Stem Cell Differentiation Controller/Monitor

Final Design Report
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Abstract

Embryonic Stem Cells (ESCs) have the capacity to differentiate into every cell type in the body, and therefore can theoretically be used to generate cells and tissues to cure a variety of diseases. Our client in the Odorico Lab (Department of Surgery) has derived foregut-committed cell lines from ESCs (which correspond to progenitor cells of the gut region that develops primarily into pancreas) and would like to differentiate these ESCs into insulin-producing pancreatic beta-like cells. These cells could replace or supplement transplanted donor beta cells. We have fabricated and began testing a no-flow microfluidic gradient generator that will allow our client to discover what affect the growth factor concentration has on differentiation. We have begun testing our device and will continue to improve upon our design next semester as we will be continuing this project.

Problem Statement

Embryonic stem cells (ESCs) have the capacity to differentiate into every cell type in the body, and therefore can theoretically be used to generate cells and tissues to cure a variety of diseases. Our client in the Odorico Lab (Department of Surgery) has derived foregut-committed cell lines from ESCs (which correspond to progenitor cells of the gut region that develops primarily into pancreas) and would like to differentiate these ESCs into insulin-producing pancreatic beta-like cells. These cells could replace or supplement transplanted donor beta cells. The mechanisms required to differentiate ESCs into these pancreatic cells is currently unknown, and this device would aid in researching such mechanisms. Our client would like to test a large number of growth factors for their ability to affect conversion of these precursor cells to mature insulin-secreting cells. In addition, a recapitulation of the 3-dimentional embryonic environment to prompt cells to adopt a pancreatic cell fate, perhaps using a Matrigel substrate, is desirable. A small scale cell culture using microfluidics to set up growth factor gradients is one approach that could be successful.

Client Biography

Dr. Browning and Dr. Kahan both serve as associate scientists in Dr. Jon Odorico's laboratory in the Department of Surgery at the University of Wisconsin – Madison. The Odorico laboratory's primary interest is in using embryonic stem (ES) cells to study pancreatic islet development. They are searching for a deeper understanding of precisely how insulin secreting beta cells and other endocrine cell types within mammalian pancreatic Islets of Langerhans are specified from embryonic

foregut endoderm, or what complement of transcription factors direct this fate choice. Dr. Kahan has recently isolated a stem cell line which she calls “EndSCs” which appear to be committed to forming endoderm, the tissue from which the pancreas is derived. These cells may already be part way toward becoming pancreas. The cells now need to be exposed to a variety of different growth factors in varying concentrations to establish what will cause the desired differentiation.

Erwin Berthier is a graduate student in Dr. David Beebe’s laboratory in the Department of Biomedical Engineering at the University of Wisconsin – Madison. Erwin has much experience with fabrication and testing methods related to no-flow microfluidic devices and has served as a valuable resource over the course of the semester.

Background

Beta Islet Transplantation

Type I diabetes affects millions of people worldwide and leaves sufferers dependent upon exogenous insulin. The problem is that this exogenous insulin does not mimic the blood glucose control provided by islets. A treatment method that is currently used to alleviate this dependence is Human Islet Transplantation (see **Figure 1**).

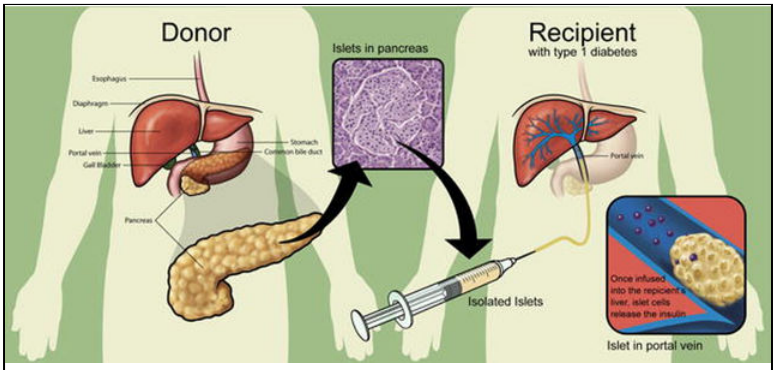

Figure 1: Human Islet Transplantation method. A current method being used to treat insulin dependence is the transplantation of beta-islet cells from a cadaver to a living recipient [1].

The procedure involves removing islet cells from a donor cadaver and implanting them

into the pancreas of a living recipient. While this procedure has shown high success rates, the large number of islet cells required (~1 million) and the lack of donor cadaver tissue limits the amount of patients who can have the procedure [1]. If these islet cells could be grown in the laboratory using stem cells, this would open this transplantation to a wider number of patients.

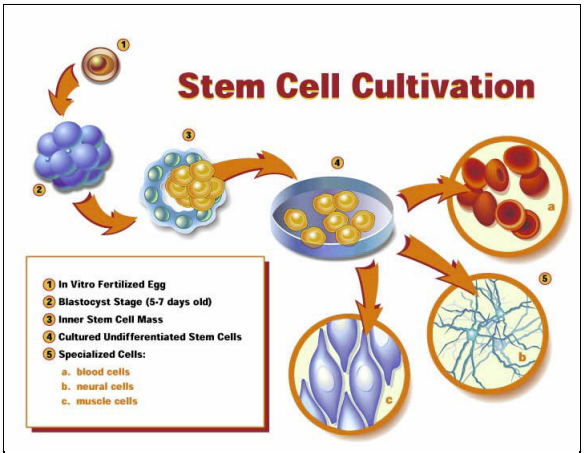

Figure 2: Stem cells are typically cultivated from blastocyst stage embryos. These undifferentiated cells fate can then be altered by exposing them to specific growth factors [2].

Stem Cells

Embryonic stem cells (ESCs) have the capacity to differentiate into most every cell type in the body. Therefore they could theoretically be used to generate a tissues and organs that could be used to help alleviate certain conditions. A cells fate is determined by what factors it is exposed to at this undifferentiated stage (see **Figure 2**). By altering the concentration and type of factor an undifferentiated cell is exposed to, researchers may be able to drive a cell towards a chosen fate. Our clients have derived stem cells that are foregut committed. This implies that they are already on their way to becoming pancreatic tissue. They have an idea of which growth factors may drive the desired differentiation, and it would now be beneficial to test a variety of different concentrations of these factors. Hence the need to a continuous gradient to which these stem cells can be exposed.

Microfluidics

Microfluidics is the study of the behavior of small amounts of liquid on the order of nano- or picoliters that flow through channels on the micrometer scale. There are a

variety of advantages to performing biological studies on such a minute scale. Microfluidic devices allow for high throughput studies by increasing replications, while simultaneously decreasing the amount of reagent volume necessary. A field that arose in the 1990s, this “lab on a chip” idea is beginning to see use in a variety of scientific applications (e.g. capillary electrophoresis, polymerase chain reactions, assays, migration studies, etc.) [3]. One of the most advantageous properties of these devices in the manner in which side by side streams flow. Parallel streams flowing at a large scale will quickly mix and lose their uniform characteristics. However, when minute liquid streams flow side by side they maintain their individual properties and mix only by diffusion. The property is called laminar flow, and it gives microfluidic devices the ability to create concentration gradients by flowing minute steams of varying concentrations side by side in their channels [4]. Fluidic gradients have a wide variety of applications that could give insight into cell behavior and development.

