Device to monitor and control the differentiation of stem cells into pancreatic beta-islet cells

COLLEGE OF ENGINEERING
UNIVERSITY OF WISCONSIN-MADISON

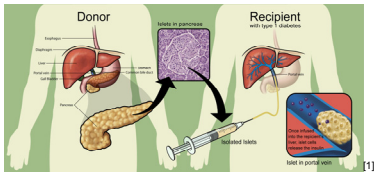
Jonathan Baran[1], Dhaval Desai[1], Kyle Herzog[1], and Timothy Pearce[1].
Advisor: Dr. Naomi Chesler PhD[1], Client: Dr. Victoria Browning PhD[2]
1 Department of Biomedical Engineering, 2 Department of Surgery

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 progenitor cells into insulin-producing pancreatic beta-like cells. These cells could replace or supplement transplanted donor beta cells. Our client would like to test the effect of a variety of growth factors on the differentiation of these stem cells. A continuous gradient of known concentrations would be ideal to determine the concentrations that can induce the desired differentiation.

Background & Motivation

Current Method to treat Type 1 Diabetes:
Human Islet Transplantation



[1]

Figure 1. Human Islet Transplantation
β-islet cells from a donor are transplanted to a recipient with Type I Diabetes.

Problems with Human Islet Transplantation:
- Shortage of Donors
- Complications during transplant (e.g. immune reaction)

Possible Solution:
Stem Cells
- Can be differentiated into various cells given the right conditions
- Client has derived foregut-committed cell line

No Flow System

- Linear gradient forms via diffusion between source and sink
- Variety of gradient profiles can be created using different channel geometries
- Allows for autocrine/paracrine signaling
- Low reagent volumes required

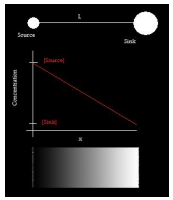


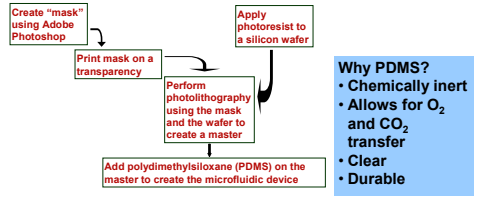
Figure 2. No Flow System
A linear concentration gradient can be generated using a no flow system.

Design Criteria

- Capable of holding ≥100 cells (1000-5000 would be ideal)
- Capable of withstanding immunofluorescense
- Maintain gradient for 7 to 28 days
- Able to withstand 7-28 day incubation period at 37^0 Celsius
- Minimal amount of growth factor required
- Cells preferably embedded in Matrigel construct
- Total cost ≤ $500

Preliminary Design and Testing

Creating Microfluidic devices



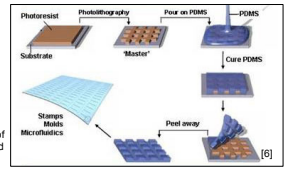


Figure 3.
This figure outlines the basic procedure of soft lithography. This procedure was used to create our microfluidic device.

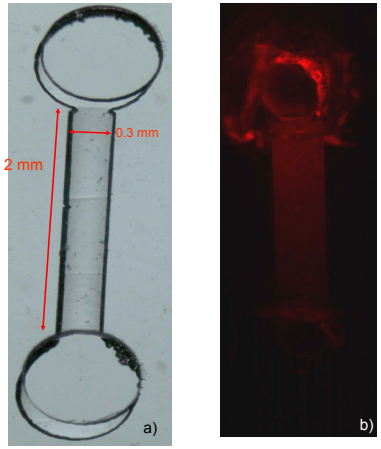
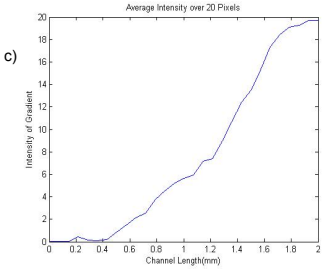


Figure 4.
a. Microfluidic Device
This figure shows the bottom layer of the device. The channel seen in the figure is filled with Matrigel. The two ports at the top and bottom of the channel serve as access ports. An additional layer of PDMS is placed on the top of this layer to create a source and a sink.
b. Device Testing using Dextran
This figure shows a complete gradient formation between the source and the sink. Fluorescently-labeled Dextran was used to emulate the growth factors to be used by our client. The gradient shown here generated in approximately 4 hours.
c. Concentration Gradient Image Quantification
Using MATLAB, the intensity of the fluorescence over the length of the channel was measured. The intensity is proportional to the concentration of the molecule. The intensity of the sink was used as the zero reference for the data.



