Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays

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ABSTRACT

Neurodegenerative diseases, such as Parkinson's Disease, result for the loss of neuronal structure or function. Our client, Dr. Randolph Ashton, is seeking to eventually use differentiation of human pluripotent stem cells to treat these diseases. However, before this can be done he must first have a means of determining what soluble factors promote stem cell differentiation into neurons. He has given us the challenge of finding a way to integrate cellular microarrays with microfluidic platforms so that various concentrations of soluble factors can be tested in a high-throughput fashion. We first focused on generating concentration gradients with microfluidic devices. Of the current options available, the Christmas Tree, Source/Sink, Universal, and Microjet gradient generators appeared to be the most relevant to this project. After quantitatively comparing these alternatives in a design matrix, it was decided that the Christmas Tree gradient generator was the best suited to meet all of the requirements of our client. As a result, it is being pursued as our final design. Illustrator drawings have been made of this design, and after COMSOL simulation analyses are performed to confirm adequacy we will begin to develop a prototype.

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INTRODUCTION

Client Description

Our client, Dr. Randolph Ashton, is an Assistant Professor in the Biomedical Engineering Department at the University of Wisconsin – Madison. His research focuses on regenerative stem cell medicines and therapies and how to transition these from the laboratory to clinical applications.

Background and Motivation

Microfluidic devices utilize small channels containing small amounts of solvent, sample and reagents. This makes them valuable for micro-scale high-throughput assays [1]. Typically, these devices are fabricated from poly(dimethylsiloxane) (PDMS) for several reasons including its flexibility, inexpensiveness, and optical transparency [1]. Most important, however, is its ease of fabrication and ability to form micron-scale details. The first step in fabricating a PDMS microfluidic device is the generation of a transparency using a photoplotter. This transparency is then used as a photomask during ultra violet (UV) photolithography to create a master. In this procedure, a photocurable epoxy (typically SU – 8) is spin-coated onto a silicon wafer and subsequently exposed to UV light under the photomask. Any epoxy that is exposed to the UV light will crosslink, while the epoxy that is not cured is easily solubilized using a developer and simply washes away. This procedure creates a mold from which PDMS microfluidic devices can be made [1].

One promising field of microfluidics is the generation of complex gradients. When cells are exposed to these gradients, their induced responses can be monitored to determine how they are influenced [2]. This approach has the potential to enhance the understanding of which combinations of soluble factors induce specific cellular responses and lead to cancer or stem cell differentiation.

Human pluripotent stem cells (hPSCs) are capable of differentiating into any cell type within the human body. This characteristic makes them advantageous for regenerative therapies; however, many of the extracellular cues responsible for hPSC differentiation into the various cell lineages are still not completely understood. Our client aspires to use high-throughput methods, particularly cellular microarrays, in conjunction with a microfluidic device to examine induction of stem cell differentiation with varying concentrations of soluble factors.

Dr. Ashton has previously established the production and efficacy of these microarrays, which consist of hundreds to thousands of separate cellular colonies plated on a microscope coverslide [3]. The method for fabricating these microarrays can be seen in Figure 1; an example of previously fabricated microarray is shown in Figure 2. This microarray approach is beneficial because many conditions can be tested simultaneously when incorporated with a gradient-generating microfluidic device. The cells in the microarray can be exposed to varying concentrations of soluble factors and changes in stem cell response (i.e. protein expression, gene expression) can be examined using immunofluorescence and polymerase chain reaction (PCR). This approach also allows for the selection of a specific colony,

which can then be expanded using standard cell culture techniques [3].

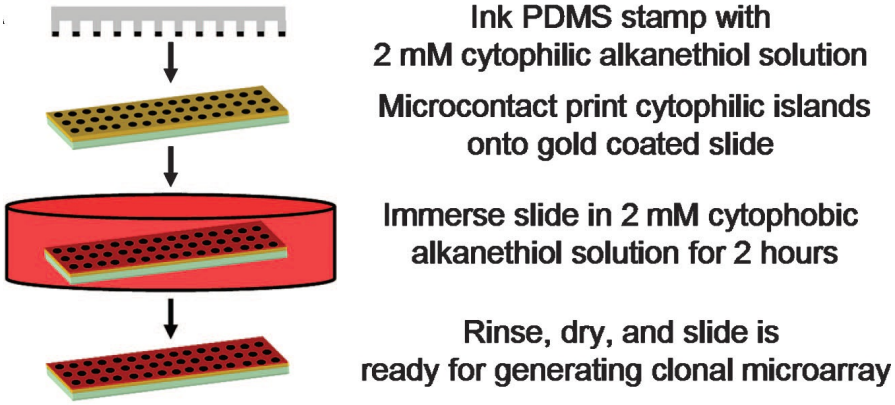

Figure 1: Fabrication of Cellular Microarrays. Individual cell colonies, or pixels, are present in the cytophilic regions, while the cytophobic regions maintain colony isolation. Depending on the colony area, hundreds to thousands of individual pixels can be created on a single microscope coverslide [3].