The device layout and material components must be compatible with the conditions favorable for biological studies. As these devices were originally based on microelectronic devices, similar fabrication materials (silicon and glass) were originally used. The problem is that these materials are expensive, difficult to work with, and impermeable to gasses necessary for cell viability such as oxygen [4]. Currently polydimethylsiloxane (PDMS) is used to circumvent these issues. PDMS devices can be custom made to fit a variety of study needs quickly and at low costs. It is also permeable to many necessary gasses and is low in toxicity. These devices are fabricated using photolithography and soft lithography in sequence (see **Figure 3**).

In our research we found two different types of microfluidic devices that are typically used to create a fluidic gradient: Flow systems, and no-flow systems. In a flow system, inlets of flowing fluids of varying concentration are split and recombined in microchannels to form a gradient across the cell culture area (see **Figure 4**). A syringe pump is typically set at a constant flow rate to maintain uniform fluid flow in the system. The advantages of such a system are rapid gradient generation, large amounts of past research, and flowing fluids may aid in nutrient replenishment and staining for imaging. As can be expected, this system is not without its disadvantages. The flowing fluids may cause shear forces that could adversely affect cellular development. The flow may also affect autocrine/paracrine signaling (cell to self/cell to cell) and allow signaling in only one direction. This lack of signaling does not accurately mimic the *in vivo* environment of natural development. And finally, the flow will require high reagent volumes which will pose a monetary burden to the researcher [3].

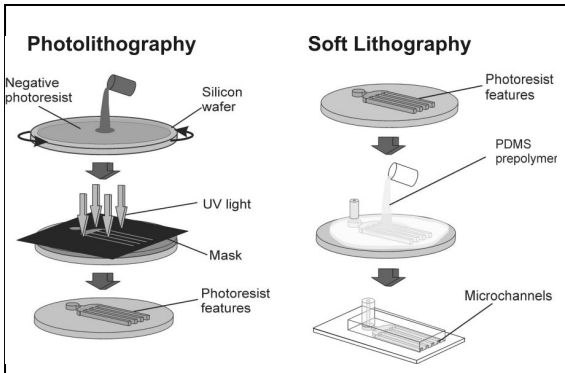

Figure 3: Photolithography is a method of patterning the photoresist using light to form the desired pattern. This 'master' print can then be used to shape the PDMS using a technique knows as soft lithography [4,5].

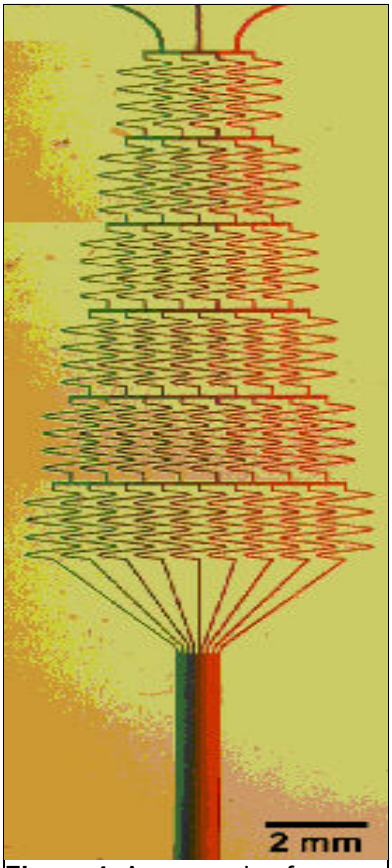

Figure 4: An example of a 'branched flow' microfluidic system in which minute streams run side by side with a small amount of diffusion occurring between streams. This allows a gradient to be created from only a few solutions of varying concentration [6].

Alternatively to this flow, diffusion itself can be used to establish a gradient in a 'no-flow' system (see **Figure 5**). This system consists of a highly concentrated 'source' and a dilute 'sink'. As the concentrated source material diffused through the microchannel to the sink, a gradient is created. The gradient profile is dependent upon the channel geometry, with a linear channel resulting in a linear gradient. One advantage of such a system is that cell to cell signaling is not inhibited and it is also possible to imbed your cells in a three dimensional hydrogel structure in your channel. These two features together create an environment that mimics the *in vivo* environment of natural development. Although the gradient does take longer to reach a steady state, the gradient is more stable than a flow system because it does not rely on flowing streams which could be disturbed by even small amounts of disturbance. A problem with a no-flow system is that less research has been performed, and more experimental testing would be required to determine device properties. Staining for imaging may also pose a problem, as the slow rate of diffusion may not bring staining materials to the cells within an adequate time frame [7].

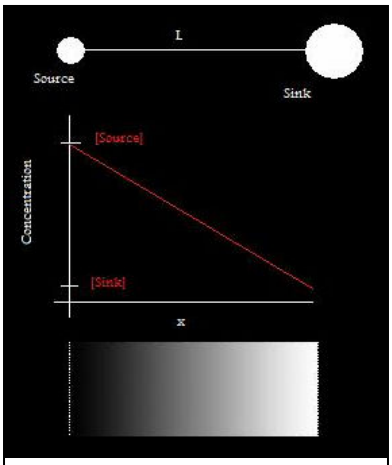

Figure 5: In a no-flow microfluidic system a gradient is generated from the highly concentrated source to the less concentrated sink. The profile of this gradient is dependent upon the channel geometry.

Design Constraints

Our client provided us with a number of constraints that our device must abide by. The first of these constraints dealt with cell capacity. The minimum amount of cells the device must hold is one-hundred, but a capacity or one-thousand to five-thousand would be more ideal. Assuming that a stem cell is 10 micrometers in diameter, five-

thousand cells would occupy less than half a picoliter. Therefore, housing five-thousand cells in our device should be a non-issue even with cells imbedded in a gel construct.

The device must also be compatible with imaging and immunofluorescense requirements. The main imaging barrier to consider would be the thickness of the substance composing the device. As stated previously, most microfluidic devices are made suing PDMS. PDMS is capable of being imaged through; therefore this should be a non-issue in our device. The setup most also allow immunostaining upon completion of any differentiation study. The stains required must be capable of reaching the cells within a time frame specified. Following staining the cells will then need to be imaged and, depending upon the material in which they are embedded, this may require slicing the material into slices of a thickness which can be properly viewed under a microscope.

The gradient generated in the device must be maintainable for seven or more days within the 37 degree Celsius incubator in which differentiation studies are performed. The length of the microfluidic channel will directly relate to how long the gradient takes to develop and may also effect how difficult it is to maintain. Other factors such as the concentration of the sink, the height of the channel, and the cross-sectional area of the input port will also affect time parameters related to gradient maintenance.

The most costly element of the experimental process will be the growth factors and therefore it would be ideal to minimize the amount required. Our clients asked up to keep the total costs this semester to under $500, which was not an issue as we were

able to obtain all necessary from materials from Dr. Justin Williams, Dr. David Beebe, Erwin Berthier, and Dr. Browning.

Design Alternatives

Flow System

Laminar flow of fluids in microchannels allows for parallel streams of fluid to move next to one another without turbulent mixing. Our flow design utilizes this unique characteristic to maintain stable gradients of growth factors perpendicular to the flow direction.

