Lead Screw Specifications

motor	speed (in/sec)	load (weight in lbs.)	duty cycle (min)
horizontal	3	~1 (axial)	~1
vertical	1	16	~1

The motors also have a torque requirement that will be explained in the next section entitled, "Motion Transfer and Torque."

Motion Transfer and Torque

After consideration of a variety of motion transfer systems it was decided that the device would use lead screws with nuts to position the sub frames containing the pipettes in the proper position. Lead screws were chosen because they allow for precise control of the nut relative to the motor revolutions. A ball screw mechanism would be more

precise, but due to cost associated with it the extra precision was not worth the additional cost. An Acme lead screw assembly was chosen because these lead screws have large amounts of engineering information on them and they are relatively inexpensive and readily available.

The use of a stainless steel (SS) lead screw with a plastic nut was decided upon for a number of reasons. This combination requires no lubricant. Lubricant could contaminate the cells being worked with. Using no lubricant also allows for easy cleaning and sterilization. The parts can be easily wiped down with soap and water to clean them, and then sterilized with 70% ethanol.

A technical representative from Nook Industries (Cleveland, Ohio) was consulted with to find the proper size lead screw for the specifications required. It was determined that the Acme lead screw that would work the best would be a ½" lead screw with two threads per inch. The threads per inch could be increased to increase accuracy, but this would lead to a higher PV value, or the unit pressure on the nut (P) and surface velocity at the nut/screw interface (V). The ½" SS lead screw with two threads per inch and a plastic nut would meet the specifications of both the horizontal and vertical motion. This would also allow for easy replacement if one nut broke you would not have to have different size nuts on reserve.

Once the motion transfer system was chosen, the torque that each motor needs to produce can be calculated. This can be calculated by using the torque formula in Figure 17: Torque Equation below.

Torque–Forward Drive

$$T_f = \frac{LP}{2\pi e_f}$$

T_f = Forward drive torque, in.-lbs.
L = Lead, in./rev.
P = Load, lbs.

Figure 17: Torque Equation

The alternative is to just look up the lead screw nut assemble number for torque to raise one pound.   For this particular lead screw nut assemble, 0.107 (in-lb) of torque is required to raise one pound. So, for the vertical motor multiple 0.107 times 16 lbs. This gives 1.7 in-lbs. of torque needed to raise the vertical sub frame. This number is then doubled to 3.4 in-lbs. to account for friction and give some wiggle room. The same approach is used for the horizontal lead screw assemble. 0.107 times 1 lb. is 0.107 in-lb of torque, times two yields .214 in-lb of torque.

Controlling the Device

Stepper motors were chosen as the means to moving the device in the x and y directions. The motors that were chosen for this device are from Lin Engineering out of Santa Clara, California. The SilverPak series was chosen for both motors because it is a package of motor, controller and driver and they are easily programmable. For the x direction the SilverPak 17C series was chosen. The stepper motor is a NEMA 17 1.8° Bipolar step motor, part # 4118M. It has a maximum holding torque of 45 oz-in. For the y direction, the SilverPak 23C series was chosen because it has more holding torque at 294 oz-in. The higher holding torque was needed for the y direction because it has to be able to withstand the weight of the x direction motor assembly, lead screw and pipette apparatus. This stepper motor is the NEMA 23 1.8° Biopolar step motor, part # 5718L. The calculations for finding the holding torque were described in the above sections.

The program that is going to be used to control the motors is supplied by Lin Engineering and has simple commands that allow it to travel certain distances. The coding is done through alphanumeric commands that account for the number of rotations

the stepper motor is doing. An example of the program is shown in Figure 18. Figure 19

shows the flow chart created by the team which outlines the steps the program must

follow.
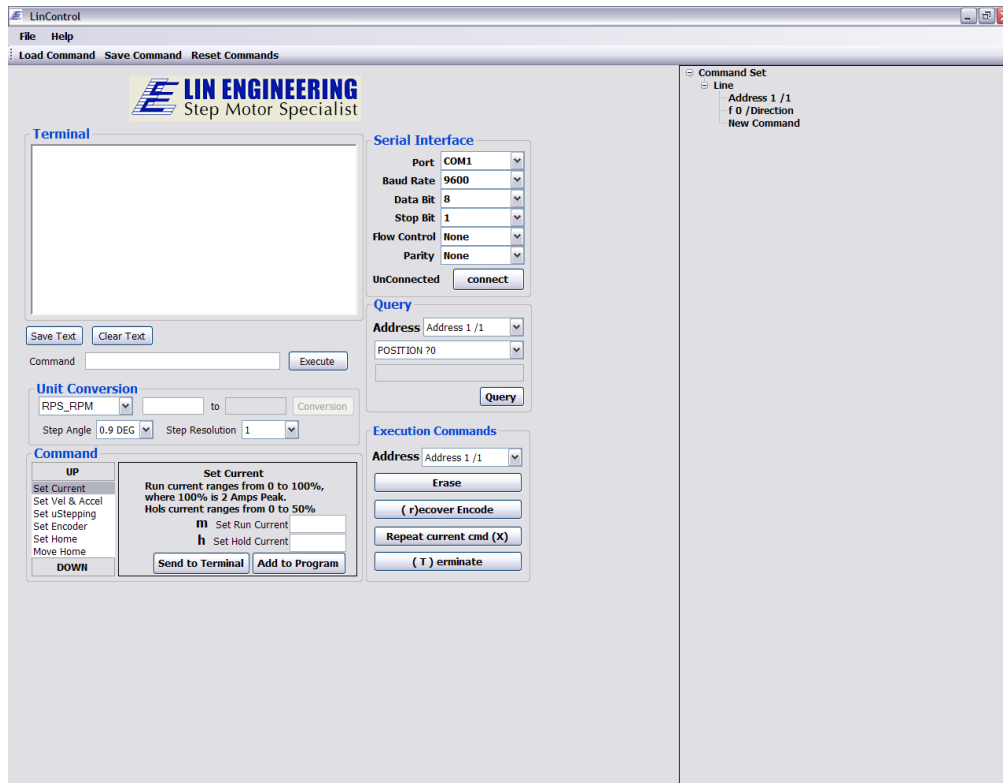
Figure 18: Screen Shot of Stepper Motor Program
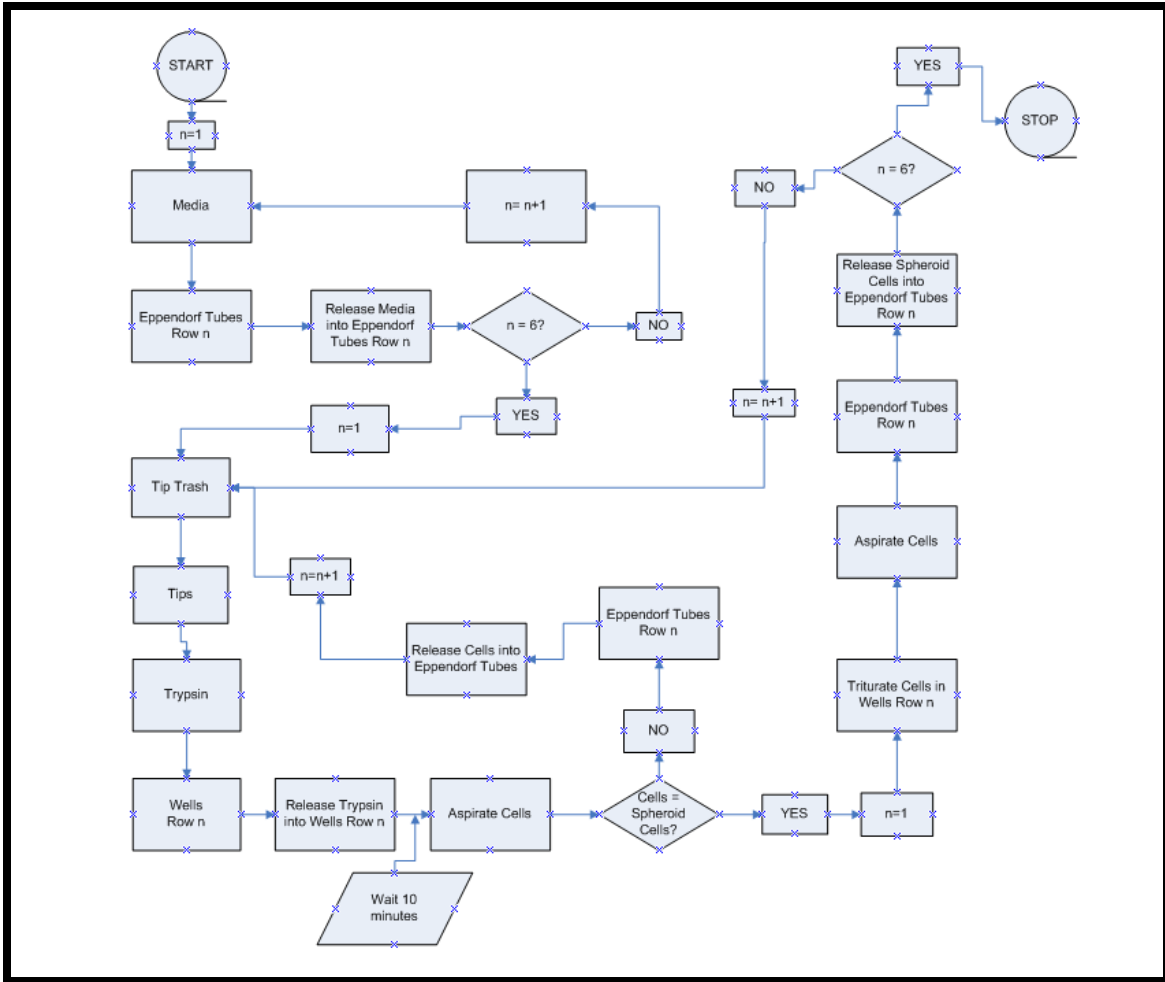
Figure 19: Program Flow Chart

The entire program is completely outlined for the 17C motor and is shown in

Appendix 4. The program for the 23C motor was not completed because testing of the

device had not been done. In order to completely write the program, timing of the

syringe pump and the amount of time it took for the shelf of the device to be raised and

lowered would have to be taken into consideration. This testing could not take place

because both of the stepper motors malfunctioned. The 17C stopped turning in the

counter clockwise direction and the 23C would continuously seize up and would have to

be manually pushed to start running again. Lin Engineering had been contacted with

these problems and Technical Support was unable to resolve them. If these problems can

be resolved, then programming of the 23C motor can be completed with specific timings to allow for the isolation process to be completed.

