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Microfluidics Fabrication and Testing for a Novel BioMEMS Cell Counting Device

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by

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Undergraduate honors thesis under the direction of

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Abstract

This project presents the research being conducted using poly-(dimethylsiloxane) (PDMS) to fabricate microchannels from poly-(methylmethacrylate) (PMMA) master molds for the design of a microfluidic system used in determining cell concentrations of samples. The final cell counter will consist of a PDMS microchannel sealed to a PMMA base chip containing fibers for optical detection. The main focus of this work is microfluidic systems in poly-(dimethylsiloxane) (PDMS) and poly-(methylmethacrylate) used in the construction of the microfabricated cell counter. Syringe pumps were connected to the cell counter via a series of tubing to precisely control flow rates. The counter was initially tested using microspheres instead of cells to optimize the flow rate of the microspheres. Research was conducted on current cell-counting methods and devices, and it was determined that a cell counter that is small and does not harm the viability of cell is needed. Optical detection was then chosen as the means of counting cells because this would not damage the cells' viability as some stains or electrical potentials utilized in other devices can. The average diameter of HeLa cells (20-25 microns) was used to design the flow channel dimensions. The channels were then fabricated from PDMS and examined using microscopy and MetaVue, an image analysis program, to ensure that the channels were accurate to the master mold. Once the molds of the chips were fabricated, flow velocity of microspheres was determined. The velocity was tested using syringe pumps by infusing the spheres into the channel, and by applying only suction to the end of the channel, and by applying both infusion and suction forces. This study will determine the optimal parameters of syringe pump control to achieve particle velocities for optical detection.

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Background to Design Project and Thesis

Need

Counting cells in a tissue culture or a blood sample is a routine yet critical assay carried out in clinical and research laboratories today. Blood cell counts are taken for physicals and general checkups, before surgery, and during emergency room visits. Red and white blood cells and platelets are routinely counted. Increases in red blood cell counts indicate to dehydration and smoking, and decreases indicate to anemia. An increased number of white blood cells indicates the presence of infection or inflammation or leukemia, while a decreased number indicates a viral infection. An increased or decreased number of platelets can indicate the presence of an autoimmune disease (Evans 2004). Mammalian cells are routinely counted for properly seeding cell-culture experiments. As an example, Wallace et al. counted primary cells "on the day cultures were seeded" (Wallace 2001). On days two, four and six of post-culture seeding, cell samples were recounted for determining cell growth.

Figure 1. Coulter Counter currently used for cell counting (Coulter 2004)

Currently, the Coulter Counter is the most widely used device for measuring both the size and concentration of cells in a sample. Wallace et al.'s counts were performed using a Coulter Multisizer II particle counter, and viable cells could not be counted in the study (Wallace 2001). This method is based on measurable changes in electrical resistance produced by nonconductive particles (cells) suspended in an electrolyte such as saline (Coulter 2004, Brunnschweiler 1999).

Each cell passes through between two electrodes displacing an equivalent volume of electrolyte, which is measured as a pulse in voltage between the electrodes indicating the size as well as presence of a cell (Coulter 2004, Brunnschweiler 1999). However, while highly accurate, the Coulter counter has the electric current flowing between the electrodes, which ranges from 30 μA - 6000 μA (Coulter 2004). Johnson et al. (1987) found exposure to 300-400 μA currents enhanced DNA synthesis by 20% relative to control groups in rat osteosarcoma cells. This increased rate of cellular proliferation could lead to undesired cellular growth and increased rate of genetic mutation. Also, the temperature inside the cell increases, which could lead to denaturation of some proteins. This was supported by Norimura et al.'s (1993) findings that cellular physiological and growth abnormalities occurred in human T-lymphocytes when exposed to magnetic fields of 4 Tesla. The typical dimensions of the Coulter Counter are about eighteen inches tall, sixteen inches deep, and twelve inches wide and weighs about thirty pounds (Coulter 2004). This equipment is rather large and bulky, and not easily transported.

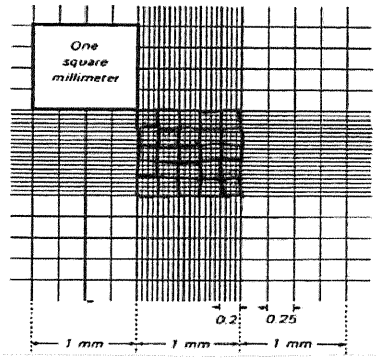


Figure 2. Hemacytometer example (Hansen 2001)

The hemacytometer is "an etched glass chamber with raised sides that will hold a quartz coverslip exactly 0.1 mm above the chamber floor with a total counting surface area of 9 mm^2" (Hansen 2001). It is a widely used device to count cells but is subject to user error and is not recognized by scholarly journals as a valid counting method. The NucleoCounter uses fluorescent dye, propidium iodide (PI), to count mammalian cells that are already dead in a culture. PI is a dye that stains the cells' nuclei and the fluorescent signals from the stained nuclei are detected

that are already dead in a culture. PI is a dye that stains the cells' nuclei and the fluorescent signals from the stained nuclei are detected (NucleoCounter 2004). For counting cells that are alive, the sample and a lysing buffer are mixed, which effectively makes the cells membrane permeable to the PI dye (NucleoCounter 2004). However, this method destroys the viability of the cells from the culture. The Nucleocounter is also too large for transport at ten and a half inches tall, fifteen inches long, and nine inches wide (Wilson 2005). The design of my senior design group uses optical detection to measure cell concentration accurately, noninvasively, and nondestructively (Lee 2003, Sheetz 2002) unlike the previous methods of cell detection. Our design implements BioMEMS (Biological MicroElectroMechanical System) technology as an inexpensive and convenient (McDonald 2002) way to obtain accurate cell concentrations.