Our design has five inputs all converging to a central channel. Flow is driven by the use of syringe pumps capable of providing constant flow rates. The five inputs will hold concentrations of growth factors of 100%, 75%, 50%, 25%, and 0%. Similar to the figure which has two inputs, after the input channels converge they will flow alongside one another and the growth factors will diffuse between streams, smoothing the gradient. Based on the flow rate, diffusion will smooth the gradient different amounts. Modeling and testing will need to be done to determine the final gradient across the entire device. **Figure 6** shows our flow system design.

Cells would be grown on the bottom of the channel, perhaps on a thin layer of matrigel to improve their viability, morphology, and physiology. In order to deposit a thin layer of matrigel in the channel, the matrigel would be flowed into the channel completely filling it. Water would then be flowed through the channel, hollowing out the center and leaving a thin coating of matrigel on every side of the channel. Cells could

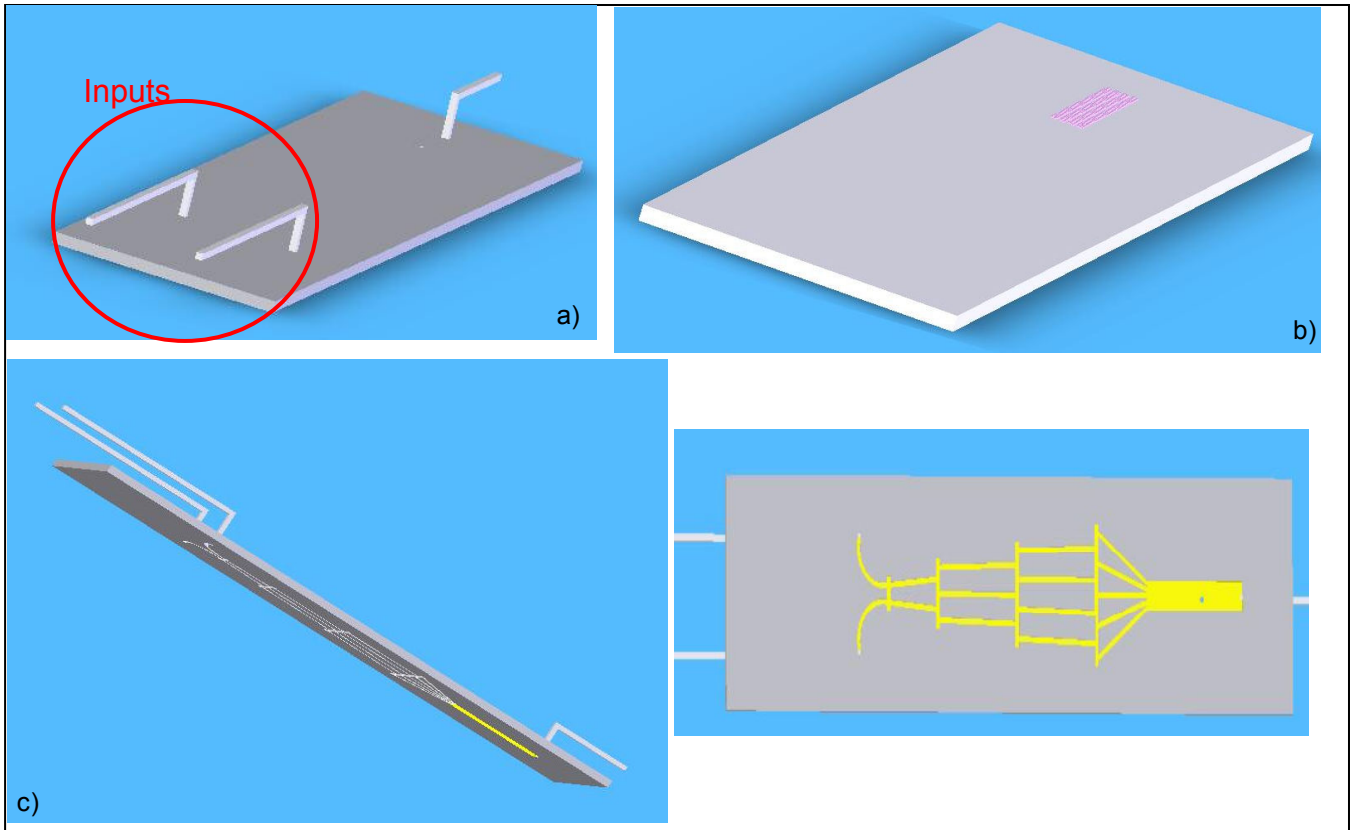

Figure 6: Flow System
a) Top view of the PDMS device. The two inputs lying close to each other are connected to the syringe pumps.
b) The device will rest on top of a glass slide that has cells mounted on a small slab of Matrigel as seen here.
c) and d) Bottom view of the PDMS device. The PDMS device allows the convergence of fluids from the inputs all the way to the cell source. Laminar flow is exhibited by this setup.

then be flowed into the channel and allowed to adhere before the growth factor gradient was established.

The biggest benefits of this design come from the fact that flowing fluids allow for rapid fluid exchange. Nutrient and growth factor concentrations will never deplete as new solution will constantly be provided to the cells and waste will quickly be removed. Characterization of the cells by immunohistochemistry and staining could utilize the flow to wash, stain, and provide antibodies efficiently to the cells.

The flow design suffers from both technical and biologic problems. The system requires a rather complex set-up with five syringe pumps and tubing all converging to a small area. Because of the precarious set-up and volatile nature of flow systems, once

flow was started the system could not be touched without drastically affecting the gradient which was in place. A huge amount of expensive growth factor would be needed for even short term experiments of 1 hr, making the desired long term experiments extremely costly. A flow system does a poor job in mimicking the *in vivo* cell environment. A flow system would expose the cells to unnaturally high shear forces as well as remove any of the autocrine and paracrine signals the cells may be expressing.

Dropdown System

The unique feature of this design is the use of a variably permeable membrane to create the concentration gradient of growth factor. The dropdown system uses two permeable membranes to separate a microchannel into three compartments. The top compartment would be for the input for a source solution of media and growth factor by syringe pump. The bottom compartment of this device would be for the input for a sink, or zero concentration of growth factor. The roof of this compartment and the floor of the top compartment would be made from a variably permeable membrane with pores in a pyramidal pattern. The middle compartment would hold the stem cells and matrigel construct. The concentration gradient will be generated as growth factors from the top compartment pass through the permeable membrane and diffuse through the matrigel construct to the bottom compartment. **Figure 7** shows our dropdown system.

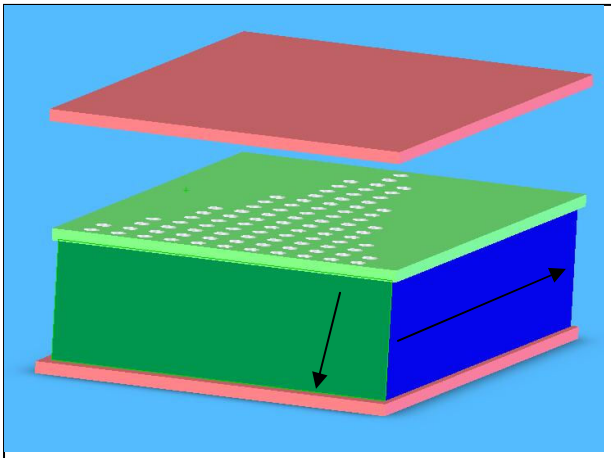

Figure 7: Dropdown System
The variable membrane (light green) allows for gradient formation in two directions shown with arrows. The source is located above the membrane and contains growth factors and other nutrients. The sink is located below the Matrigel.