Syringe Pump

In order to transfer materials inside the device during the isolation process a means of transportation was needed. It was decided that a syringe pump would be able to aspirate and discharge material as well triturate the cardiogenic cells in order to isolate them from the spheroid body. The MQP group consisting of Michael Fakjarzadeh, Gregory Fredette and Emily Martin had used a syringe pump to complete their project entitled "A Scaffold for Cardiac Regeneration." Because both this project and their project were advised by Professor Gaudette, they were able to share this program for this project. This program was developed in LabView 8.1 and the Front Panel of this program is shown in Figure 10. It has not been altered to fit the needs of this device, but this problem will be addressed in the Future Recommendations section of this paper.
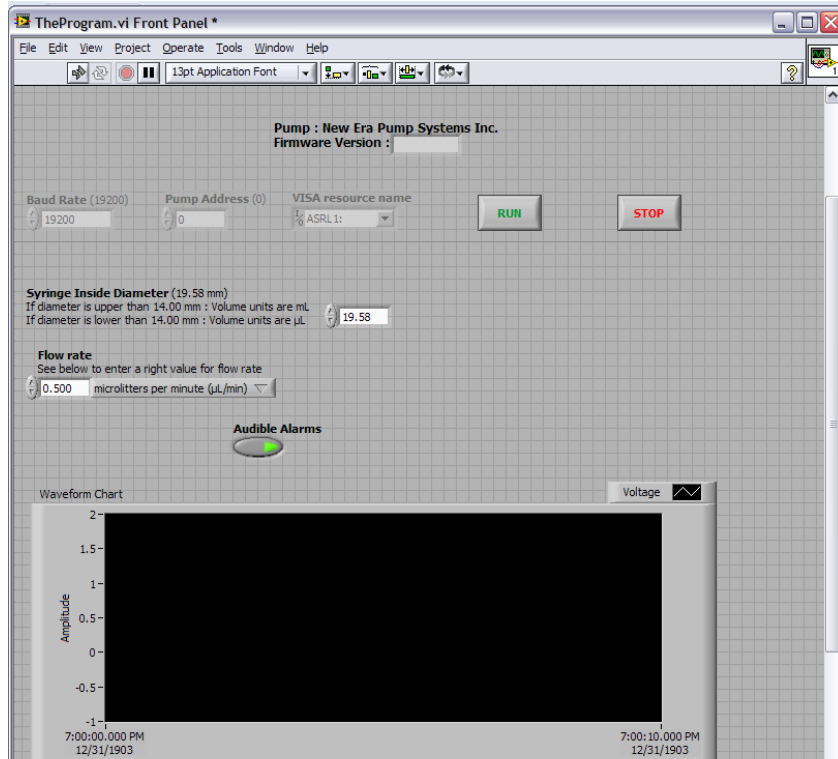
Figure 20: Front Panel of LabView 8.1 Program

Experimental Methods

Non-automated Testing

In order to test the efficiency of the device, the team completed manual experimental tests in the laboratory. Spheroid bodies were grown using the hanging droplet method and plated in a 24 well plate after 3 days.

The initial testing for proof of concept of the device started with the culture of Human Mesenchymal stem cells. The passage 9 cells were thawed in a 37°C water bath for one minute. These cells were aspirated out of the initial tube and added drip-wise to a culture flask. 8mL of DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% P/S was added to the flask and the flask incubated for 4 days.

After 4 days, the cells in the flask were trypsinized with 6 mL of trypsin for 7 minutes, aspirated and added to a 15 mL vial holding 4 mL of 10% FBS media. The cells were spun down in the centrifuge at 3000rpm for 5 minutes. The media was poured off and 1 mL of DMEM supplemented with 20% FBS and 1% P/S was added drip-wise to the cell pellet. A cell count was conducted and showed that there were approximately 1,810,000 cells in the vial.

1st count = 89 cells

2nd count = 92 cells

89 + 92 = 181 cells

(181 cells/10 squares) x 10^4 x 10 x 1mL = 1,818,000 cells/mL

15,000 cells/20 µL = 1,810,000 cells/x

x= 2413.33 µL or 2.413 mL

Add 1.413 mL of 20% FBS media to the cell suspension

1,810,000/2413 µL = 500,000/y

Y = 666.6 µL

2413-666.6 = 1746.4 -666.6 = 1079.8

Add 666.6 µL cell suspension to two new cell culture flasks and add 8mL 10% FBS media

1079 µL/20 µL = 53 spheroids

20µL droplets of media were placed onto every other circle on the lid of a 96 well plate. The plate was carefully flipped over and laid on top of the bottom section so the droplets were hanging upside down. Every day for 3 days, the lid was flipped over and another 20µL droplet of 20% FBS media was added to each droplet.

On the third day, round spheroids had formed and the lid was flipped over one final time. The media was aspirated off of each spheroid and rinsed with a 20 µL droplet of PBS. The spheroids were drawn up and released into the center of individual wells of a 24 well plate (every other well). The empty wells contained sterile PBS to provide moisture within the container and prevent drying. After 8 hours, drying out within the wells was observed, so 200 µL of 20% FBS media was added to each well to prevent cell death as a result of drying. Every day for 2 more days, 50 µL of 20% FBS media was added to each well that contained a spheroid. On the third day after the cells had been plated, testing was conducted.

Using P1000 pipette, 100 μL 20% FBS 1% P/S media was added to 5 eppendorf tubes. Using the P1000 pipette, varying amounts of trypsin were added to the wells that contained spheroids with cells coming off. Since there were a limited number of spheroids with cells coming off, the tests were conducted carefully under the microscope while continuous observation took place.

Automated Testing

Automated testing would utilize the same testing procedure of the non-automated testing but will utilize the syringe pump that is available in Gaudette's Laboratory. This will insure that the results of the non-automated testing would be reproducible by the syringe pump. Once this testing is complete, testing of the device would occur. This would include running the LabVIEW® program that was controlling the syringe pump in line with the stepper motor programs and making sure that the precision of the pipette tips is accurate and that all of the systems are working together, the syringe pump, the motors and the software. This testing was not completed due to technical difficulties with the stepper motors and time constraints of this project. This setback was described in the Motion Control section and will be addressed in the Future Recommendations section of this paper.

Results

Testing was conducted in the wells containing plated spheroids. Using the P1000 pipette, 200μL of trypsin was added to a well containing a spheroid (all wells also contained 200μL of media) and the well was observed for several minutes under the microscope. After 20 minutes, neither the free cells nor the spheroid still had released from the bottom of the plate. 300μL of trypsin was added to another well containing a spheroid and the well was observed for several minutes under the microscope. After 10 minutes, neither the free cells nor the spheroid had released from the bottom of the plate. 500 μL of trypsin was added to another well containing a spheroid and the well was observed for several minutes under the microscope. After 5 minutes, the free cells had released from the bottom of the plate. Two more tests were completed repeating the addition of 500 μL of trypsin to a well containing a spheroid, and the result was the same in both cases. It took only 5 minutes for the free cells to release from the bottom of the well. Using a P1000 pipette, the free cells and media were aspirated from the well and transferred into the eppendorf tube containing 100 μL 20% FBS 1% P/S media. Using the P1000 pipette, varying amounts of trypsin were added to the spheroid starting with 100 μL every 30 seconds. After 2 minutes the spheroid released from the bottom of the plate. Manual trituration took place for one minute and the cells appeared to have isolated into single cells under a 4X magnification in the microscope. During each step of the experiment, the cells were observed for cell isolation. Table 11 shows the results of this testing. Figure 21 below is a photograph of a spheroid with cardiogenic cells coming off that was taken during this testing.

Table 11: Results of Cell Testing

Well #	Media	Trypsin	Observations
1	200µL	200µL	No changes, even after 20 minutes
2	200µL	300µL	No changes
3	200µL	400µL	Few cells released from the bottom of the plate around the spheroid after 10 minutes
4	200µL	500µL	Cells released from the bottom of the plate around the spheroid after 5 minutes. The cells were aspirated off and 100 droplets of trypsin were added every 30 seconds until the spheroid released from the plate bottom at 2 minutes. The spheroid was triturated for 1 minute once per second and the spheroid appeared to have isolated into single cells under the 4X magnification.
5	200µL	500µL	Test was run a second time with same results
6	200µL	500µL	Test was run a third time with same results
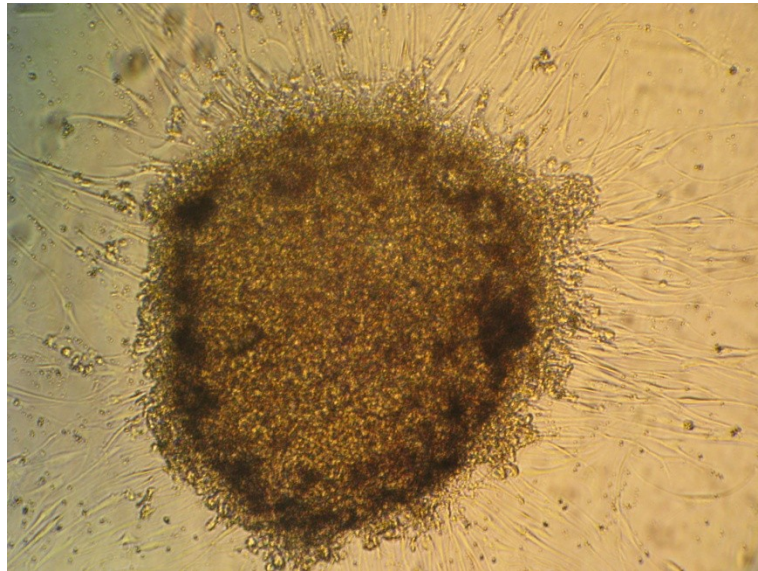
Figure 21: Photograph of Spheroid at 4X

Analysis and Discussion

Through the cell testing outlined in the section above, it was found that if 500 µL of trypsin is added to a well of a 24 well plate containing a spheroid and 200µL of media, then the free cells can be isolated without disturbing the spheroid body. It was also found that if 500 µL of trypsin is added to the spheroid body once the free cells are aspirated, it will take approximately 2 minute for the spheroid to release from the bottom of the well. Futhermore, if the spheroid is triturated for one minute after releasing from the bottom of the well, the spheroid body can be isolated into single cells. This device will be able to utilize the results of this manual testing in order maximize cell isolation.