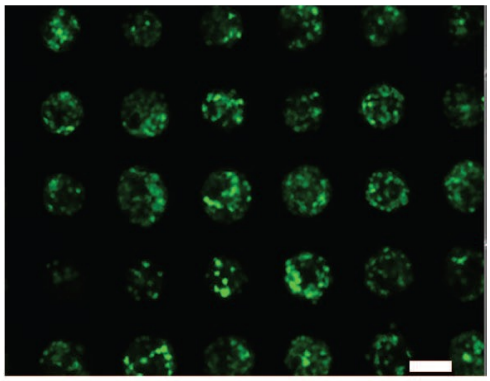

Figure 2: Cellular Microarray Pixels. Adult rat neural progenitor cell colonies expressing green fluorescent protein on a microarray. Scale bar = 100 μm [3].

Dr. Ashton is specifically interested in studying the differentiation of hPSCs into midbrain dopaminergic neurons as an eventual treatment for Parkinson's Disease (PD). The National Institute of Health estimates approximately half a million Americans currently suffer from PD with another 50,000 new incidences occurring annually [4]. There is currently no cure for PD, but regenerative therapies using differentiated midbrain dopaminergic neurons from hPSCs have been successful in mice as well as initial human clinical studies [5]. Dr. Ashton's goal is to use high-throughput methods to better understand midbrain dopaminergic neuron

differentiation from hPSCs and enable the large-scale production of midbrain dopaminergic neurons to become a treatment for PD.

Design Requirements

The requirements for this project are outlined in the Product Design Specifications in Appendix A, and a few key constraints are highlighted here. In order for the microfluidic platform to fit precisely onto the microscope stage, it must be 158 mm in length and 105 mm in width. The platform must also contain an opening that is 61 mm in width and 25 mm in length so that the cellular microarray can be integrated into it. Since the microscope uses a piezoelectric positioning system, the final device, which includes the microfluidic platform, microarray, and cell media must weigh less than 0.5 kg.

Ergonomically, the platform must be easily incorporated and removed from the microscope stage and the microarray must be easily integrated with and detached from the platform. Our client also wants the microfluidic platform to maximize discrete concentration conditions as well as the number of cellular colonies on the microarray. These specifications will allow for the determination of the soluble factor combinations and concentrations necessary to induce specific differentiation of hPSCs. Lastly, the designed microfluidic platform must be capable of maintaining a reliable gradient throughout the 1- 10-day time period that experiments are run for.

Current Devices

Many research efforts addressing stem cell differentiation and expression incorporate the effects of soluble factor concentration gradients on cell response. Within these efforts, multiple devices have been developed to generate concentration gradients on a cellular scale for high throughput analysis. For example, Beebe *et al.* developed a device that uses a diffusional transport of particles from source to sink to establish a concentration gradient [6]. This method, along with others such as the Christmas Tree, Microjets, and Universal will be discussed further as design alternatives for gradient generation in the final design [7-9].

Despite the wide use of gradient analysis, to our current knowledge no device exists that combines gradient generation with the specific requirements outlined by our client. The notable requirements that are novel in combination are the development of a flowing concentration gradient of multiple soluble factors across a removable microarray for long-term cell culture.

DESIGN ALTERNATIVES

Christmas Tree

The Christmas Tree was first developed by Jeon *et al.* [9]. The number of inputs into the device can be varied for convenient alteration in gradient generation. The device is composed of a channel of networks consisting of horizontal channels, serpentine (or vertical) channels, and the broad channel, as shown in Figure 3A. Fluid with varying concentrations can be pumped through the separate inlets, as

shown in Figure 3B [2, 7]. The resistivity of the horizontal channels can be neglected because the resistivity of the serpentine channels dominates due to their greater length and narrower width. This is because resistance is proportional to length divided by width. All serpentine channels have the same dimensions, thus the same resistance and fluid flux; this allows for a well-defined flow throughout the network. The device generates a gradient when two fluid streams of differing concentrations enter the serpentine channels. The streams run parallel to each other and mix by diffusion creating one homogenous stream with a new concentration. The length of the serpentine channels must be designed to ensure adequate diffusive mixing of the two streams occurs. Mixing is dependent of flow rate, the diffusivity coefficient, and residence time within the serpentine channel [2, 7].

Using this design for gradient generation, the cellular microarray would be located within the broad channel. Dertinger *et al.* reported that the width of the broad channel and sagging of the PDMS did not interfere with fluid flow [2]. However, Dertinger did not conduct long-term analysis of the device's function and reliability. The use of this design would allow for the development of a well-defined gradient with simple control over fluid flow rate to vary the shear stress placed upon the cells. The flow rate can be varied to reduce the shear stress on cells within the broad channel. Spatial resolution of soluble factor concentrations range from 2-20 μm [7]. However, along with the well define spatial resolution, the width of the broad channel would limit the number of cellular pixels available for analysis.