The dropdown system has three major advantages. For one, the dropdown system sets up a growth factor gradient in two dimensions which allows one experiment to test twice the number of gradients. Also, using the permeable membrane separates the bulk flow of solution from the stable gradient decreasing the likelihood that moving the device during an experiment would drastically alter the established gradient. Little work has been done to develop a variably permeable membrane for small molecules so it is possible that the design would be patentable.

While the idea of a dropdown system is relatively simple, the construction of the device could be quite challenging. Fabrication of the membrane would likely require precise micromachining or some other microfabrication technique capable of producing features smaller than 10 micrometers. Material selection for the membrane is also important. The material needs to be biocompatible over a period of 4 weeks to avoid affecting the stem cells and preferably non-reactive to the media, nutrients, and growth factors to avoid adsorption into the material. Growth factors generally range in size from 10-100 kDa necessitating a variety of membrane diameters if many growth factors will be tested. If a molding or machining technique is used, a new device with different dimensions would need to be made, a potentially costly and laborious process.

No Flow Design: Construction

No Flow System Overview

The design we decided to pursue was the no flow system. This system takes advantage of diffusion and high resistance to generate a linear gradient between a source and a sink. The source is the place where a known concentration of growth

factors is added. The sink contains no growth factors. The success of this device underlies in the ability of growth factors to diffuse from the source, through the channel, and into the sink. However, the growth factors experience high resistance as they enter the channel. This enables the formation of a concentration gradient in the channel over time. The long period of time needed to generate the gradient makes the fluid flow in the channel negligible. Therefore, such a system allows for autocrine/paracrine signaling and requires low reagent volumes.

Channel Dimensions

The channel dimensions chosen for our design were dependent on two constraints: cell density and cell viability in microchannels. Our client wished to seed between 1,000 and 5,000 stem cells per channel, with a density of around 8,000 to 40,000 cells per microliter of matrigel. Cells which are farther than 1 millimeter away from a source of nutrients are often time necrotic. These two constraints led us to design channels with a height of 200 microns, length of either 1 mm or 2 mm, and width of 150, 300, or 450 microns. In total, we constructed six different channels ranging from 30 to 180 nanoliters.

Channel Fabrication

A combination of photolithography and soft lithography was used to create the channels. The first step in photolithography was to use Adobe Illustrator to design the mask used during exposure to define the channel dimensions. The channel design was then printed by a high resolution printer onto an overhead transparency. SU-8, a negative photoresist, was put onto a silicon wafer using a spin-coater to ensure a known, uniform thickness of 200 microns. The mask was placed on top of the silicon

wafer and exposed to UV light, cross-linking the polymers. Developer was then used to dissolve away all of the uncross-linked photoresist, leaving a mold for the soft lithography step.

Polydimethylsiloxane (PDMS), a silicon based polymer, was poured over the mold creating the microchannels. To create the rubber like material, a 10:1 ratio of liquid monomer and curing agent are mixed together, poured onto the mold, and heated to transform the liquid into a solid. After curing, the PDMS channels were pulled off the mold and placed onto a glass slide. PDMS was used because it is chemically inert, low cost, permeable to gases, and clear. The biggest drawback of PDMS microchannels is the inherent hydrophobicity which leads to the adsorption of hydrophobic molecule like growth factors into the bulk material.

A second layer of PDMS with reservoirs cut out with a scalpel was added on top of the first to hold the source and sink solutions. The reservoirs were approximately 2x2x2 mm for the source and 10x10x2 mm for the sink.

No Flow Design: Testing

Experimental Procedures

Experiments were carried out to validate the possibility of creating a gradient of growth factors in our microchannels and to determine the diffusion coefficient of matrigel and the diffusing molecule.

The PDMS channels were first filled with matrigel provided to us by our clients. A micropipette was used to deposit a small volume of liquid matrigel onto on port of the channel. Suction was applied to the other port, moving the matrigel throughout the

channel, completely filling it. Matrigel is a liquid below 0 degrees Celcius but begins to solidify at slightly higher temperatures. At 37 degrees, the matrigel is completely solid. In order to avoid premature solidification of the matrigel in the channel, the matrigel, pipette tips, and channels were kept on ice during filling. Once the channels were filled they were place on a 37 degree hot plate to complete the solidification process.

In an attempt to verify the formation of a gradient in the channels, fluorescently labeled Dextran was used as the solution for the source rather than a growth factor. Dextran is a complex polysaccharide of variable length. Since growth factors are around 25 kDa in size, we used a Dextran with a know size of 10 kDa with was attached to a small (>1 kDa) fluorescent molecule known as Texas Red. Using equations taken from [7], the source was filled with 10 microliters of 100% fluorescently labeled Dextran while the sink was filled with 200 microliters of reagent grade water. The channels were then put into a Petri dish and put under the fluorescent microscope. To delay evaporation of the source and sink liquid, water was added to the bottom of the Petri dish and the top was sealed to the bottom using scotch tape.

Imaging of the channels was done using a combination fluorescent microscope/camera with the green filter. Pictures were taken every 15 minutes for the first hour and then approximately every hour until no further change was observed.

Tim-lapse testing

Using Dextran labeled with Texas Red, the gradient formation of the channel was tested. The Dextran was added to the source in 3/10 ratio, 3 parts Dextran to 10 parts water and pure H20 was added to the sink. Initially the pictures were taken in 15 minute

intervals over the first hour and then pictures were taken each hour following. The following series of images was obtained. (see **Figure 8**).