This device completely automates the process of isolating cardiogenic cells. This will allow any laboratory utilizing this device to maximize productivity of trained personnel. This will reduce the amount of time that the technicians spend isolating the cells and will also eliminate human error during the process. Human error is reduced because the system is fully automated and the device is both accurate and precise so the inputted values are delivered every time. This will in turn increase the amount of viable, cardiogenic cells that will be produced.

This device can easily be installed by one person into the bio-safety cabinet as it weighs less than 40 lbs and only needs a 24V DC power supply to power the device. The device can easily be moved as it is only 3 square feet and this enables it to be moved from location to location and shared between different laboratory spaces. Once the motors have been programmed to the customer's needs, there is no need for additional PC support. The process is saved in the motors' memory and will begin the isolation process each time the device is powered up. Another benefit of the compact size of the

device is that it is able to fit into a bio-safety cabinet which will ensure the sterility of the device, materials and cells. This will allow for maximum cell viability.

This device is extremely versatile. The base plate has machined holes that are able to house different inserts for a variety of uses. For example, this device is customized to effectively isolate cardiogenic cells from a standard 24 well plate that the spheroid bodies are grown in. New inserts can be machined to accommodate different size plates, pipette tips and eppendorf tubes. This allows for this device to automate a number of different isolation processes and could also be used to make dilutions of various solutions used in the laboratory. Speeds, pipetting times, and amounts of fluid being transferred can be altered because the motors and syringe pump programming can be adjusted to satisfy the user requirements.

This device organizes the large quantity of materials that are used in the isolation process. All of the supplies needed are strategically located within the base plate of the device in order to maximize the efficiency of the process and decrease contamination. Because everything is securely contained in the base plate there is less chance of accidental spilling which cuts waste and prevents contamination.

Furthermore, this device is cost-effective.   It is able to be manufactured for approximately $1200 and is constructed out of materials that are commonly found in industry. This is significantly less than other automated devices on the market that range from $15,000 to $25,000. For example the Precision ™ Microplate Pipetting System from BioTek® (Winooski, Vermont) was quoted to be $15,300, not including software and supplies. This is only designed for 8-channel liquid transfer capability, and therefore does not have the versatility of the device designed through this project. This quote can

be seen in Appendix 5. In addition to the cost effectiveness of this device it is also easily

assembled and can be programmed to fit any laboratory's needs.   The many benefits of

this device make it an invaluable addition to any cell research laboratory.

Conclusions

The objective of this Major Qualifying Project was to develop a method for isolation of cardiogenic cells produced through spheroid body formation. Through the team's cell testing, a method for isolation of cardiogenic was discovered and a device was constructed that has the potential to fully automate this method. As described in the client statement, this device will allow for maximized cell isolation within a completely sterile environment. Future testing of this device will produce results on whether or not the device has the ability to retain cardiogenic potential.

This device has been designed for manufacturing. It is easy to assemble, made out of materials commonly used in industry, and is able to fit into a bio-safety cabinet to maintain sterility. The device is fully automated to save the user time and reduce human error. It is the start of producing cardiogenic cells from the laboratory bench to the patient bedside. These cardiogenic cells could be delivered to patients to potentially treat cardiovascular disease in the future.

Future Recommendations

For the purposes of the device working as effectively as indicated, we recommend that two types of tests be conducted on this device. It should undergo mechanical testing to ensure that the motors are in sync with the syringe pump, and to test the accuracy and precision of the device's movements in both the x and y directions.

We also recommend that additional cell testing be completed in order to verify that this device maintains cell viability. Due to time constraints, cell viability testing was unable to be completed. This can be completed through a live/dead cell stain.

The syringe pump program needs to be written in such a way that it will pick up the directed amounts of media and trypsin, triturate at the correct speeds, and do this in the correct timing sequence.
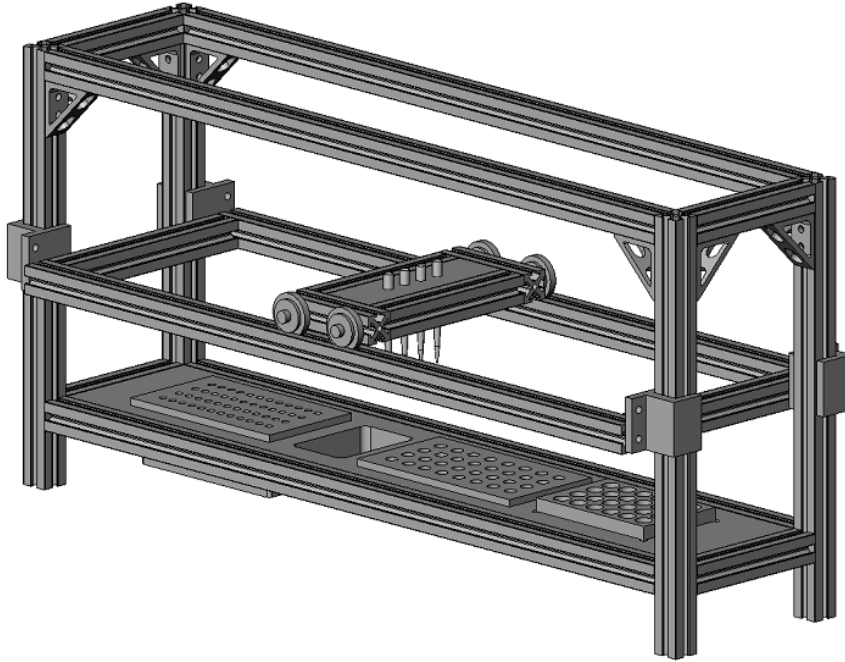
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APPENDIX 1: Step by Step assembly

First please open and lay out all parts. Please check to make sure you have all the parts you are supposed to. Compare parts to the bill of materials.

ITEM NO.	PART NAME	QTY.
1	base plate 2	1
2	beam 28.5 inch	4
3	beam 5.5 inch	4
4	beam 15 inch	4
5	beam 27.875 inch	2
6	24 well plate metric back to inch	1
7	corner bracket	8
8	linear bearing	4
9	beam 7.968 inch	2
10	wheel	4
11	beam 4 inch	2
12	beam 6.253 inch	2
13	collector insert 3	1
14	pipette base holder	1
15	pipette holder top	1
16	pipette large	4
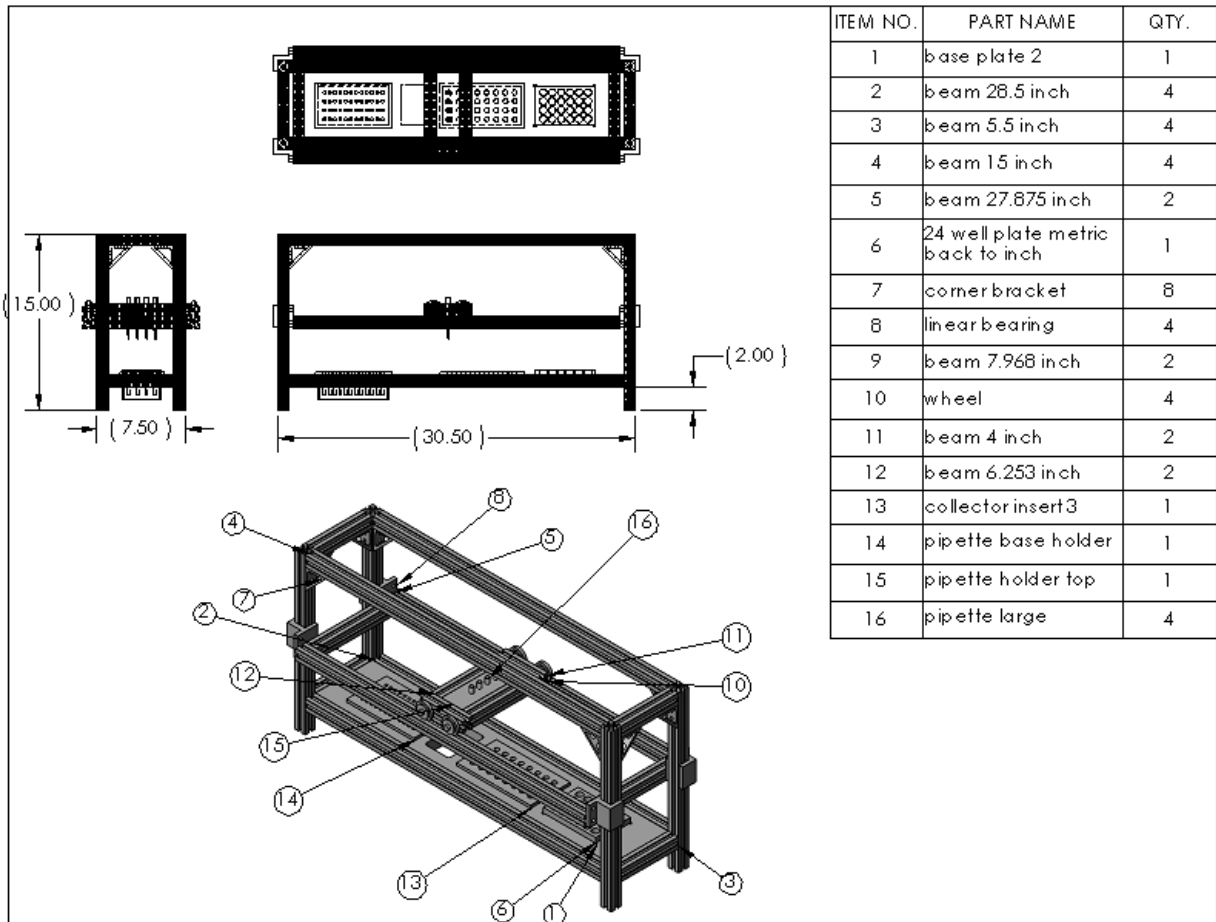
Tools Required