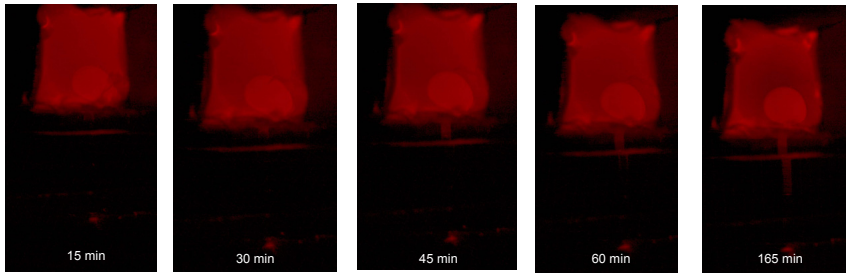


Figure 5. Time lapse gradient generation
To ensure the diffusion of Dextran through our Matrigel-filled device, a time-lapse experiment was setup. The source of our device was filled with a known concentration of fluorescently-labeled Dextran. The movement of Dextran through the channel over time was verified by taking images at various time-points as seen in the figure. Concentration gradient formation is clearly seen over time.

Modeling our Device

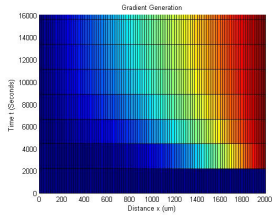
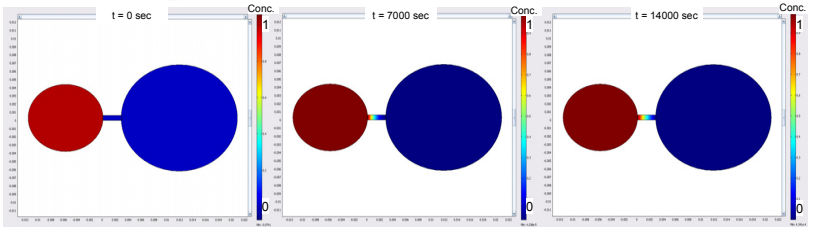


Figure 6.
a. Modeling the gradient formation of the microfluidic device using MATLAB (left)
The progression of the gradient formation over time can be modeled with MATLAB. The program takes into account variables such as the growth factor diffusion coefficient and channel dimensions. The gradient stabilizes after approximately 4 hours (~14,400 seconds)
b. Modeling the gradient formation of the microfluidic device using COMSOL (below)
COMSOL offers another theoretical method to model the gradient formation. The device was drawn in the software. The diffusion coefficient and channel dimensions were specified. The gradient stabilizes in about 4 hours. (Source concentration=1 M, Sink concentration=0 M)



Future Work

- Validate sustainability and stability of gradient with Dextran
- Calculate diffusion coefficients for growth factors
- Validate sustainability and stability of gradient with labeled growth factors
- Check for cell viability
- Ensure compatibility of the device with analysis techniques

References

[1] Islet cell transplantation. Wikipedia, The Free Encyclopedia. 16 Sep 2007 http://en.wikipedia.org/w/index.php?title=Islet_cell_transplantation&oldid=158190546. 16 Oct 2007
[2] Stephanie Watson. How Stem Cells Work. 11 Nov 2004 http://science.howstuffworks.comstem-cell2.htm. 2 Dec 2007.
[3] Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL. 2005. Human nueral stem cell growth and differentiation in a gradient generating microfluidic device. Lab Chip. 5(4): 401-406.
[4] Whitesides GM. 2006. The origins and the future of microfluidics. Nature. 442: 368-373.
[5] Generating Microgradients. Harvard University. 19 Oct 2006 http://www.mrsec.harvard.edu/research/nugget_12.php. 2 Dec 2007.
[6] Soft Lithography. Nanoterra. 20 Dec 2005 http://www.nanoterra.com/soft_lithography.asp. 2 Dec 2007

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