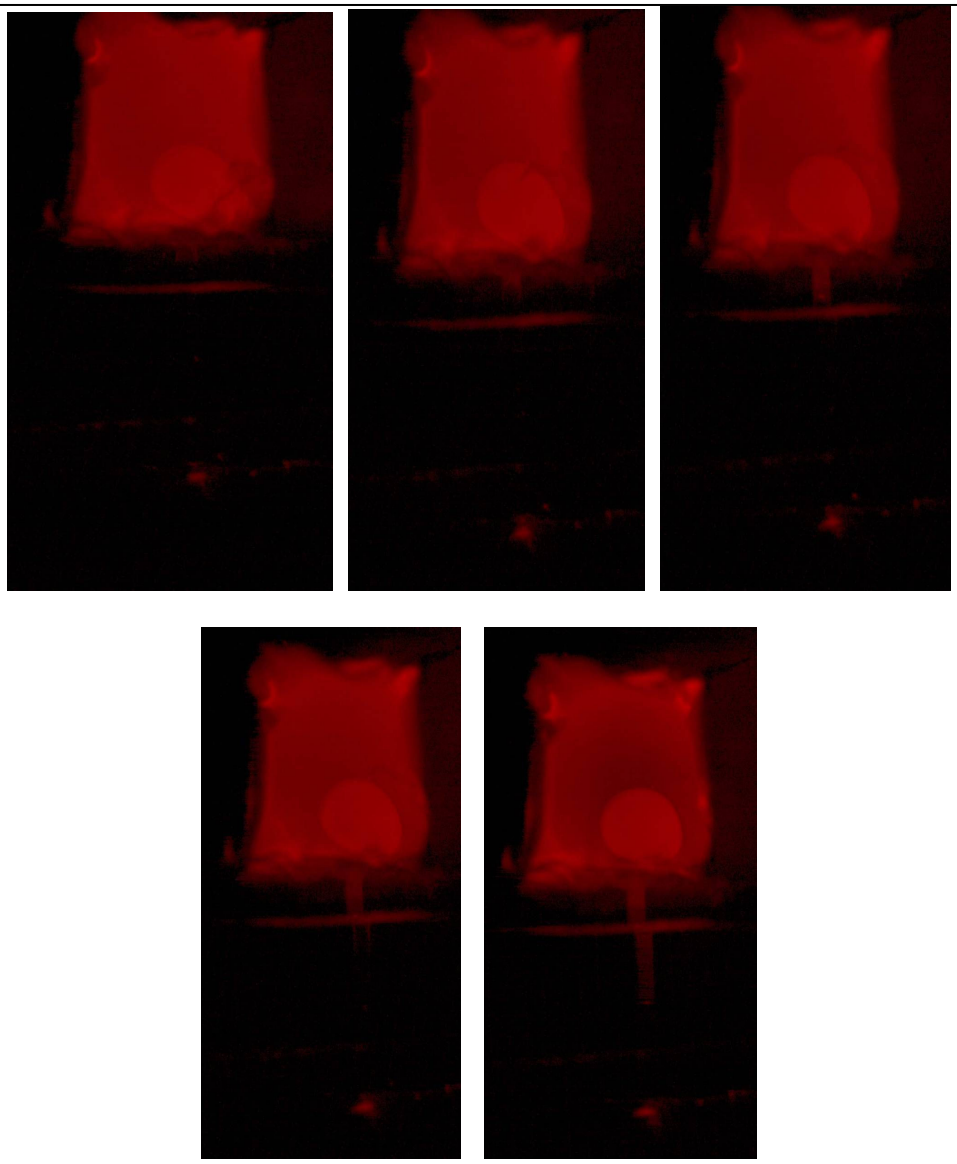

Figure 8: The time-lapse photographs show the gradient formation. A) At 15min B) At 30min C) At 45min D) At 60min E) At 165min

As can be seen over time a gradient formation has began. The final time for the gradient to form was 4.2 hours. Note, the red bar of light which can be seen over the channel is due to the refraction of light caused by the additional layer of PDMS. In our initial testing the second layer was constructed in two separate pieces, one area for the

source and one for the sink. Once this imaging issue was discovered, our setup changed to create the source and sink out of a single slab of PDMS. A completely formed gradient can be seen below. (see **Figure 9**).

Figure 9: Fully formed concentration gradient

Image Processing with MATLAB

In order to quantify the data, MATLAB image processing was used to determine if the gradient was linear. Since the intensity of the light emitted by the fluorescence is proportional to the concentration of Dextran, by analyzing the intensity of the image conclusions can be drawn about concentration profile of the channel.

The image in **Figure 9** was analyzed using the MATLAB algorithm. The algorithm uses the rgb2gray function, which converts a standard RGB image into a grey scale image and finds the intensity of each pixel. The signal was referenced with the sink intensity. The signal was then averaged over the length of the channel and the graph in **Figure 10a** was obtained. In order to obtain a more accurate representation, the resulting output was average over 10 pixels, which corresponds to 30μm in actual length. The results are displayed in **Figure 10b**.

As can be seen by Figure 10, a concentration gradient has been verified to exist. However, this is only one sample gradient so more need to be analyzed in order to verify the consistency of the results. Regardless, this is a major step in the overall scope of the project, as gradient formation in principle can be applied to a microfluidic channel.

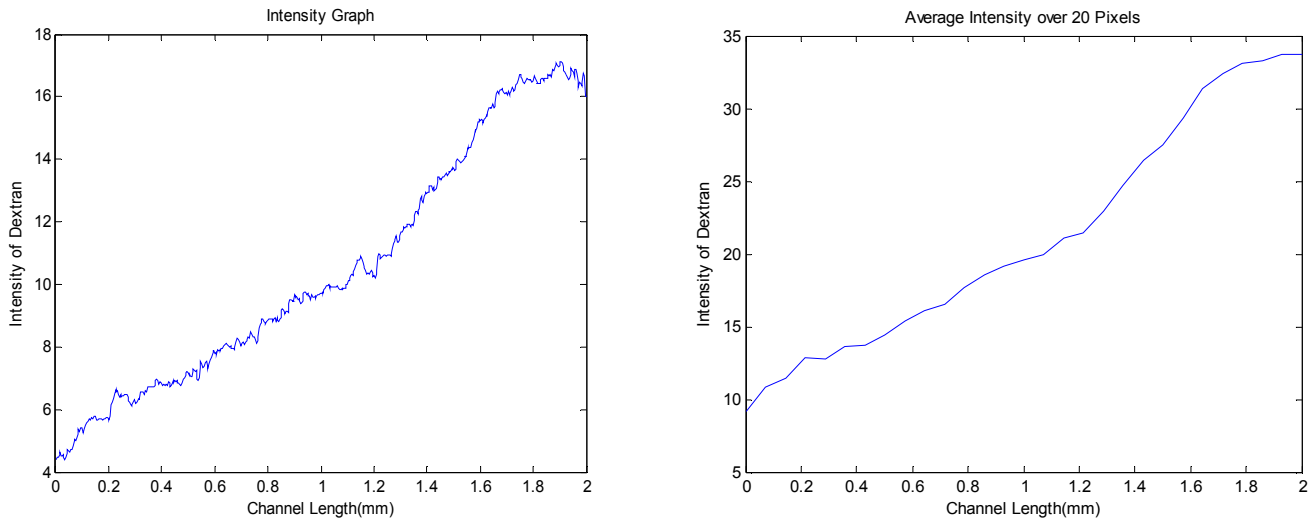

Figure 10: a) Intensity of Dextran in the channel, using the intensity of each pixel. B. b) Intensity of Dextran in the channel, averaging the intensity over 20 pixels.

Modeling our device

Once the results of the experiments had been obtained, two systems were used to model the behavior and determine the diffusion coefficients. The two different systems used were the MATLAB model and the COMSOL model.