- Phillips head screw driver
- Allen wrench
- Pliers

Assembly of individual sub assemblies

Sub assembly one, roller cart

ITEM NO.	PART NAME	QTY.
1	wheel	4
2	beam 4 inch	2
3	beam 6.253 inch	2
4	pipette holder top	1
5	pipette large	4
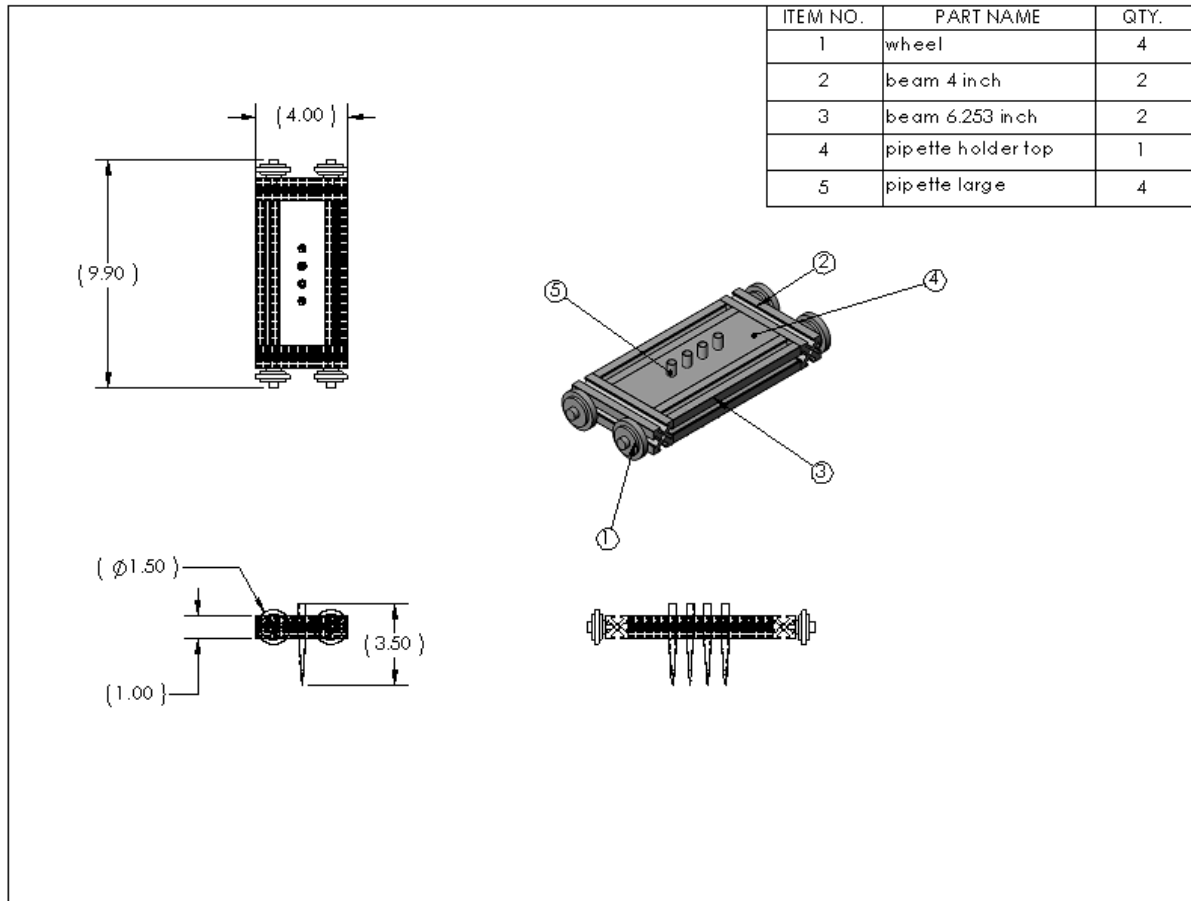
Fasten the 4" and 6.253" pieces together as shown in the picture using the double anchor fasteners. Make sure that you slide in two 80/20 nuts onto the underside of each of the 6.253" pieces. Once the rectangle is formed you can attach the support beams to the underside of the square using ¼-20 screws that are ½ inch. Also make sure to slide two 80/20 nuts onto the opposite side of the aluminum and four 80/20 nuts onto the other side where the nut bracket will be attached. Now you can drop it the pipette holder. The pipette tips can then be placed into the pipette tip holder. The nut bracket can now be attached to the cart frame with ¼-20 screws that are ½ inch long. The pipette cap can then be secured over the pipette tip tops. This is secured using two more of the 4 inch

support beams attached with ¼-20 screws into the 80/20 nuts. Next attach the wheels

onto the four inch pieces. Wheels should be attached one inch from the ends. Place the

pipette holder top piece in place.

Sub assembly two, outside frame

ITEM NO.	PART NAME	QTY.
1	beam 15 inch	4
2	beam 28.5 inch	2
3	beam 5.5 inch	2
4	corner bracket	8

To assemble the outside frame but together the parts shown in the asembly. Make

sure to tighten all the screws securely. The top peieces should be relatively level. Make

sure when assembling the top rectangle portion to slide the 5.5 inch cross section bars

into place. Make sure that each of the middle 5.5 inch cross section bars each have two

80/20 nuts slide into them. Next attache the 23 mounting plate to these cross bars with ½

inch long 1/4-20 screws. These screws should be low profile so the fit into the

countersunk holes. Once this is complete you can attach the NEMA 23 silverpak motor

to the motor mounting plate. Next attach the drive pulley to the motor shaft.

Sub assembly three, slider

ITEM NO.	PART NAME	QTY.
1	beam 27.875 inch	2
2	linear bearing	4
3	beam 7.968 inch	2
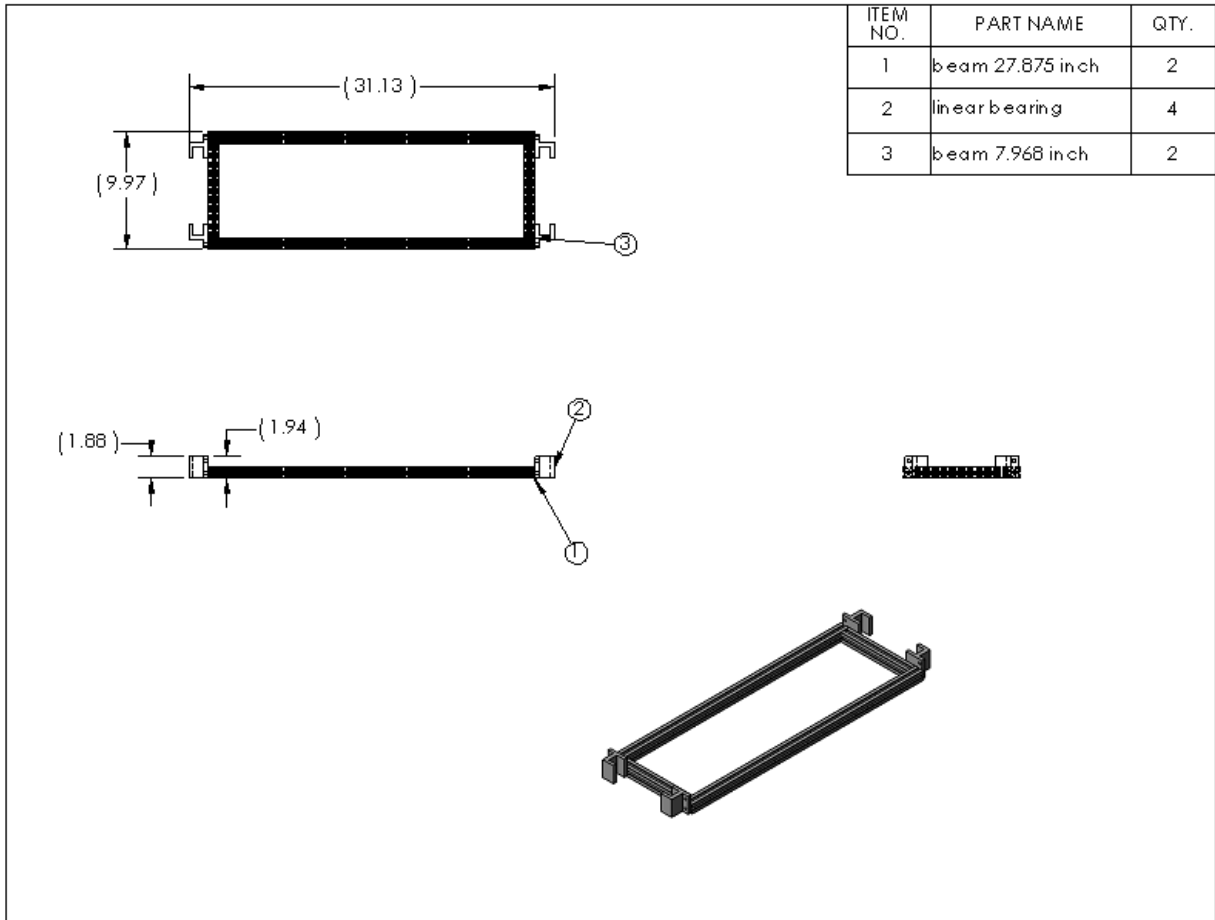
Attach the 27.875" bars to the 7.968" bars using the double anchor fasteners.

Before these bars are fully attached to form the square frame make sure to slide two

corner bracket assemblies onto each 7.968" bar. Also make sure to slide the U shaped

support assembly onto the 7.968" bars before securing them to the 27.875" bars. To the

7.968" bars with the small corner brackets and screws provided.

Now slide the slider assemble onto the upside down outside frame. Now slide the

linear bearings onto the outside frame. Make sure they are aligned properly. Attach the

linear bearings to the ends of the 27.875" bars with the provided screws. You may need

to use washer to achieve the desired fit. Also make sure to attach the corner gusset supports between the 27.875" bars and the linear bearings. The linear bearing slider assemble should be able to move vertically with ease. Now you can attach the mounting bearings to the top of both the U shaped support assemblies. Make sure to slide two 80/20 nuts onto both vertical sides of one of the U shaped supports. Make sure that the nuts are facing the outside of the frame. Now you can attach the 17 mounting plate to the U shaped support.