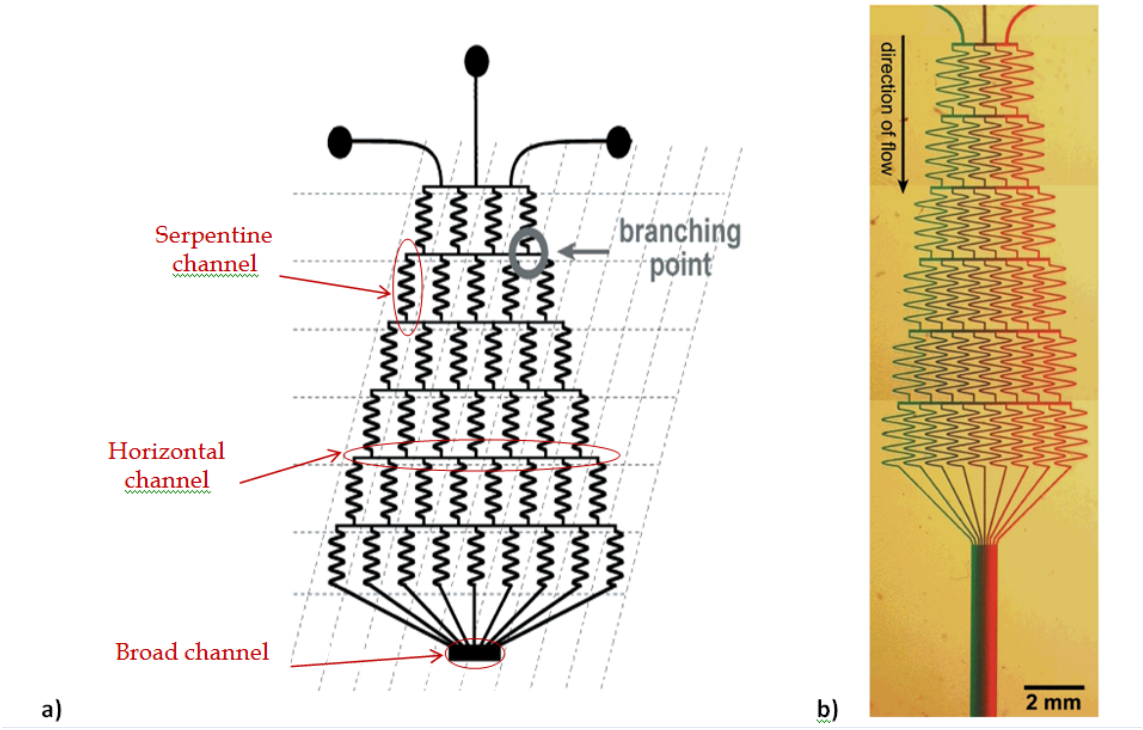

Figure 3: Christmas Tree Alternative. A: The Christmas Tree design contains serpentine channels, horizontal channels, and broad channels. At a branching point, the fluid leaves the serpentine channel and splits within the horizontal channel. **B:** The gradient is established using red and green dyes. The gradient is maintained in the broad channel by laminar flow. Mixing between laminar streams occurs by diffusion and lessens the accuracy of the gradient with time [2, 7].

Source/Sink Gradient Generator

The second design alternative for gradient generation is the Source/Sink Gradient Generator. This device is synthesized of PDMS using soft lithography and rapid prototyping. The Source/Sink Gradient Generator is assembled in three layers bonded together using plasma oxidation [10]. As shown in Figure 4, the top layer of the device contains a source, while the bottom layer contains a horizontal channel and relatively large sink region. The top and bottom layers are separated by a high resistance membrane constructed from polyester [10]. The high resistance of this membrane allows particle diffusion but resists fluid flow between the layers of

PDMS. This device therefore establishes a concentration gradient without fluid flow between the input and output.

To establish a concentration gradient, soluble factors injected into the source region diffuse across the porous membrane into the channel by mass action diffusive transport [10]. Once in the channel, particles continue to diffuse toward the low concentration sink region, creating a concentration gradient within the channel. The concentration is highest nearest the source region and decreases with distance along the channel. To maintain the gradient and prevent equilibration, desired concentrations are maintained in both the source and sink regions by flowing fluid streams. This is also aided by the large relative volume of the sink region, which prevents accumulation of particles in the channel [10]. The channel where the gradient is constructed, however, does not experience any fluid flow. Because of the nature of gradient generation in this method, a transient period occurs when the gradient is being established. This may be problematic in a cell culture system where media in the channel must be changed. However, this approach may be beneficial for cell-cell communication but it also limits the transport of nutrients and waste to and from the cell, respectively.