MATLAB

The MATLAB model was created using the PDEPE function in MATLAB, which allows for the solving of a system of partial differential equations. The following equations were used to create the model.

$$\frac{\partial c}{\partial t} = D\Delta^2 c$$

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

$$c(x,0) = 0$$

$$\frac{\partial c(0,t)}{\partial t} = KA_m(C_0 - c)$$

$$\frac{\partial c(L,t)}{\partial t} = -DA_c(c - C_\infty)$$

$c = Concentration$

$D = Diffusion\ Coefficient$

$K = Partition\ Coefficient\ of\ Membrane$

$A_m = Surface\ Area\ of\ Membrane$

$C_o = Inital\ Concentration$

$C_\infty = Final\ Concentration$

$A_c = Cross\ Sectional\ Area\ of\ Channel$

Since the coefficients were unknown the values were modified to explain the experimental data and the solution to the differential equations is seen in Figure 4. As can be seen the experimental concentration of the gradient becomes stable at approximately 4 hours (14,400 seconds). By changing the values for the diffusion coefficient, a MATLAB model was created that followed the experimental data. As can be seen in the appendix, a diffusion coefficient of 78.67 x 10^{-6} meters^2/sec was found to best model the experimental results.

Since the diffusion coefficient was now found, the time of source/sink replenishing can be determined through the equation determined by Vinay *et. al.* [7]:

$$\lambda = (V_s L_t)/(D_{gel} A_c)$$

$V_s = Volume\ of\ Solution$

$L_t = Total\ Diffusion\ Length$

$D_{gel} = Diffusion\ \text{Coefficient}$

$A_c = Cross\ Sectional\ Area$

$$\lambda = (10 * 10^9\ \mu L^3 * 2100 \mu m)/(78.67 \frac{\mu m}{s} 30000 \mu m^2) = 8.9 Ms$$

$$= 103\ days$$

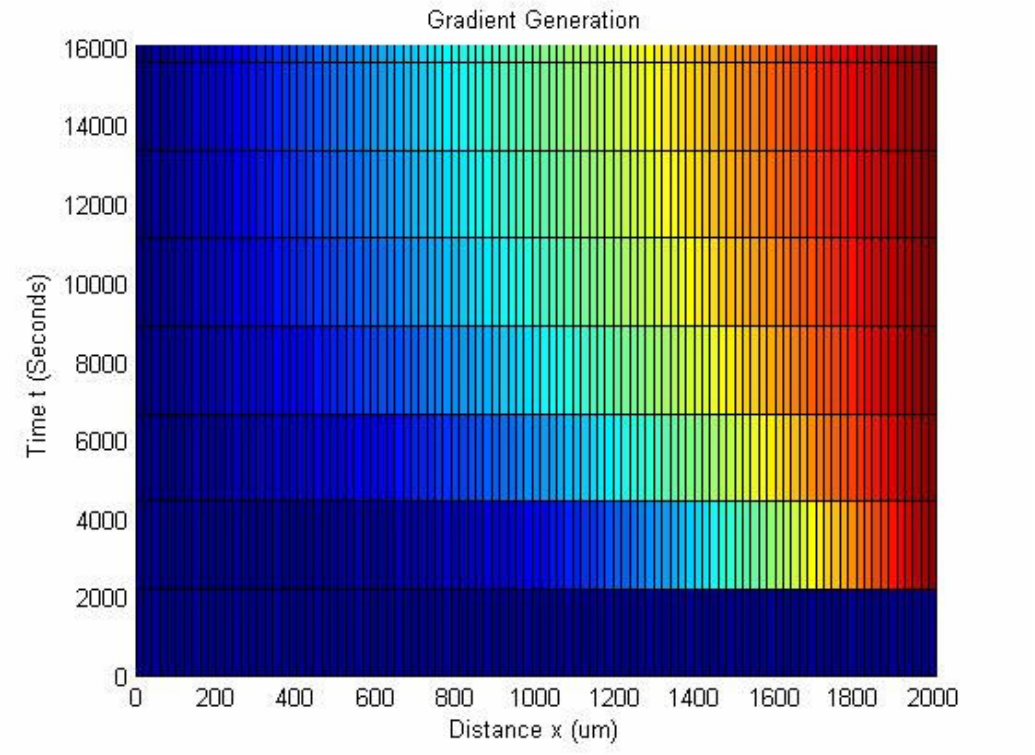

Figure 11: Solution of the partial differential equations which model the microfluidic channel formation.

COMSOL

COMSOL Multiphysics is a software offered by the COMSOL group. The software allows users to define device geometry, specify the forces and properties of the device, meshing, solving, and post-processing of the results [10]. We were referred to this software by Erwin Berthier, who has used this software to model microfluidic devices for his research project.

COMSOL has several different design modules that the user can choose from. For our case, as advised by Erwin, we used Convection and Diffusion mode. This mode allows the user to draw a design with its true dimensions, input domain and boundary conditions, and carry out diffusion analysis.

Our device was drawn in COMSOL. The sink and the source were drawn as two ellipses. The size of the sink and the source were drawn to about 50 times the area of the channel. Sink and source sizes above 50 times the area of the channel did not change the calculations conducted by COMSOL. The channel dimensions were 2 mm in length and 0.3 mm in height. The concentration of the source was set at 1 Molar and the concentration of the sink was set at 0 Molar. The diffusivity coefficient of our compound was set at 8.0×10^{-6} meters^2/sec, which is the diffusion coefficient found by our MATLAB program.

After these values were specified, a mesh was initialized. The creation of this mesh is necessary as the COMSOL program uses this mesh to carry out its calculations. After the mesh was created, the time parameters were specified. COMSOL was asked to determine the concentration gradient formation from time, t = 0 seconds to t = 20,000 seconds (~5.6 hours). The system was solved by COMSOL and postprocessing was carried out to generate an animation of the gradient formation.

Figure 12 shows the device that was drawn in COMSOL and concentration gradients at three different time points. The gradient took about 4 hours to setup in our model. This matches our MATLAB modeling of the channel as well as what we observed during testing. Therefore, COMSOL is yet another tool that we will use extensively to model our devices for future tests.

