Kaleidoscope

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Microfluidic for Testing Mechanical Properties of Cancer Cells

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Introduction

Metastatic cancer is the cause of ninety percent of cancer-related deaths.[1] While great strides have been made in cancer research, there is still much to learn about the mechanisms tumor cells undergo in the metastatic cascade. As illustrated in Figure 1, the metastatic cascade begins with tumor cells detaching from the primary tumor, at which point there are two main options: tumor cells either enter the lymphatic system or enter the vasculature. The focus of this study is the metastatic cascade with respect to the vasculature. Once in the bloodstream, hematogenous dissemination occurs. The tumor cells travel through the bloodstream until they either adhere at a distant site or die. If they adhere, they are then able to extravasate and form a secondary tumor.[2] In order to better understand metastatic cancer we must be able to model it accurately. Creating a more physiologically relevant model will better shed light on the cellular interactions taking place *in vivo*. We were able to create a microfluidic device to more stably model flow patterns in the body. Previously, we used a parallel plate flow chamber, which was inconsistent in its functionality. The microfluidic device enables eight trials to be run simultaneously as opposed to one at a time. As well, variation from trial to trial has been decreased by creating a chamber that has constant dimensions, rather than one that varies from trial to trial based on the vacuum pulled.

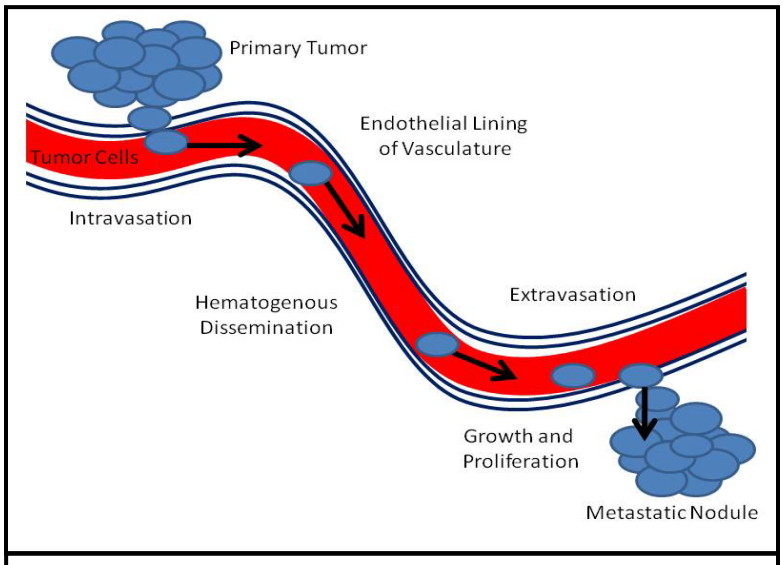

Figure 1: Metastatic Cascade

Methodology

Mask Fabrication

The design used for the mask was developed by Chau et. al. and altered slightly to better fit our needs.[3] With the design, the fluidic device can achieve multiple shear stresses at once by joining two reservoirs with channels of various lengths, as in Figure 2. The pressure drop across the channels is constant because of common pressure in the entrance and exit reservoirs; thus the flow rate in each channel dictates the respective shear stresses. The flow rate in each channel varies based on the length of the channel. Using ratios of the channel lengths, the shear stresses could be predicted for any given flow rate. The final design consisted of eight channels of four different lengths.

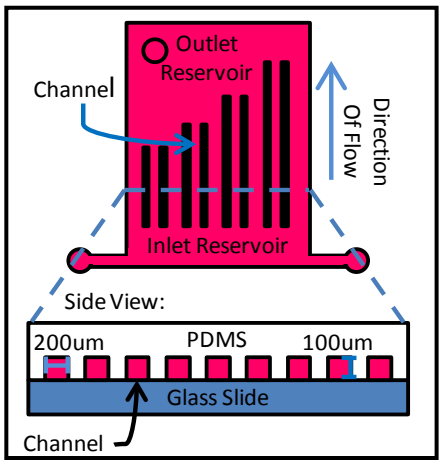

Figure 2: Microfluidic device design

A few adjustments were made to the original design, one of which being the ratio of channel lengths. The original design had shears ranging from 0.7-130 dynes/cm^2. For our purposes the shear stresses only needed to range from 1-15 dynes/cm^2 so the relative lengths of the channels had to be recalculated. As well, we need replicates of the trials, so our design had four lengths with two channels at each length. Calculations to determine the shear stress at the wall of the channels were done using the following equations. The flow rate of each channel was calculated using the following equation:

$$Q_{total} = \sum Q_i$$

Where Q_{total} is the overall volumetric flow rate

Q_i is the volumetric flow rate in each individual channel

Then with the flow rate in each channel, the shear stress at the wall can be calculated with the following equation:

$$\tau_w = \frac{6\mu Q}{wh^2}$$

Where τ_w is the shear stress at the wall

μ is the viscosity of the fluid

Q is the volumetric flow rate

w is the channel width

h is the height of the channel

Knowing the desired shear stresses – 1, 2, 5, and 10 dynes/cm^2 – and the flow rates we were capable of achieving with the peristaltic pump, channel lengths were designed to be 3, 6, 15, and 30mm long. Once the design was generated in AutoCAD, a photomask was made by FineLine Imaging.

Chamber Development

First a mask must be made on which polydimethylsiloxane (PDMS) chambers can be cast. The process begins by spincoating a layer of SU-8, a negative photoresist, onto a glass slide. After the SU-8 is spun to an even height across the slide, the slide with SU-8 undergoes a 70 minute soft-bake at 95°C. Next, the mask aligner is used to cross-link the SU-8 into a permanent pattern using ultraviolet (UV) light; areas exposed to UV light is dictated by the photomask. Finally, there is a post exposure bake, followed by washing steps that removes all the undesired SU-8 that was not cross-linked because it was not exposed to UV light in the mask aligner. The masks were produced in the CeNSE microfabrication lab and all of the protocols were developed with the help and guidance of Dr. Chuck May.

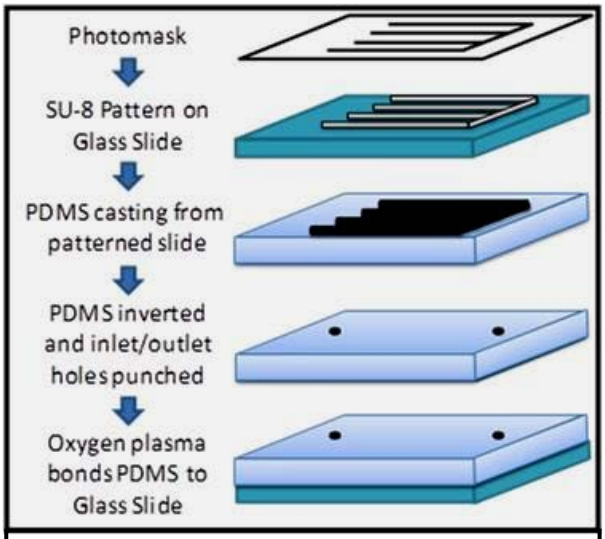

Figure 3: Overview of microfluidic fabrication

The second step in this process is to cast the microfluidic chamber using the mask. Twenty grams of degassed PDMS, mixed with 10% by mass curing agent, is poured onto the mask and then allowed it to solidify for 70 minutes in an oven at 65°C. Once set, the PDMS is removed from the oven, peeled off the

mask and inlet and outlet port holes were punched through the PDMS. To create a chamber, the PDMS was permanently attached to a glass slide with plasma oxygen. The slide and PDMS were exposed to plasma oxygen at 600microns for 90 seconds and then immediately pressed together, creating a permanent bond between the glass slide and PDMS chamber. Once the chamber is bound to the glass slide, inlet and outlet ports are inserted into the punched holes and PDMS is cast around the ports to permanently bond them in place.

Seeding Cells

The final step in device fabrication is cell growth on the channel floors under flow conditions. This will create an environment which mimics the vasculature of the body. In such a setting, cancer cell adhesion and detachment to endothelial cells can be tested. After complete assembly of the microfluidic device, it must be sterilized by pumping a 10% bleach solution through the chamber, followed by a 70% ethanol solution, and then cell media should be pumped into the chamber and allowed to set overnight to draw any remaining toxins out of the PDMS. Finally, fresh cell media should be pumped through the chamber and then aspirated out.