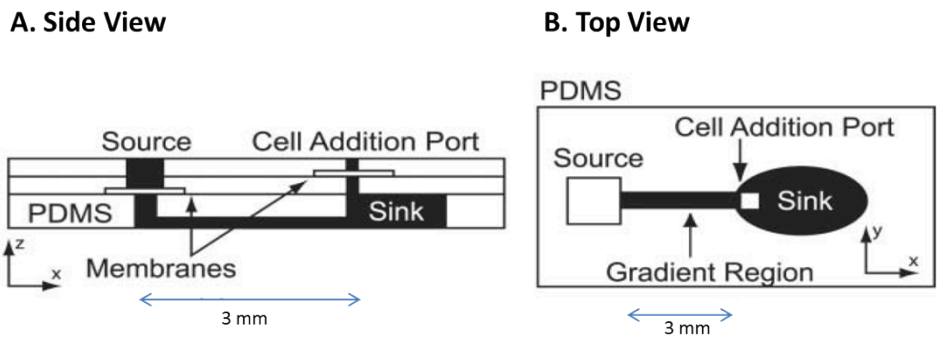

Figure 4: Source/Sink Gradient Generator. A: Side view of a multilayered source/sink orientation. The top layer (source) is separated from the bottom layer (sink) with a high resistance membrane. **B:** Top view layout of Source/Sink Gradient Generator. The gradient is generated in the channel connecting the source and the sink [10].

Universal Gradient Generator

The Universal Gradient Generator was designed using some of the same principles as the Christmas Tree alternative. As seen in Figure 5, two or more input fluid streams enter the mixing channel where numerous flow dividers function to control the splitting and diffusive mixing of these streams in order to regulate the lateral transport of soluble factors and create a gradient. The output is an arbitrary monotonic gradient with various discrete concentrations that are dependent on the concentrations of the input solutions. Mathematical calculations can be performed to determine the proper placement of the flow dividers, and altering their locations can achieve a variety of user-defined gradient profiles [9].

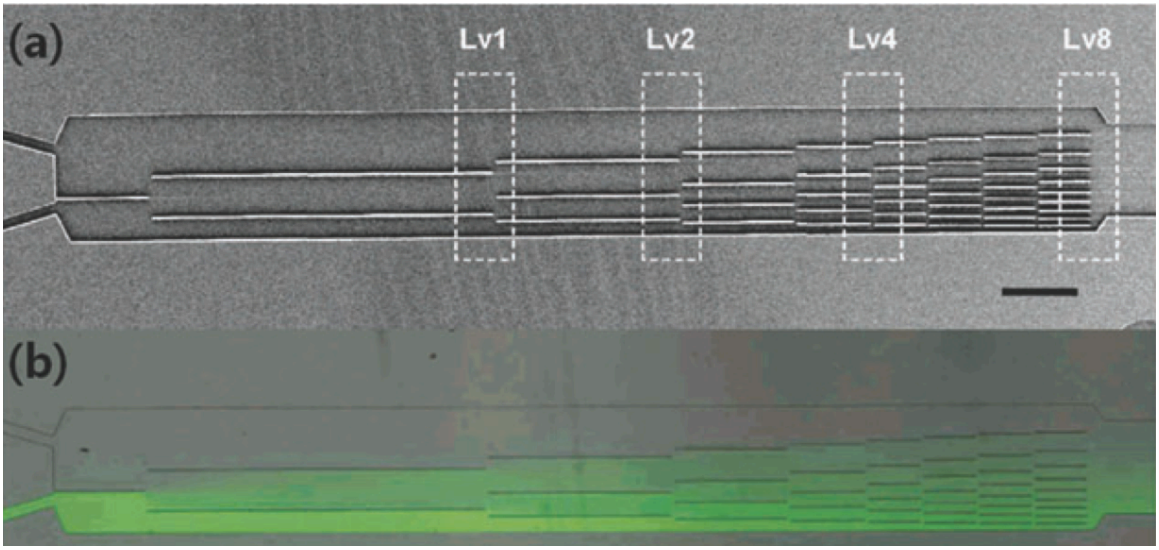

Figure 5: Universal Gradient Generator. A: This particular universal gradient generator has eight levels of flow dividers, which can be seen in this with scanning electron micrograph. **B:** Distribution of fluorescein isothiocyanate within the channel can be observed with fluorescence imaging. Scale bar is 500 μm [9].