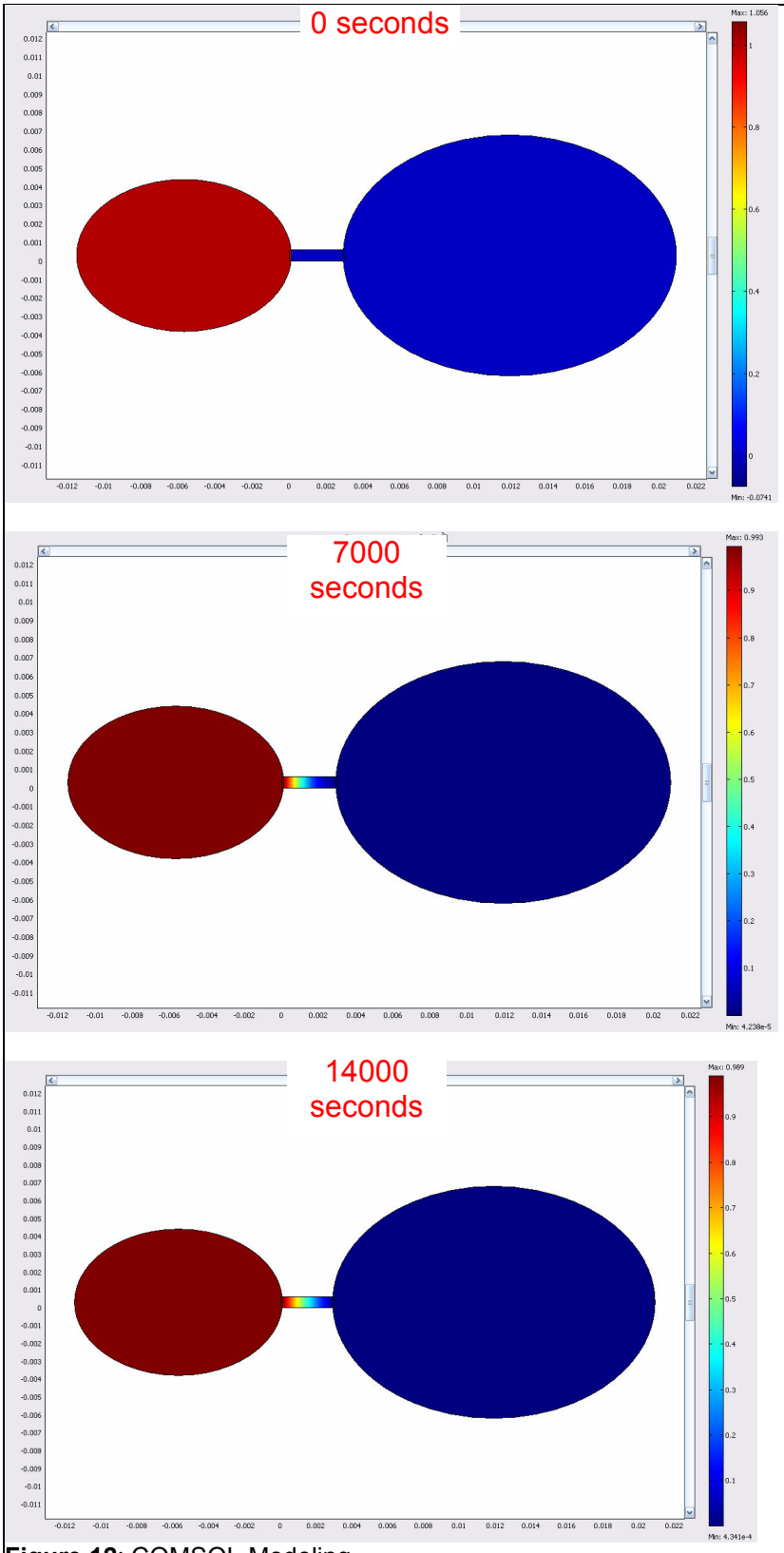

Figure 12: COMSOL Modeling
COMSOL Multiphysics software was used to model our microfluidic device. A time-lapse analysis of our device showed that it takes about 4 hours for our device to set up a concentration gradient over the length of the channel. The above three images show the gradient formation at a) 0 seconds, b) 7000 seconds, and c) 14000 seconds. The red circle signifies the source with a concentration of 1 M and the blue circle signifies the sink with a concentration of 0 M. The other colors represent intermediate concentrations as seen in the scale to the right of the channel.

Project Expenses

The following is a list of the materials used for our project and their respective costs.

- Mask printing – donated by Erwin Berthier (estimated cost <$3/mask)
- 3 inch silicon wafer – donated by Erwin Berthier (estimated cost $10/wafer)
- SU-8 – donated by Justin Williams (estimated cost <$1)
- PDMS – donated by Justin Williams (estimated cost <$1 per master)
- Matrigel – supplied by client
- Dextran labeled with Texas Red – donated by Erwin Berthier

Ethical Considerations

Ever since the derivation of a human embryonic stem (ESC) cell line by Jamie Thompson and coworkers in 1998, there has been widespread controversy over the ethics of the technique. Until recently, starting a stem cell line required the destruction of a human embryo and the ethical debate has revolved around this issue. Proponents of using ESCs for research have two main arguments. One of the arguments suggests that the utility of embryonic stem cells - for regenerative medicine, the capability to alter approaches to understanding and treating diseases and to alleviate suffering – outweighs the cost of the research in terms of the embryonic life. A second argument reasons the value of an embryo should not be placed on par with the value of a child or adult because the embryo has no capability of existing outside the womb and is simply a cluster of undifferentiated cells no more 'human' than skin cells. Opponents to ESC research raise objection to the destruction of something that is inherently human in nature. They reason that an embryo should be considered a human life and therefore should not be destroyed to further research. A second argument is that alternative therapeutic options should be explored because of potential to get comparable, or even better, results. Adult stem cells obtained from sources like umbilical cord blood and

bone marrow can differentiate into a variety of different tissue types quicker and with less error.

A second ethical dilemma surrounding our project is the cost to benefit ratio of the research. Diabetes affects 20.8 million people in the US, about 7% of the population and quicker and is associated with an increased risk for a number of serious, sometimes life-threatening complications [8]. Luckily, diabetes can be effectively monitored and controlled with current therapeutic strategies. With effective, existing strategies for diabetes already in place, the question must be asked if more research into this subject is warranted. In order to pursue further research using ESCs, huge sums of money must be granted to the researchers who hope to improve the quality of life of those suffering from diabetes. It can be argued that the money granted to these researchers could better be used for solutions to other, more serious, health problems like malaria which kills 1 million people and affects 350,000-500,000 more each year [9]. Also, the potential treatment that is being researched will likely be expensive. A costly treatment for a non life threatening disease would probably benefit only those people wealthy enough to afford the treatment. Perhaps the best solution to this cost to benefit dilemma is that an appropriate amount of money should be granted by the government after careful consideration of the previously mentioned issues.

COMSOL was used without a license. We have discussed this with our advisor and we realize the ethical dilemma of this notion. If any testing results related to COMSOL are to be published, a license will be obtained.

Future Work

While we have established a linear gradient in our microfluidic channel, there is much future work necessary in the upcoming semester. Our most immediate goal will be to perform gradient tests of longer duration using the Dextran. This will allow us to calculate diffusion coefficients, determine the time to steady state, and test how long the gradient can be maintained for. When we have calculated these values in relation to the dimensions of our test channel, we will have to decide on final dimensions for the channel, source, and sink to obtain optimal results. Next we will have to establish a maintainable gradient using fluorescently labeled growth factors. While the Dextran gives us a theoretical idea of how the growth factors will diffuse and set up, it will still be necessary to ensure that the growth factors themselves actually do behave as predicted.

Perhaps the most important element of work yet to be done will be testing for cell viability in the channel. Although it would be beneficial to use actual stem cells to test this viability, this may not be realistic due to the limited availability of SCs. We will most likely use a more readily available cell that is known to have similar biological requirements. An important aspect will be sufficient nutrient delivery. This may be an issue in our one channel source-sink set up as the nutrients would need to diffuse through the Matrigel, and delivery time may become an issue. Future testing and literature research as to how other laboratories have delivered nutrients in no-flow devices will help us to remedy this potential obstacle.

A final element that will have to be considered will be the devices compatibility with analysis techniques (e.g. immunostaining). Upon completion of the study, cells will

have to be exposed to a stain necessary for imaging. Again the time necessary for this stain to diffuse through the Matrigel and affect all cells may be an issue and additional channels may be required to minimize this diffusion time. When all cells have been exposed, the Matrigel will need to be removed from the channel and sliced into individual elements of thickness compatible with standard imaging techniques. While this is a problem that is currently in the distant future, we will need to be sure to take this into consideration when altering our current system set up.