To seed cells in the chamber, Attachment Factor (Cell Systems) is be pumped through the chamber with a syringe and then aspirated out. The chamber is allowed to dry before a suspension of cells at a concentration of $1x10^6$cells/mL is then syringed into the chamber. Cells are permitted a 6 hour static period to firmly adhere to the bottom of the chamber. Once the cells adhere to the chamber, they need a continuous supply of nutrients. The nutrients are supplied in the form of media. To constantly feed media through the chamber, tubing is run from a media reservoir, through a peristaltic pump and to the chamber inlet port where it finally reaches the cells. From there, tubing leads the media back out into a waste container.

Results & Discussion

A fabrication process was developed and a microfluidic device was successfully fabricated, as seen in Figure 4. The device contains two inlet ports, one is a perfusion inlet to provide nutrient-rich media to the endothelial cells growing on the slide, and the second is for introduction of a second cell population, such as cancer cells to test their adhesion to an endothelial monolayer. A phenol dye was used to monitor flow patterns as it was pumped into the chamber to ensure that the fluid was dispersed evenly to each channel. The inlet reservoir was long enough that the fluid spread across the width of the chamber before filling the length of the reservoir, so fluid reached each channel at approximately the same time. As well, the oxygen plasma adhesion of the PDMS to the glass slide was effective. With the phenol dye it is evident that there are not any leaks around the perimeter of the chamber. Phenol dye was left in the chamber overnight to ensure that leakage would not occur after longer periods of time with the same positive results. The microfluidic was successfully produced and capable of flowing fluid through the chamber.

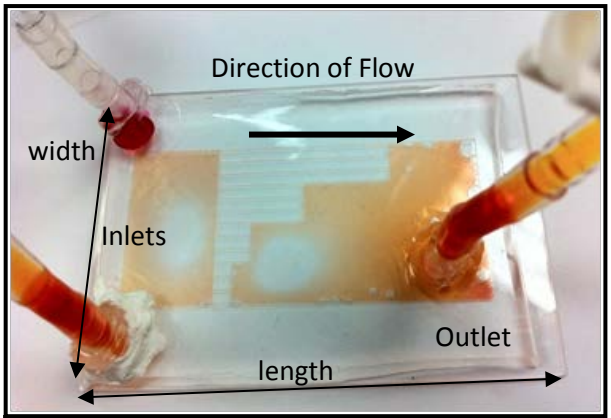

Figure 4: Microfluidic with a dye to display channels

The channel widths were measured to validate the fabrication process. The goal channel width was 200µm, using AxioVision software on a Nikon microscope the PDMS channel widths were measured to be 200-215µm wide, as seen in Figure 5. The slightly wider portions of the channel are likely due cross-linking of SU-8 around the pattern edges under the mask where ultraviolet light should not have reached. This would lead to slightly less SU-8 being removed during the washing step of the mask fabrication; thereby causing the mask to cast channels slightly wider than desired. The range of channel widths encompassed the original goal and the maximum deviation was less than 10% different from the goal. The PDMS edges of the channels appear to be well defined.

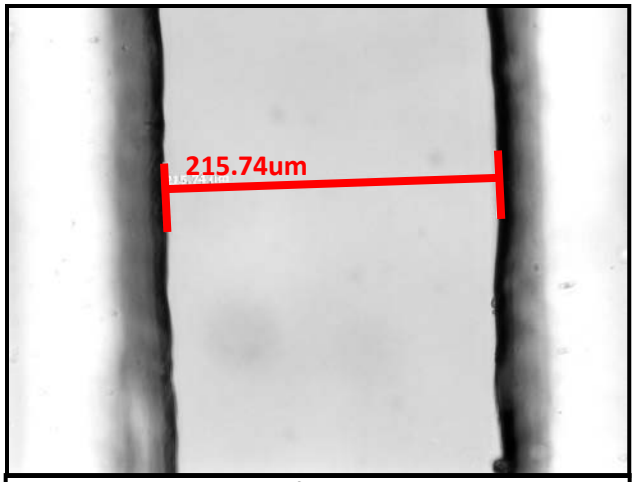

Figure 5: Measurement of channel widths between the PDMS walls.