One advantage of this design is that it does not have the same dead space that is associated with the serpentine channels of the Christmas Tree alternative. Adding more inlets and rearranging the flow dividers can allow for extensive manipulation of the gradient profiles that are generated. However, like the Christmas Tree, it requires significant volumes of input solutions in order to establish the concentration gradients and the cells within the microarray are still subjected to fluid flow [8]. A further challenge that this alternative would have in regards to our intended application is that scaling the output up to the size of the microarray would necessitate the mixing channel to be a significantly greater length. This could make both fabrication of the device and implementation with the microscope stage difficult.

Microjets Device

The final design alternative that was considered is the Microjets Device, shown in Figure 6. Microfluidic jets connect the source and sink compartments to the central cell culture reservoir and actively control the input of solutions into this area through pneumatic pressurization. The relative and absolute amounts of pressure that are applied through these microjets can be manipulated in order to alter the gradient profiles that are generated over the cultured cells. Within the cell culture area, no appreciable fluid flow occurs, so the gradient is established purely through diffusion [8]. Steady-state gradients can typically be formed with this alternative within 10 minutes. However, in order to maintain a reliable gradient, the source and sink outlets must be consistently supplied with fresh solutions so that the two constant concentration boundaries can be preserved. This design prevents the cultured cells from undergoing the forces associated with fluid flow and the open culture area enables gas exchange with the surrounding environment. Unfortunately, complex gradient profiles cannot be created with this method and the microjets are prone to clogging, which can decrease the accuracy of the gradients that are estabished [8].

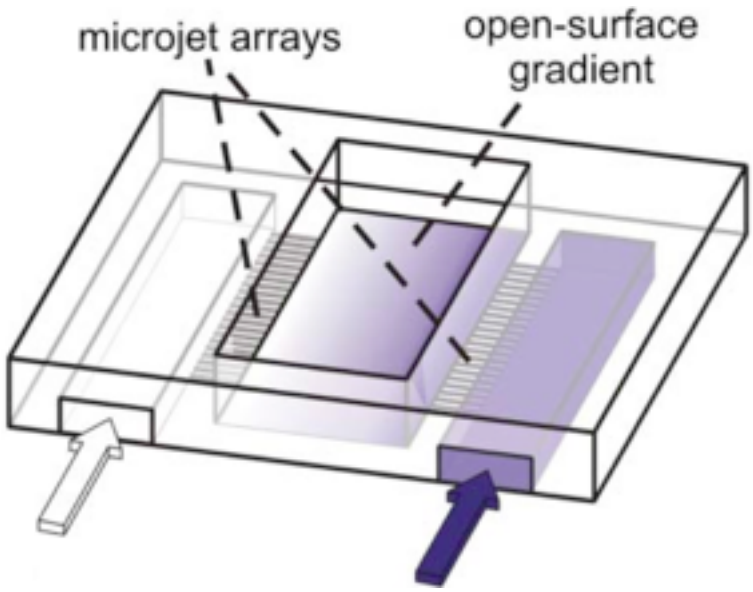

Figure 6: Microjets Device. Opposing arrays of microjects transport fluids from the source and sink chambers into the culture area, which is open to the surrounding environment. The microjects typically have cross-sections of 1.5 μm by 1.5 μm [8].

DESIGN MATRIX

We constructed a design matrix in order to compare the design alternatives and determine which one was the most appropriate for use as a gradient generator in our final design. This analysis, included in Table 1, provided a quantitative means of assessing how well each alternative suited the needs of the project. The five criteria that were used to evaluate the alternatives were Ease of Fabrication, Accuracy of Gradients, Estimated Throughput, Ergonomics, and Client Preference. Based on the results of this matrix, we chose to pursue the Christmas Tree alternative.

Table 1: Design Matrix. The weight of each evaluation category is indicated in the row headings, and the reasoning behind point allocations is described in the text. Based on the results of this design matrix, the Christmas Tree alternative will be used as the gradient generator for the final design.

Category (Weight)	**Christmas Tree**	**Source/Sink Gradient Generator**	**Universal Gradient Generator**	**Microjets Device**
Ease of Fabrication (25)	22	20	20	20
Accuracy of Gradients (25)	21	17	18	18
Estimated Throughput (20)	20	20	18	20
Ergonomics (20)	20	20	15	17
Client Preference (10)	10	0	0	0
Total (100)	**93**	**77**	**71**	**75**

Ease of Fabrication

Designing and fabricating microfluidic devices can be a relatively time-consuming process, which is why this category was allotted 25 of the total 100 points in the design matrix. Due to the high resolution required and numerous intricate steps that fabrication entails, the ideal final design would not significantly add to the complexity of this procedure. The Christmas Tree alternative was designated 22 points in this category because one member of our group has previous experience fabricating this type of device and we have numerous resources available on campus to assist with the process. The remaining alternatives only received 20 points each due to the scale of the project. Creating appropriately sized versions of these alternatives to have flow over the entire microarray while maintaining adequate aspect ratios and still fitting onto the stage of a microscope

would pose complications in creating the designs. The previously discussed design dimension requirements as well as the PDMS aspect ratio would impose difficulties during fabrications of these alternatives.