Now you can drill approximately a 1inch deep size 9 drill hole into the center end of the thread screw. Next take a ¼ inch flat end mill and remove the thread on the center line of the thread screw ½ inch from the end of the drilled thread screw. Next drill a hole onto the flatten part of the thread screw with a #36 drill bit. Drill the whole until you go into the horizontal hole which is already drilled. Next tap the hole with a 6-32 tap. Now you can friction fit the motor shaft onto the thread screw and tight the 6-32 ¼ inch set screw.

Now you can take the motor and thread screw assembly and place the thread screw through one mounting bearing then thread the nut and pass the thread screw through the second mounting bearing. Next secure the motor to the motor mount using the provided M3 screws.

Putting it together

Once this is on you can then attach the two 28.5" and two 5.5" beams with the double anchor fasteners. The bottom of these beams should be attached 2" from the bottom of Sub assembly three. Make sure that on the underside of the 28.5 inch beams you slide in two 80/20 ¼-20 nuts. Once this rectangle is assembled you can attach the

base supports with the ½ inch ¼-20 screws. Now you can drop base plate 2 into place.

Once this is completed you can place the remaining pieces of plastic into their locations.

Make sure that the base plate is positioned correctly by placing the end with the 24-well

plate cut out on the right hand side the side with the smaller stepper motor by it. Now

Sub assembly one, roller cart can be placed in its location on the middle of Sub assembly

two, slider. Slide the cart until the nut bracket it touching the nut. Now connect the nut

to the nut bracket with the provided ¼-20 screws.

You have now completed assembly of the frame. Now you can attach the pulley

string to the device. The final step is to attach the wiring to the motors and plug it in.

APPENDIX 2: CAD Drawing of Final Design

Whole Assembly

Base Plate 2 part #1

base plate 2

(26.00)
(20.98)
(1.00)
(19.73)
(1.15)
(13.10)
(R.13 TYP)
(1.00)
(2.50)
(R.13)
(8.50)
(R.13)
(9.50)
(11.85)
(.25)
(1.00)
(5.50)
(28.50)

Scale 1:10
all dimensions in inches

89

Beam 28.5 part #2

beam 28.5

(.585)

(Ø.205 Thru)

(.323)

(.087)

(1.000)

(.354)

(.236)

(.170)

(.255)

(.177)

.500

DETAIL 1
SCALE 1 : 1

(28.500)

scale 10:1
all dimensions in inches

Beam 5.5 inch part #3

beam 5.5 inch

(1.00)

{ 5.50 }

scale 1:2
all dimensions in inches
geometry is the same as beam 28.5 inch
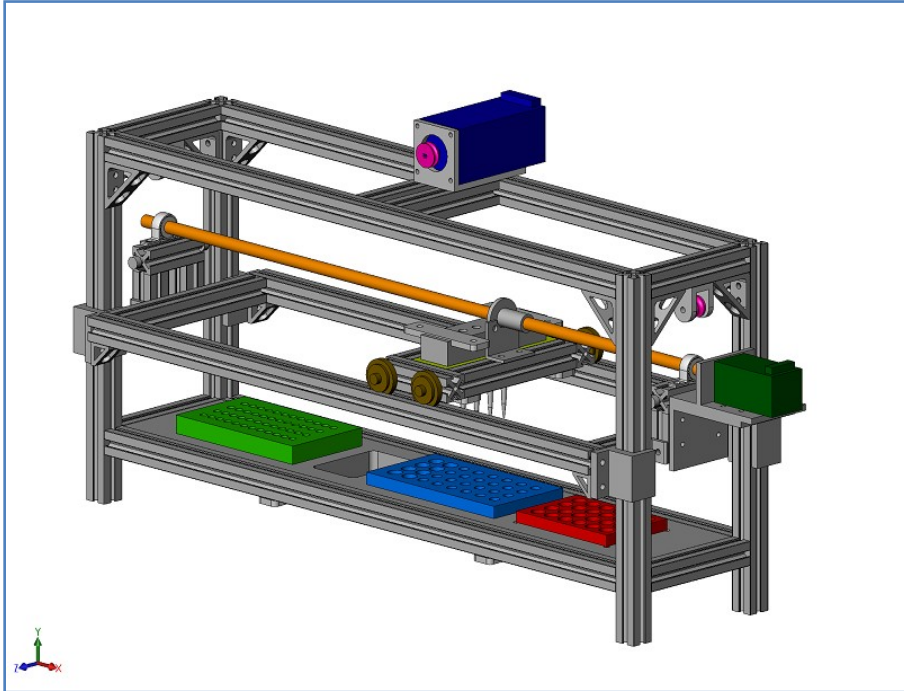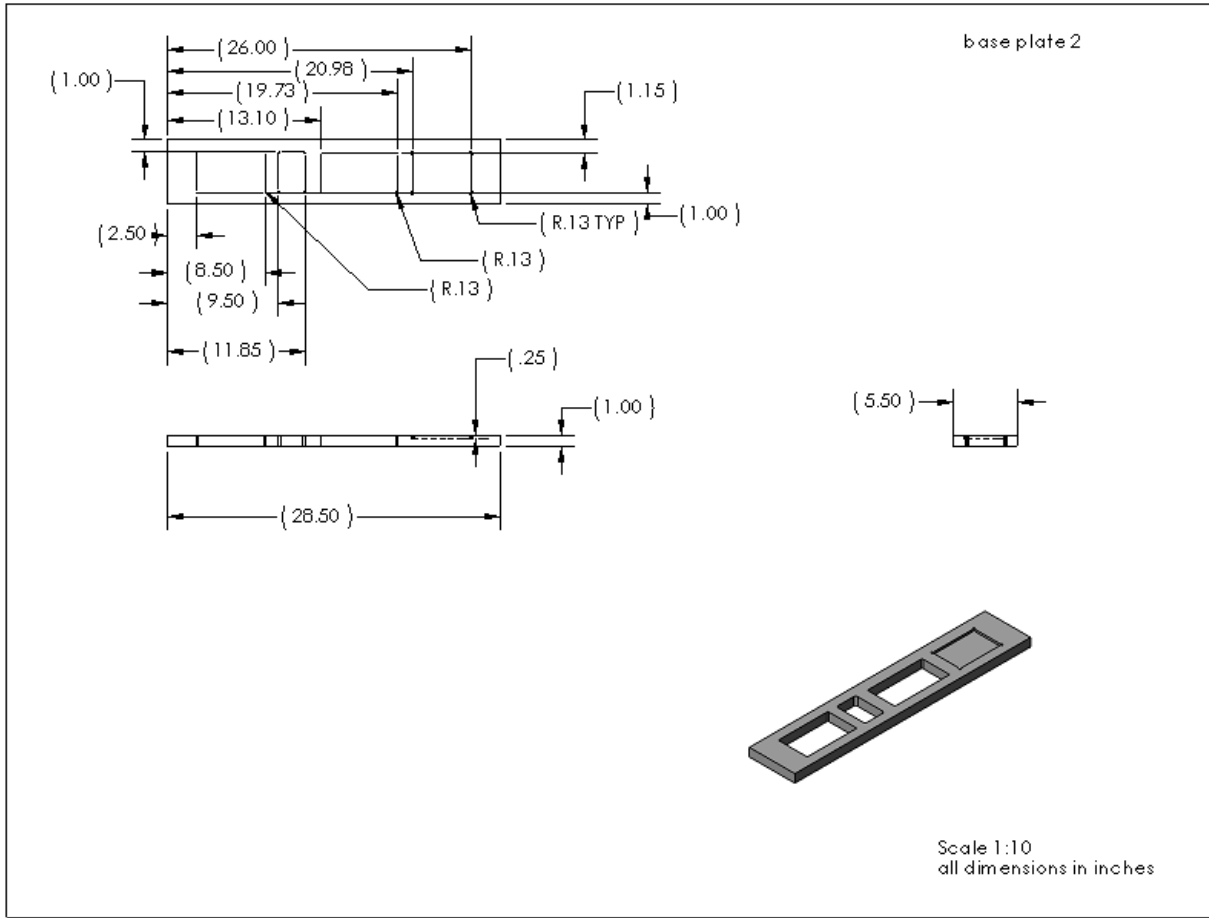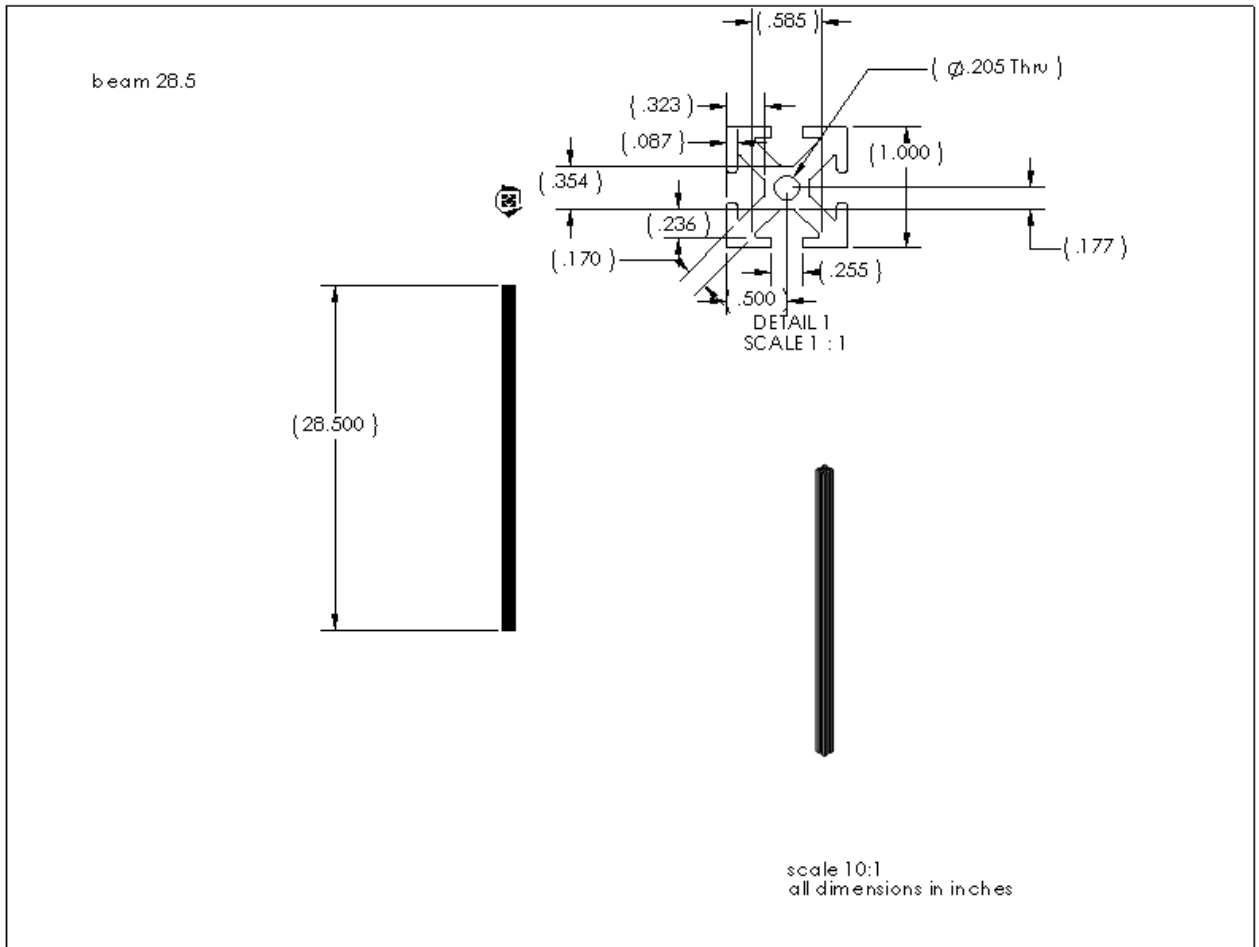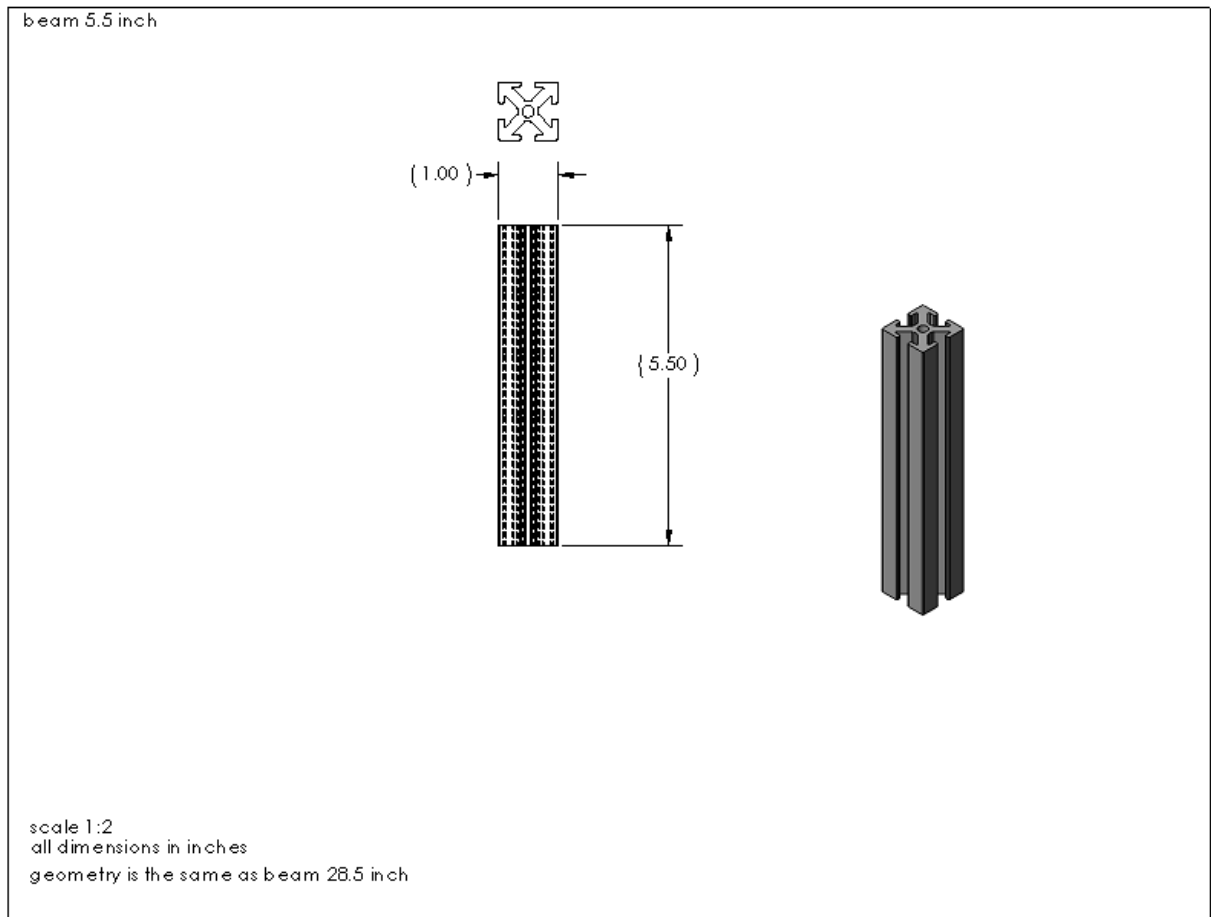
Beam 15 inch part #4