In order to determine the shear stresses each of the channels were experiencing, the overall flow rate had to be determined. The flow rate of media through the chamber is dictated by the peristaltic pump. Prior to experimentation, the peristaltic pump had to be calibrated with cell media at 37°C. A calibration curve was generated by measuring the volume of fluid pumped over a period of time at ten different pump settings. The calibration curve (Figure 6) allows calculation of the pump setting to achieve a desired volumetric flow rate using the equation:

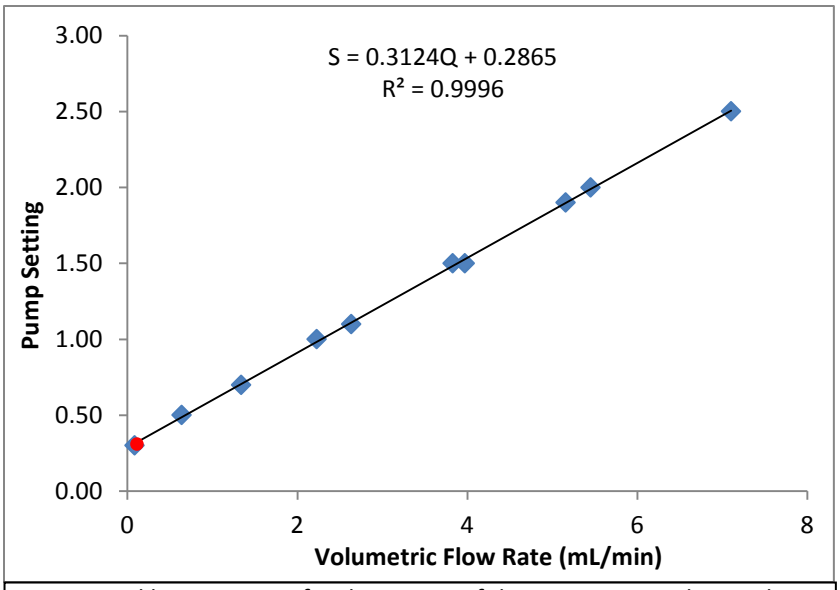

Figure 6: Calibration curve for the setting of the pump versus the resulting volumetric flow rate.

$$S = 0.312Q + 0.287$$

where S is the pump setting and Q is the total volumetric flow rate. Utilizing the calibration curve equation, the pump setting to achieve a total flow rate of 0.033mL/min thereby producing shear stresses of 1, 2, 5 and 10 dynes/cm^2 in the respective channels, should be 0.297, indicated by the red circle on the calibration curve. This setting is specific to the microfluidic devices and the fluid being pumped (cell media at 37°C). The calibration curve yielded an r-squared value of 0.9996, indicating a strong linear relationship in the data.

As well, cells were successfully seeded in the microfluidic device during the 6hr static period, indicating that the device environment is not toxic to the cells. However, multiple problems have occurred which have prevented successful cell growth under flow conditions. Endothelial cells were beginning to adhere to the glass floor of the chamber at the end of 6 hours as indicated by their spreading, but the cells were

very sparse and the cell count was low. Upon induction of flow the endothelial cells become detached from the floor of the microfluidic. To date, cells have not successfully been grown in the microfluidic device under flow conditions.

Conclusions

A microfluidic device fabrication protocol was successfully developed; the protocol yielded channels of the appropriate width and lengths, which can contain liquids without leakage for extended periods of time, and does not appear to be toxic to cells. From there, calculations and calibrations were preformed to achieve the desired shear stresses at the channel floors within microfluidic device. Looking forward, further steps must be taken to finalize the process by which endothelial cells can be cultured under flow conditions. Potential solutions may include using a higher density of cells in solution when seeding cells in the device. As well, different agents could be used to seed cells more firmly to the slide. We have been using Attachment Factor, which consist of a serum in a collagen medium; instead we could try fibronectin. Another potential fix may be that rather than turning the pump directly on, it should be ramped up to the appropriate setting. The immediate dynamic change may be too rough of a condition for the cells to survive. Along the same line, the cells may need a better developed matrix before flow is induced, so a longer static incubatory time could be another potential solution. Once cells can be cultured, the device will have a breadth of applications.

References

1. Mehlen, P.; Puisieux, A. Metastasis: a question of life or death. *Nature Rev.* **2006,** 6, 449-458.

2. National Cancer Institute. http://www.cancer.gov/cancertopics/factsheet (April 2011).

3. Chau, L.; Doran, M.; Cooper-White, J. A novel multishear microdevice for studying cell mechanics. *Lab Chip*. **2009,** 9, 1897-1902.

4. Duffy, D.C.; McDonald, J.C.; Schueller, O.J.A.; Whitesides, G.M. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* **1998,** 70, 4974-4984.