Accuracy of Gradients

The intended use of the device is to generate gradients of various soluble factors and allow them to interact with cultured cells to determine their effects on differentiation. As multiple factors may be used simultaneously, it is necessary that the gradients generated are accurate enough that the optimal levels of all the factors can be distinguished from other conditions. Accordingly, this category was weighted with 25 points. The Christmas Tree received the most points because the lengths of the channels can be easily manipulated to ensure that adequate mixing occurs within them to generate an accurate final gradient. The Source/Sink Gradient Generator and Microjets Device are unable to accurately establish complex gradient profiles and the Universal Gradient Generator can require exceedingly long channels in ensure proper mixing and gradient establishment. For these reasons, these alternatives received lower point allocations.

Estimated Throughput

One of the main goals of the project is to enable high-throughput analysis of cellular microarrays. It is desirable to be able to test multiple concentrations of growth factors simultaneously as well as maximize the number of cell pixels per condition. The Universal Gradient Generator is the only alternative that did not receive the full 20 points in this category. This is again due to the increased length of

the mixing channel that would be needed in order to establish a greater number of discrete concentrations within the gradient.

Ergonomics

The device should not be exceedingly difficult to operate, integrate with the microarray, separate from the microarray after use, or utilize with a standard microscope. Based on all of these considerations, the ergonomics category was designated 20 total points. The Microjets Device lost points in this category due to the challenges it poses with establishing the gradients. Similarly, the Universal Gradient Generator received less than the maximum amount of points due to the difficulty with incorporating it onto the microscope stage for imaging and analysis.

Client Preference

As our client has experience with microfluidics, we took into consideration which alternative he felt would be the best option for this project. The Christmas Tree alternative was the only one that he felt would be feasible and meet all of his design requirements, so we assigned the full 10 points possible to this design. The remaining options were not given any points in this category.

FINAL DESIGN

The final design prototype will be centered upon a Christmas Tree microfluidic gradient generator fabricated from PDMS. Synthesis of this device will be conducted with negative replica molding of a silicon master chip generated using soft photolithography. Unlike a conventional Christmas Tree microfluidics device,

the final design prototype will not contain output channels that converge into a single broad channel. Due to the aspect ratio for functional PDMS synthesis, each output channel will traverse the cellular microarray horizontally and house a single row of cellular pixels on the array. This is depicted in Figure 7, in which a magnified image of the channel (400 μm wide) comfortably contains cellular pixels with diameters of 200 μm. Separate horizontal channels prevent diffusive mixing between adjacent concentration streams. Furthermore, the segregated channel structure allows the researcher to analyze the contents of the cell media at each concentration. Each horizontal channel will be spaced 100 μm from adjacent channels to allow accurate synthesis. Based on this spacing, a total of 60 rows of cell pixels are feasible on the cellular microarray. The final design will therefore contain 60 horizontal channels, each with one row of cell pixels on the microarray.

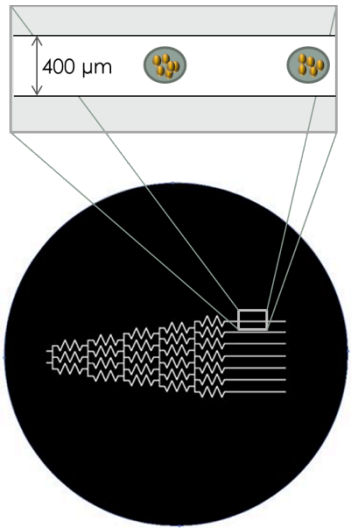

Figure 6: Christmas Tree Final Design. Christmas Tree network with horizontal segregated channel outputs, with each output channel containing a row of cell pixels from a cellular microarray. The pixel diameters are 200 μm, the channel diameters are 400 μm, and the spacing between the channels is 100 μm.

A single Christmas Tree with 60 channel outputs is not feasible due to the dimensional parameters of the microscope stage itself. The width required for a large extent of branching is greater than that allotted in the design specifications. To correct this, the final design will consist of six adjacent Christmas Tree networks, as depicted in Figure 8A. This design allows for simultaneous analysis of multiple growth factors using a single microarray of cells. Additionally, the researcher is not limited to a single concentration range. Each independent Christmas Tree network can be tailored to span a specific concentration range, enabling more refined analysis.