Conclusion

While stem cells have shown the potential to remedy a variety of diseases and conditions, there is limited knowledge of what conditions will cause differentiation into one cell type as opposed to another. Growth factor concentration is believed to play a large role in a cells fate, and exposing these SCs to a variety of concentrations would be ideal. A microfluidic device that is capable of establishing and maintaining a continuous, linear gradient of these growth factors would be a vital tool to stem cell researchers. This semester we were able to establish such a continuous, linear gradient within our no-flow microfluidic device. Using similar testing methods in the future, we will be able to determine diffusion coefficients that will allow us to define appropriate dimensions for our final device. We will then be able to test for cell viability within this device and eventually see the device employed in actual stem cell differentiation testing.

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Appendix A Project Design Specification

Stem Cell Differentiation Monitor

Updated: December 11, 2007

Team Members:

- Jonathan Baran: BWIG
- Dhaval Desai: Communicator
- Kyle Herzog: Team leader
- Tim Pearce: BSAC

Problem Statement:

Embryonic stem cells (ESCs) have the capacity to differentiate into every cell type in the body, and therefore can theoretically be used to generate cells and tissues to cure a variety of diseases. Our client in the Odorico Lab (Department of Surgery) has derived foregut-committed cell lines from ESCs (which correspond to progenitor cells of the gut region that develops primarily into pancreas) and would like to differentiate these ESCs into insulin-producing pancreatic beta-like cells. These cells could replace or supplement transplanted donor beta cells. The mechanisms required to differentiate ESCs into these pancreatic cells is currently unknown, and this device would aid in researching such mechanisms. Our client would like to test a large number of growth factors for their ability to affect conversion of these precursor cells to mature insulin-secreting cells. In addition, a recapitulation of the 3-dimentional embryonic environment to prompt cells to adopt a pancreatic cell fate, perhaps using a Matrigel substrate, is desirable. A small scale cell culture using microfluidics to set up growth factor gradients is one approach that could be successful.

Client Requirements:

- A high-throughput way to culture Endodermal SCs (foregut-committed cells) with growth factor gradients.
- Need to be able to perform antibody staining on the cells following culture to determine whether they differentiated appropriately.
- Create a three dimensional embryonic growth environment.

Design Requirements:

1. Physical and Operational Characteristics

a) *Performance Requirement:* Must be more efficient than current methods for testing the effects of growth factors. Each unit should be capable of holding at least 100 cells (1000 -5000 would be better). Must be compatible for imaging (i.e. thin enough that it can fit in microscope fixture, glass thin enough to be viewed through). Must be able to withstand immunofluorescence. Capable of setting up tests for a variety of growth factors and gradients of those GFs.

b) *Safety:* No potentially harmful materials.

c) *Accuracy and Reliability:* The gradient formed should range from 10ng/mL to 150ng/mL of a given growth factor. Due to the lengths of the experiments, sink and source replenishing will need to take place. Minimal disturbing of the

gradient is a must to obtain accurate results. Also to ensure accuracy of the system cell nutrients and waste need to be taken into account in the design. Cells need to be fed once a day and cell waste (e.g. lactic acid) needs to be expelled from the system. Also the entire system must be sterile.

d) *Life in Service:* For the duration of the study, which is currently unknown (likely 7-28 days).
e) *Operating Environment:* Should be able to withstand 37^0 Celsius environment, tissue culture conditions, and imaging.
f) *Ergonomics*: Should be relatively easy to use and clean (but probably it will be disposable, so cleaning it is not essential).
g) *Size and Shape:* Must be small enough to fit in the imaging devices as well as the incubator.
h) *Weight:* Not a big concern, due to the small size, however should be under 1 lb
i) *Materials:* Must allow cell adhesion. Must be sterile. Imaging of cells while they are growing using an inverted phase microscope is also important.
j) *Aesthetics, Appearance, and Finish:* Not important, except for imaging purposes, as stated above.

2. Product Characteristics:

a) *Quantity:* One device is required at this time, but more would be desirable in the future.
b) *Budget:* $500.

3. Miscellaneous:

a) *Standards and Specifications:* No specific standards will be required for project.
b) *Customer:* Since cell signaling is vital to the development of cells and shear force is an unwanted byproduct, a no flow system of establishing a gradient would be ideal. Also multiple growth factor gradients may be wanted by the customer
c) *Patient-related concerns:* Cells must live in a sterile environment and get adequate nutrients. Also cell waste must be expelled from the system
d) *Competition*: None

Appendix B MATLAB code

MATLAB Gradient Generation Model Code

```
function [c,b,s] = eqn1(x,t,u,DuDx)
%EQN1: MATLAB function M-file that specifes
%a PDE in time and one space dimension.

% C=1/D
% C=1/78.67
c = 1.27*10^-2;
b = DuDx;
s = 0;

function value = initial1(x)
%INITIAL1: MATLAB function M-file that specifies the initial condition
%for a PDE in time and one space dimension.
value = 10;

function [pl,ql,pr,qr] = bc1(xl,ul,xr,ur,t)
%BC1: MATLAB function M-file that specifies boundary conditions
%for a PDE in time and one space dimension.

%pl=-KAm(Co-ul)
%-KAm=-4.57*502.65E3=-600E-6
pl = ((2.3*10^6)*-10)+((2.3*10^6)*ul);
ql = 1;

%pr=DAc(ur-Cend)
%DAc=78.67*30,000=2.36E-6
pr = ((2.36*10^6)*ur)-((2.36*10^6)*150);
qr = 1;

%PDE1: MATLAB script M-file that solves and plots
%solutions to the PDE stored in eqn1.m
m = 0;
%NOTE: m=0 specifes no symmetry in the problem. Taking
%m=1 specifes cylindrical symmetry, while m=2 specifies
%spherical symmetry.
%
%Define the solution mesh
% Define the length of the channel
x = linspace(0,150,100);
t = linspace(0,5000,10);
%Solve the PDE
u = pdepe(m,@eqn1,@initial1,@bc1,x,t);
%Plot solution
surf(x,t,u);
title('Surface plot of solution.');
xlabel('Distance x');
ylabel('Time t');
```

MATLAB code for Image Processing

```
x=[1:(573)];
x=x./(573*0.5);
NewAverage=zeros(1,28);
% Normalize over the 2mm channel
x1=[0:20:560];
x1=x1./(560*0.5);

% Load image, convert to greyscale and find intensity
intensity=rgb2gray(imread('Gradient1.jpg'));
% Subtract off intensity of sink
intensity=intensity-7.0886;

% Average over length of channel
average=(sum(intensity));
average=average./147;

%Average over width of the channel
Dummy=0;
count=0;
i=1;
for j=1:560
    count=count+1;
    Dummy=Dummy+average(j);
    if (count==20)
        NewAverage(i)=Dummy;
        i=i+1;
        if (j==560)
            NewAverage(i)=Dummy;
        end

        count=0;
        Dummy=0;
    end
end

NewAverage1=NewAverage./10;


figure (1)
plot(x,average);
title('Intensity Graph')
xlabel('Channel Length(mm)')
ylabel('Intensity of Gradient')

figure (2)
plot(x1,NewAverage1);
title('Average Intensity over 20 Pixels')
xlabel('Channel Length(mm)')
ylabel('Intensity of Gradient')
```