Please see 80/20 catalogue for dimensions of Aluminum T-Slotted Framing System 1010 with an overall length of 15 inches.

Beam 27.875 part #5

Please see 80/20 catalogue for dimensions of Aluminum T-Slotted Framing System 1010 with an overall length of 27.875 inches.

24 well plate part #6

BD Falcon™ HTS 24-Multiwell Insert System and
Cell Culture Insert Companion Plates

LID:
Material:PET (Polyethylene Terepthalate)
Length: A =129.57 mm (5.101 inches)
Width: B = 86.82 mm (3.418 inches)
Height: C = 8.20 mm (0.323 inches)

INSERT PLATE HOUSING:
Material:PET (Polyethylene Terepthalate)
Length: D =127.61 mm (5.024 inches)
Width: E = 85.01 mm (3.347 inches)
Height: F = 18.14 mm (0.714 inches)

Insert Well:
Top Interior Diameter: 12.50 mm (0.492 inches)
Bot. Interior (Membrane) Dia: 6.50 mm (0.256 inches)
Bot. Exterior Diameter:10.00 mm (0.394 inches)
Total Well Depth: 18.14 mm (0.714 inches)
Well to Well Distance: 19.30 mm (0.760 inches)
Sampling Port Length: 9.50 mm (0.37 inches)
Sampling Port Width: 4.00 mm (0.16 inches)

FEEDER TRAY:
Material:PS (Polystyrene)
Length: G =127.86 mm (5.034 inches)
Width: H = 85.47 mm (3.365 inches)
Height: J = 19.94 mm (0.785 inches)
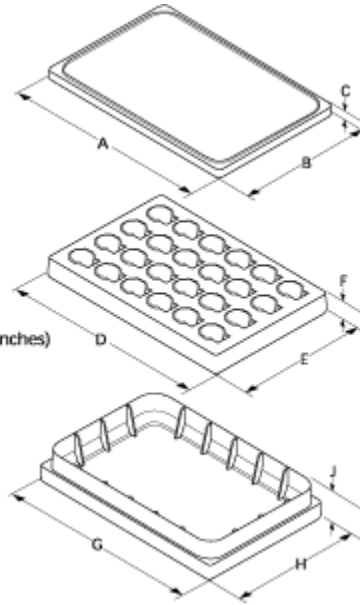
Effective Diameter of Membranes	6.5 mm
Effective Growth Area of Membrane	0.3 cm^2
Distance from Membrane to Bottom of Well	2.0 mm
Insert Height	18.14 mm
Suggested Media Volume in Insert	300-500 µl
Suggested Media in Feeder Tray	30-40 ml
Suggested Media Volume in Well	1000-1400 µl
Effective Growth Area in 24-well Plate	2.0 cm^2
Pore Density: 1 µm insert	1.6×10^6 pores/cm^2
Pore Density: 3 µm insert	8.0×10^5 pores/cm^2
Pore Density: 8 µm insert	1.0×10^5 pores/cm^2

Plate Indexing Dimensions

SAMPLING PORT CENTERLINES FROM PLATE EDGE
FOR FALCON 24-WELL PLATE

WELL CENTERLINES FROM PLATE EDGE
FOR FALCON 24-WELL PLATE
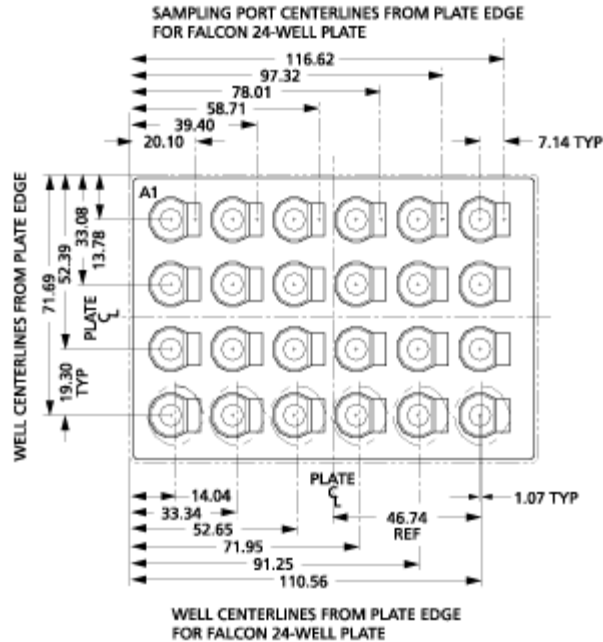
Corner Bracket part #7
Please see 80/20 catalogue for dimensions of pat # 4136- 10 S 4 Hole Inside Corner
Gusset Bracket

Linear Bearing part #8
Please see 80/20 catalogue for dimensions of part # 6715- 10 S Single Flange Linear
Bearing

Beam 7.968 inch part #9
Please see 80/20 catalogue for dimensions of Aluminum T-Slotted Framing System 1010
with an overall length of 7.968 inches.