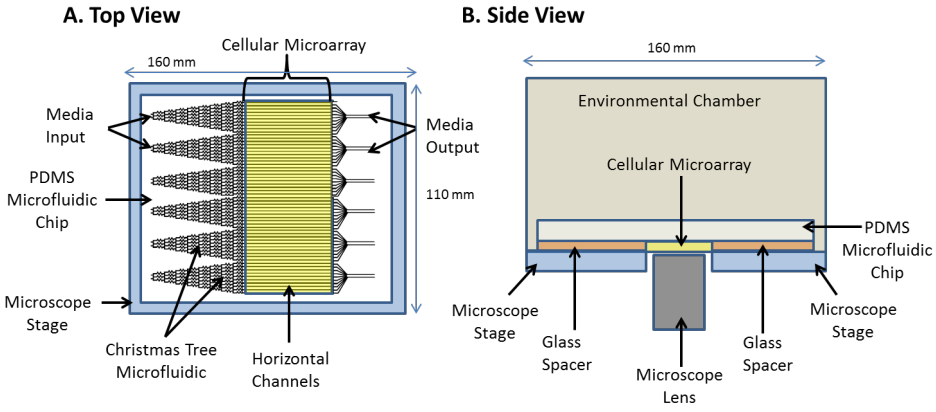

Figure 7: Integrating Design Components. A: Top view of the final design assembly. Output channels from the gradient generators flow horizontally across the cellular microarray (yellow). The entire design rests on a piezoelectric microscope stage. **B:** Side view of a potential integration scheme of the environmental chamber and microarray. The microarray is held between two glass spacers which are fused to PDMS with plasma oxidation.

Also shown in Figure 8 is the interface between the cellular array and the PDMS microfluidics device. To ensure each horizontal channel houses a single row of cells on the cellular microarray, the array must be inserted with the same orientation during each experiment. The array must also be easily removed from the

microfluidic chip for further analysis and culture after experimentation is complete. The details of this interface are part of the future work that is planned for this project, but a potential schematic showing glass spacers that perfectly flank and orient the cellular array is shown in Figure 8B.

The final design will also contain an environmental chamber to provide physiological conditions during cell culture. The integration of this component into the final design is also addressed as future work.

ETHICAL CONCERNS

As our device will be used for testing of cell culture systems *in vitro* the primary ethical concern is the collection and presentation of accurate data. Caution shall be taken to ensure that experiments are conducted precisely and accurately to maintain the integrity of the produced results. Finally, to ensure the safety of the researcher, the design should not pose any physical or chemical threats to the researcher while performing its intended uses.

FUTURE WORK

This Semester

The immediate steps to follow for development of this device are COMSOL simulation analysis and verification. COMSOL is a multi-physics software package capable of simulating diffusional mixing in microfluidics devices; this will be used to ensure the dimensions of the final design are adequate for mixing in the serpentine channels. The simulation will then be manually verified using Fick's Law of Diffusion.

After any necessary modifications and repeated simulations are complete, a photomask will be ordered from Fine Line Imaging in Colorado Spring, CO with an expected cost of $100. The device will then be fabricated in either the Dr. Justin Williams' or Dr. David Beebe's laboratory at the University of Wisconsin-Madison.

Gradient generation will be optimized to maximize accuracy; this may require changes in dimensions and a second photomask. During optimization, the team will also work on incorporating the cellular microarray into the gradient-generating fluidics device. We expect the molecular forces between glass and PDMS will be sufficient to seal the cellular microarray within the device. However, if this is not the case, small clamps will be incorporated into the bottom of the fluidics device to secure the cellular microarray in place during analysis.

Next Semester

With incorporation of the cellular microarray, an environmental chamber will be engineered to provide an adequate environment for cell survival. This will be used to maintain 5% CO_2, a temperature of 37 °C, and a humidity of 95%. Along with this, cell viability testing will be conducted to ensure cell survival. For environmental chamber modeling, Dr. Brenda Ogle's lab has an environmental scope that will be used as a model. Additionally, another design team is currently working on developing an environmental chamber; we plan to collaborate with this design team.

Finally, the device will be used for experimentation. Following completion of the prototype, immunofluorescence and PCR will be used to analyze cellular responses induced by the presence of varying concentrations of soluble factors.

Cost Analysis

The projected costs of the microfluidic gradient generator are $100. This cost entails only the estimated photomask printing. Due to the small amounts of Sylagard 184 and Su-8 Photoresist we will require to synthesize our prototype, these materials will be generously provided by the NITRO Lab, Department of Biomedical Engineering, UW-Madison. The total cost of the microfluidic prototype is feasible with our allotted budget by our client. The integration of the cellular microarray and environmental chamber will likely increase our final costs significantly, however these costs will be addressed as integration occurs, because estimates will vary greatly depending on design.

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APPENDIX

Appendix A: Product Design Specifications

Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays
(Microfludic_Platform)

Project Design Specifications
October 26, 2011

Group Members: Sarah Reichert, Anthony Sprangers, Alex Johnson, and John Byce

Advisor: Dr. John Puccinelli
Client: Dr. Randolph Ashton

Function:

Cellular microarrays contain populations of living cells that are spatially separated from one another. Because of the numerous discrete populations, these devices are beneficial in high-throughput screening applications. We aspire to expand the utility of cellular microarrays by designing a way to integrate them with microfluidic platforms that are compatible with a standard microscope stage. Along with fitting in the stage, the platforms must be able to generate concentration gradients across the field of flow, form a watertight seal, and be reusable in order for the devices to be effective. By accomplishing this, our client will be able to perform live-cell imaging and high-throughput analysis to determine how various culture conditions effect stem cell differentiation.

Client Requirements:

- A prototype microfluidic platform that can:
  - Generate concentration gradients across the field of flow
  - Form a water-tight seal with a microscope slide
  - Be reusable for multiple cellular microarrays
  - Fit on top of a microscope stage and be used for live-cell imaging

1. Physical and Operational Characteristics

A. ***Performance Requirements:*** The device must be able to house cellular microarrays and enable a concentration gradient of fluids to continuously flow, without leakage, while the arrays are imaged with a confocal microscope.
B. ***Safety:*** The apparatus cannot be harmful to the cells that it will contain or the researchers who will be working with it.
C. ***Accuracy and Reliability:*** An accurate and reliable concentration gradient must be able to be established and maintained across the field of

flow. This will ensure that the data obtained from experiments utilizing the microfluidic platform will be repeatable and that the results are truly representative of how certain conditions effect stem cell differentiation.

D. ***Life in Service:*** The platform must be able to be continuously used for the duration of various types of stem cell experiments, which typically range from 1 to 10 days in length. The molds to create the platforms should be capable of being reused for 10 PDMS devices.
E. ***Shelf Life:*** When not in use, the device mold will be stored on a laboratory shelf at 20 °C and standard pressure. It must be capable of retaining its full functionality at these conditions for up to two years.
F. ***Operating Environment:*** While experiments are running, the temperature of the apparatus will be 20-37 °C. During imaging, the laser used may increase the temperature to slightly above 37 °C; however, this change is not expected to be significant and therefore should not affect the efficacy of the device. As testing will be performed in a standard laboratory, humidity and pressure will be within the typical ranges for this type of environment. In order to sterilize the device, it will be autoclaved, temporarily exposing it to high pressure saturated steam at 121 °C, or washed with sterilizing chemicals, which may potentially be corrosive.
G. ***Ergonomics:*** The platform should be easy to use by trained researchers and should not impose any physical strain on their part to assemble or disassemble for experiments.
H. ***Size:*** The maximum dimensions for the portion of the microfluidic device that will fit within the microscope stage for imaging are 158 x 105 mm. The device should be under 2.5 cm in height to fit within the stage area.
I. ***Weight:*** The device must weigh less than 0.5 kg, the maximum recommended load for the piezoelectric microscope stage that it will be mounted on for imaging.
J. ***Materials:*** The materials used must be biocompatible, nontoxic, and able to withstand sterilization techniques such as autoclaving and the use of sterilizing chemicals. Materials that have a history of use in microfluidic devices and entail simple fabrication and design processes are ideal.
K. ***Aesthetics, Appearance, and Finish:*** The portion of the apparatus that will contain the cellular arrays must be transparent so that the cells can be properly imaged and analyzed by researchers.

2. Production Characteristics

A. ***Quantity:*** One mold that can be used to create PDMS devices for use in multiple experiments is required.
B. ***Target Product Cost:*** If the device does not require temperature control for effective usage, then the target total manufacturing costs should be less than $5,000. If temperature control is required, the costs will likely need to be between $10,000 and $20,000.

3. Miscellaneous

A. ***Standards and Specifications:*** There are no federal regulations that need to be met for this device; however, as the apparatus will be used with cultured cells, it must adhere to standard cell culture protocols.
B. ***Customer:*** Intended customers for this device will desire a microfluidic platform that can be easily applied, removed, and reused on cellular microarrays. Other devices in the competitive market are not removable and thus limit the potential for expansion of cell lines after experimentation.
C. ***Patient-related Concerns:*** Induced pluripotent and embryonic stem cells will be seeded in this apparatus. As a result, it must be able to be sterilized between uses. There are no concerns regarding data storage or confidentiality involved with this project, as the subjects are not patients.
D. ***Competition:*** Several research efforts have used PDMS microfluidic devices to deliver soluble factors to cells and establish concentration gradients (pioneered by Whitesides *et al*., 2000). One notable competing device developed for a similar research goal was patented by David J. Beebe *et al.* in 2007. This apparatus is titled *Microfluidic platform and method of generating a gradient therein*, and implements a single microfluidic channel with porous membranes and source/sink action to generate a gradient of particles. A comparable device using a source and sink gradient bridge titled *Microfluidic gradient devices* was developed and patented by Noo Li Jeon *et al.* in 2011.