Wheel part #10
Please see 80/20 catalogue for dimensions of part # 2281- 10 S Roller Wheel

Beam 4 inch part #11
Please see 80/20 catalogue for dimensions

Beam 6.253 inch part #12
Please see 80/20 catalogue for dimensions of Aluminum T-Slotted Framing System 1010
with an overall length of 6.253 inches.

Collector insert 3 part #13

Collector insert 3   Scale 1:2
All dimensions in inches

(.2500)

(1.0000)

(7.1240)
(6.3740)
(5.5765)
(4.7790)
(3.9815)
(3.1840)
(2.3865)
(1.6282)
(.8307)
.2500)

(R.1250)

(.7835)
(1.5435)
(2.3035)
(3.0635)

(3.8470)
(3.5970)

27/64 .4219 ↧ 1.0000
(.2500)
7/16 .4375 ↧ 1.0000
(6.8740)

first two columns from left are 7/16
the next six columns from left are 27/64

94
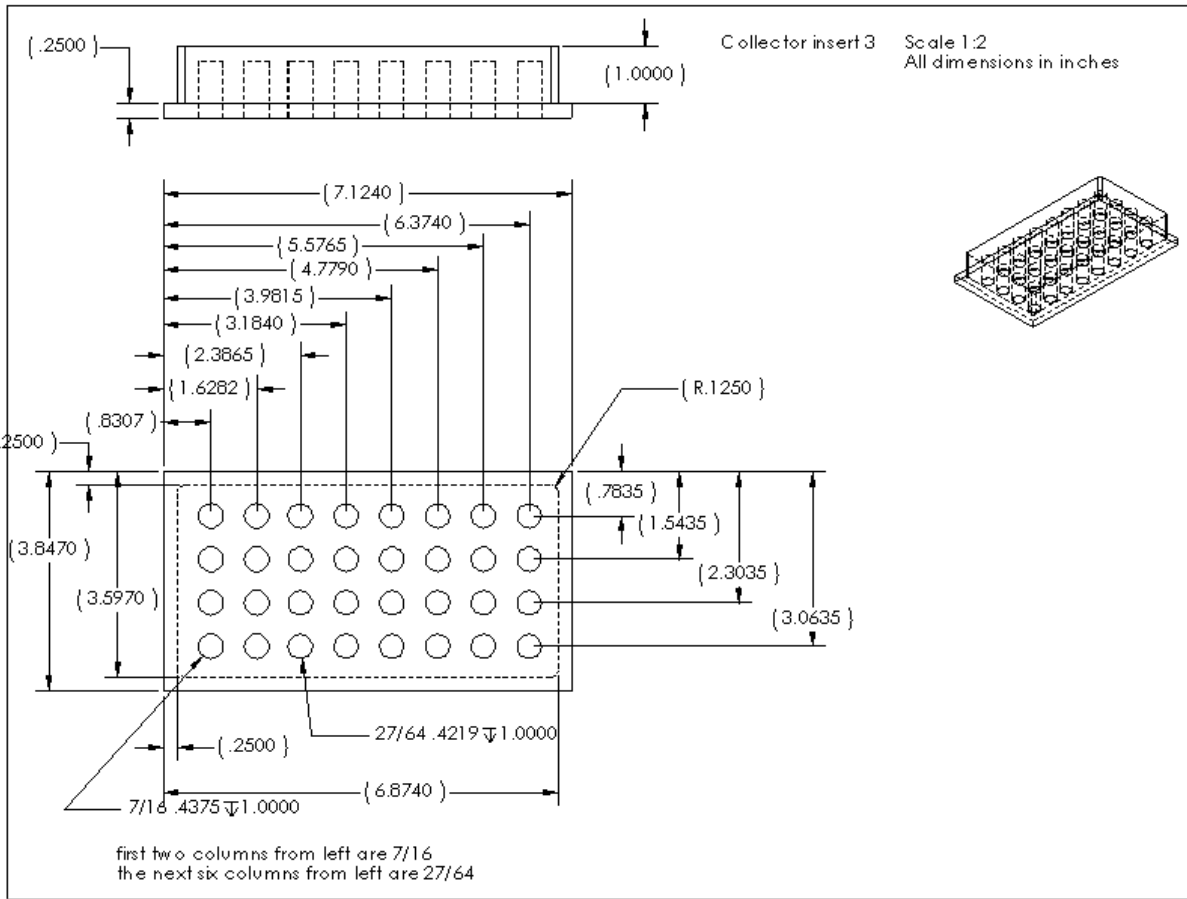
Pipette base holder part #14

pipette base holder

(2.0000)

(.2500)

scale 1:2
All dimensions in inches

(5.7500)
(5.3000)
(4.8500)
(4.4000)
(3.9500)
(3.5000)
(3.0500)
(2.6000)
(2.1500)
(1.7000)
1.2500
(.8000)

(1.5435)

(2.3035)

(3.0635)

(3.3470)

(3.8470)

(R.1250)

(6.0000)
(6.5000)

F .2570 ⊽ 1.9500
same for all holes
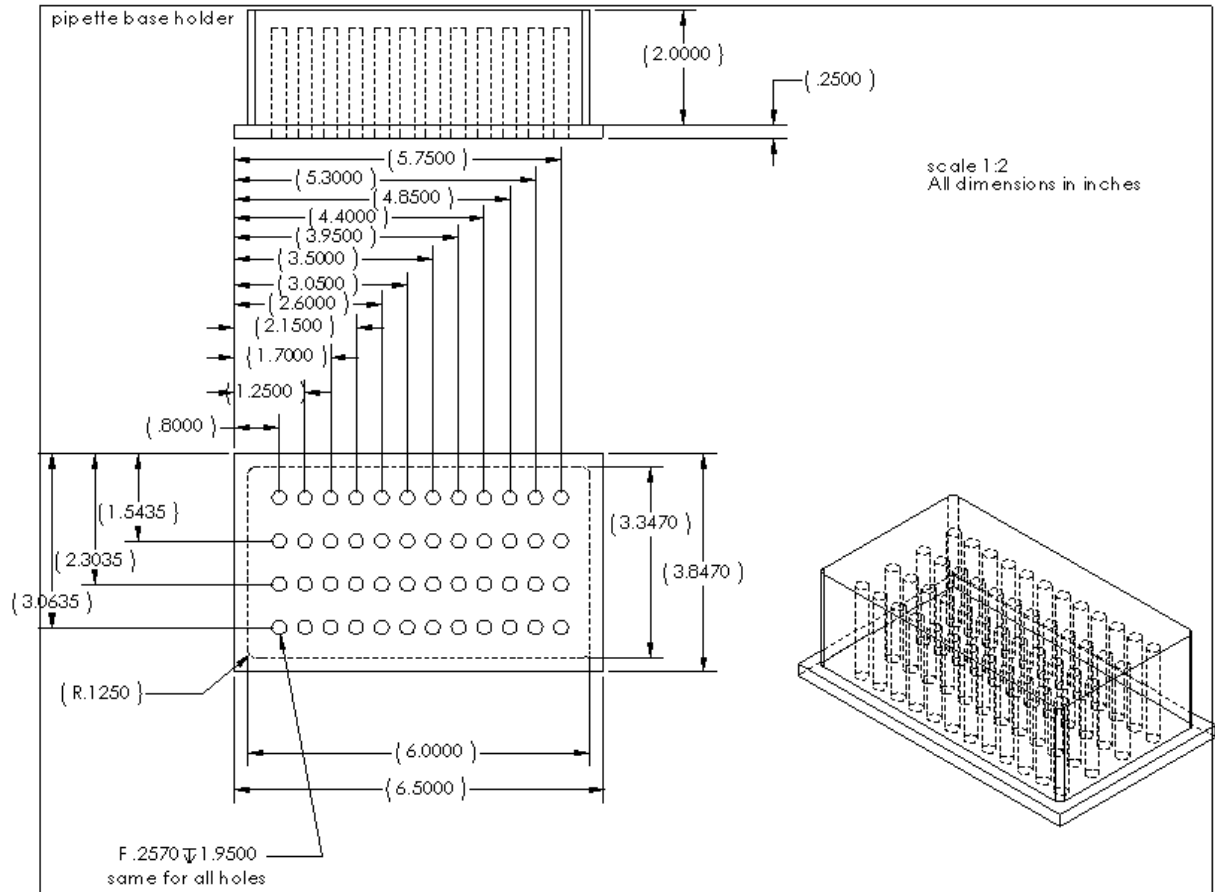
Pipette top holder part #15

pipette holder top

scale 1:2
All dimensions in inches

(1.00)

(4.34)
(3.58)
(2.82)
(2.06)

(1.00)

(2.00)

Ø .34
.3390 R thru TYP

(6.25)

Pipette large part #16

pipette large

scale 1:2
all dimensions in inches

{ Ø.03 }

{ Ø.05 }

(Ø.17)

(Ø.13)

(Ø.34)

DETAIL A
SCALE 4 : 1

(Ø.30)

(1.00)

(2.90)

(3.50)

23 Mounting Plate

4X TYP F Ø.257   .500+.05   23 Motor Mount

(1.250)   2.250+.1

.250+.1

1.500+.1

.250

2.500+.1   .250

4.000+.25

.195 ±.005   (1.860 ±.005)

.195 ±.005   930 ±.005

(1.860 ±.005)   Ø1.500+.1

(4X TYP ø.200 +.05)

.930 ±.005

All dimensions in inches

17 Mounting Plate

motor mount 17

M3 ∅.126 +.05
∅.875+.1
.500+.1
.225 ±.005
.225 ±.005
(4X TYP F ∅.257)
.300+.05
1.000+.1

2.850+.1
1.500
.250
.250
1.700+.1
.600
.500
2.750+.1
.250
4.000

4X TYP F ∅.257
.300-.05
.300-.05
1.500+.1
.250+.1
1.700+.1

All dimensions in inches

scale 1:2

Nut Bracket

.3750-.05
#7 .2010 for 1/4-20 tap
1.0000+.05
.5000
.7500
∅.2570
.1250
1.4925
.1250
1.0000+.05
1.7500+.1
.8750
.2447
.3163
1.4925
.3685
1.3402
.3750
.5000+.05

scale 1:1
all dimensions in inches
nut bracket

99

Support 4

support 4
all dimensions in inches
scale 1:2

(3.000 ±.005)

(R.200)

(1.000 ±.1)

(2X TYP ⌀.257)

(4.000 -.25)

(.250 ±.05)

Support 7.5

(R.200)

(6.500 ±.005)

(2X TYP ⌀.257)

(1.000 ±.1)

(7.500 -.25)

(.250 ±.05)

support 7.5
all dimensoins in inches
scale 1:2

Tip Remover

Pipette Cap

Appendix 3: Testing Procedure

Experiment: Testing Proof of Concept

Materials:
24 well plate with plated spheroids (10 days old)
P1000 pipette and tips
P200 pipette and tips
20% FBS 1% P/S Media
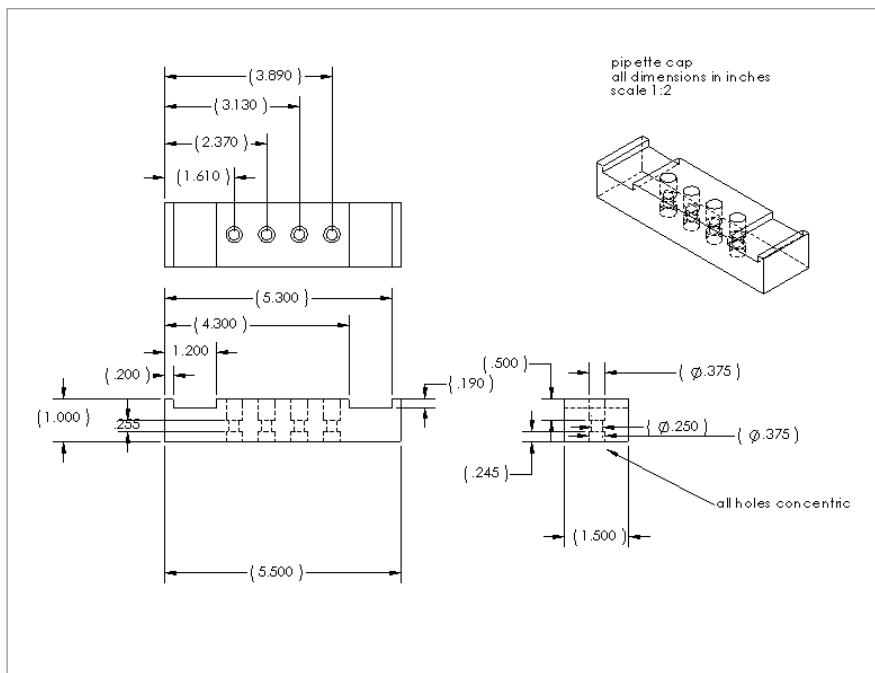Trypsin
10 eppendorf tubes

Procedure:
1) Using P1000 pipette, add 1 mL 20% FBS 1% P/S Media to an eppendorf tube
2) Using the P1000 pipette, add .187 mL trypsin to one well
3) Let stand for 5 min
4) Using a P200 pipette, remove free cells and media from the well and transfer into the eppendorf tube containing 1 mL of media (do not remove spheroid)
5) Using the P200 pipette, add 20 µL 20% FBS 1% P/S Media to the spheroid
6) Let stand for 1 min
7) Using the P1000 pipette, add .187 mL trypsin to the spheroid
8) Let stand for 5 min
9) Using a new P1000 pipette tip, add 1 mL 20% FBS 1%P/S Media to the well
10) Triturate manually for 2 minutes
11) Plate the cells on a slide and observe under a microscope for cell isolation

Appendix 4: Stepper Motor Programs

Stepper motor program for 17C motor that is controlling the x direction:

/1j2V2000D4000P4000D8000P8000D12000P12000D16000P16000D20000P20000D240
00D24000P44000P8000D24000D42000M600000P30000P24000P10000D26000D42000
M600000P42000P24000P14000D30000D46000D42000M600000P30000P28000P16000
D32000D42000M600000P30000P28000P18000D34000D46000M600000P30000P32000
P20000D36000D46000M600000P30000P32000P22000D36000D50000M600000P30000
P36000P24000D36000D50000M600000P30000P36000P24000D38000D54000M600000
P30000P40000P28000D38000D54000M600000P30000P40000P26000D40000D58000
M600000P30000P44000P32000D40000D58000M600000P30000P44000D20000

APPENDIX 5: Automatic Trituration System Quote

::BioTek®

Quotation # 214530

P.O. BOX 998, HIGHLAND PARK, WINOOSKI, VT 05404-0998, USA
TEL: 888/451-5171 Outside the U.S.A.: 802/655-4740, FAX: 802/655-7941
URL: www.biotek.com, E-mail: CustomerCare@biotek.com

Quotation to:

Contact:	Amber Deangelo (adangelo@wpi.edu)
Enterprise:	WORCESTER POLYTECHNIC INST
Address:	100 Institute Rd
	Worcester, MA 01609
	USA

Your Local Representative is:

Sales Group:	BioTek
Rep Name:	Brian Murphy
Phone #:	(888) 451-5171 x540
	(508) 839-0559
Fax #:	(802) 655-7941
E-mail:	murphyb@biotek.com

Phone #: 508 855 3605   Fax #:

Date	Expiration	Terms of Payment	Terms of Delivery	Customer #
04/24/2008	06/23/2008	Net 30	Fob Origin Ppd And Add	221609

Special Notes:
Expected Shipment Date is 30 days ARO
Warranty is 1 year parts and labor on units and 90 days on software

Qty	Part #	Description	Disc	List Price	Net Price	Amount
1	PRC384	Precision™ Microplate Pipetting System - 8-channel liquid transfer capability.	10.00 %	$15,300.00	$13,770.00	$13,770.00
1	PRCPWR V2	Precision Power™ PC Software Version 2: Graphical user interface (GUI) for program creation under Windows 98, NT, 2000 and XP. Package includes software on CD, User's Guide, and two user licenses.	10.00 %	$1,650.00	$1,485.00	$1,485.00
1	SVCIQT9	Installation Qualification and Initial Training	100.00 %	$750.00	$0.00	$0.00
		The Liquid Handling IQ and Training service includes unpacking and setup of the instrument, following authorized BioTek procedures. Package contents are verified against the purchase order, and Bill of materials. Instrument configuration and proper operation is confirmed. Results are recorded in the IQ checklist. Initial training on the use of the instrument and/or basic overview software is included. Price cannot be guaranteed if not purchased at same time as instrument.				

Required Item Total: 15,255.00

Service and Support Packages for PRC384

Item	Description	Amount
SVCONFWU	FIELD WARRANTY UPGRADE	$400.00
SVCONBDA	BASIC DEPOT SVC AGREEMENT	$1,295.00
SVCONBFSA	BASIC FIELD SVC AGREEMENT	$1,795.00

Optional Item(s) (1 ea) for PRC384

Item	Description	Amount
98195	PIPETTE TIP LABCON 200 ROBOTIC	$101.00

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Print Date: 4/24/2008

BioTek®

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TEL: 888/451-5171 Outside the U.S.A.: 802/655-4740, FAX: 802/655-7941
URL: www.biotek.com, E-mail: CustomerCare@biotek.com

Pete Wimble
Customer Care Specialist

Required Item Quote Total: 15,255.00

105

::BioTek ®

P.O. BOX 998, HIGHLAND PARK, WINOOSKI, VT 05404-0996, USA
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URL: www.biotek.com, E-mail: CustomerCare@biotek.com

::BioTek

BioTek Warranty and Support Agreement Options

Warranty Options

1. **Standard Depot Warranty – included no additional charge**

   The BioTek standard warranty for all products is depot repair. Customers send their instruments to our depot facility in Winooski, VT. Coverage includes repair parts and depot labor. BioTek pays for return shipping to the customer (ground) or overnight for an extra charge. Normal turn around time is 5 business days. If needed, a loaner is provided until instrument is repaired (loaner is no charge).

2. **Field Warranty Upgrade – price varies by model (SVCONFWU)**

   Customers may elect to upgrade their depot warranty to a field service warranty for a fee. With this warranty a field service engineer (MPS) is dispatched to your site instead of repair at the depot. Coverage includes repair parts, travel and labor. If needed, a loaner is provided until instrument is repaired (loaner is no charge).

Field Service Agreements

1. **Basic Field Service Agreement – price varies by model (SVCONBFSA)**

   Field service engineer (MPS) is dispatched for repair service. Coverage includes repair parts, travel and labor. This basic agreement does **NOT** include Planned Maintenance (PMs.) If needed, PMs are available at a 25% discount.

2. **Comprehensive Field Service Agreement – price varies by model (SVCONCFSA)**

   Field service engineer (MPS) is dispatched for repair service. Coverage includes repair parts, travel and labor. If needed, a loaner is provided until instrument is repaired (loaner is no charge). The comprehensive agreement **INCLUDES** one Planned Maintenance call per year (PM). Note – no charge loaners are provided for repairs only and does not apply to PMs.

Depot Service Agreements

1. **Basic Depot Agreement – price varies by model (SVCONBDA)**

   Customers send their instruments to our depot facility in Winooski, VT. BioTek pays for return shipping to the customer (ground) or overnight at an extra charge. Coverage includes repair parts and depot labor. The basic depot agreement does **NOT** include PMs. If needed, PMs are available at a 25% discount.

2. **Comprehensive Depot Agreement – price varies by model (SVCONCDA)**

   Customers send their instruments to our depot facility in Winooski, VT. BioTek pays for return shipping to the customer (ground) or overnight at an extra charge. Coverage includes repair parts and depot labor. If needed, a loaner is provided until instrument is repaired (loaner is no charge). The comprehensive agreement **INCLUDES** one Planned Maintenance depot call per year (PM). Note – no charge loaners are provided for repairs only and does not apply to PMs.

Priority Partner Program

Priority Partner Program – price varies by model (SVCONPPP)

BioTek provides EVERY customer FREE technical support. Customers who sign up for this program get "first in line" for priority help and in depth technical and application support. In addition, they are entitled to various discounts on value added services (test plate certification, PMs, OQs., etc. per service option matrix). Ask a BioTek representative for details.