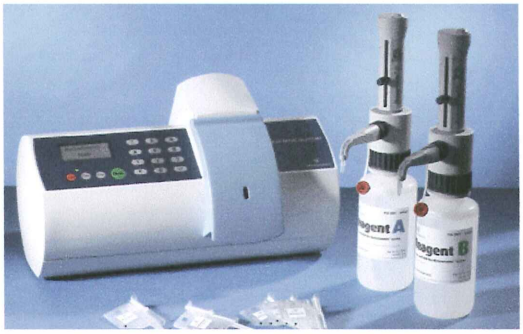


Figure 3. NucleoCounter picture (NucleoCounter 2004)

MicroElectroMechanical Systems Applicability

Using MEMS applications is a relatively new field of research and technology. Currently used in "laboratory-on-a-chip" applications, MEMS is an ideal method for cell counting because it minimizes a laboratory bench scale procedure to a chip-scale assay (Brunnschweiler 1999, McDonald 2002). The small size of chip-scale analyses allows the devices to be portable and easier to

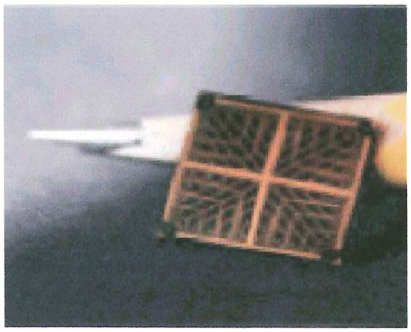


Figure 4. Depiction showing scale of MEMS device (Brunnschweiler 1999)

electrophoresis, and DNA microarrays, which have proven accurate, efficient, and useful in laboratory settings (McDonald 2002, Jackson 2004, Burns 2004).

Current MEMS cell counting techniques include electrical impedance (Brunnschweiler et al. 1999), detection by lasers (Lee 2002), electrokinetic voltages (Cui et al. 2002), and fluorescent dyes (Jackson 2004). Brunnschweiler et al. (1999) and Nieuwenhuis et al. (2004) each designed an on-chip particle counter using the Coulter principle of measuring change in electric resistance. As previously discussed, the Coulter principle does not allow for the maintenance of cell viability. Both methods combined microfluidics with electronics. Cui et al. (2002) designed and fabricated dielelectrophoretic lab-on-a chip, which uses traveling wave dielelectrophoresis to induce particle or cell movement, and cells were then detected optically and fluorescently. However, dielelectrophoretic induced flow uses an array of electrodes to create a non-uniform electric field, which causes polarizable particles to move. Their optical detection method employed sending and receiving light across a flow channel via optical fibers. The cells were injected into the chip by syringe pumps and were detected by measuring the changes in intensity of the light recorded by a photodiode as the cells passed between the optical fibers. However, they used red laser light and fabricated too large a flow microchannel because their output graph showed only changes of less than ten percent in light intensity. Though the testing beads were detected in the flow channel, clear distinction of individual particles was not possible due to large size discrepancy between channel dimensions of 200 microns wide and particle size or 10 microns and 45 microns. The other detection method used, fluorescence, was effective, but once the cells are dyed, they cannot be used again. Jackson et al.'s (2004) overall objectives were the same as

ours, to design and fabricate a microfluidic device that is low cost and portable for diagnostic applications. However, they used complex techniques of hydrodynamic focusing, which uses a sheath fluid to define the diameter of the sample fluid being pumped through the microchannel. Their optical detection also relied on the use of lasers. Lasers run the risk of causing biological damage, most commonly from negative thermal energy effects, such as protein denaturation even if for submicrosecond exposures in some instances (Holt). These current methods may harm the viability of the cells, while our design will employ optical detection methods to avoid harming the cells (Lee 2003).

Cell Counter Design

The designed cell counter will flow the cells through a thirty-micron micorchannel, designed for HeLa cells, and past an optical detection region. A light emitting diode (LED) will emit light through an optical fiber across the channel and a photodiode (PD) will receive the light through another optical fiber on the other side of the channel. The photodiode will read the intensity of the light as constant when no cells are present between the fibers. As a cell passes through the channel between the two optical fibers, light will be absorbed and scattered by the cell, and the photodiode will detect a lower intensity of light. The output of the photodiode will connect to a data acquisition card and be read by the computer program LabVIEW. The device will be tested using polystyrene microspheres and HeLa cells. In prior research, this method was tested using

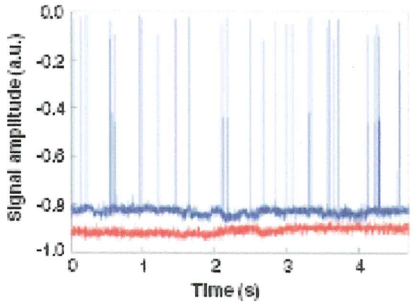


Figure 5. Graph of signal amplitude vs. time for bead testing (Lee 2003)

hydrodynamic focusing by Lee and Lin (2003). Lee and Lin (2003) also found a clear output signal with the microsphere testing, where the constant amplitude shows the absence of microspheres and the spikes in amplitude indicate the presence of microspheres. Their output using a dilute whole blood sample was less defined as the microspheres but still demonstrated the effectiveness and robustness of optical counting. The variations in amplitude are attributed to the different cells in whole blood, white and red blood cells, and lymphocytes (Lee 2003). Also, cell fragments from broken cells would cause the amplitude noise to occur.

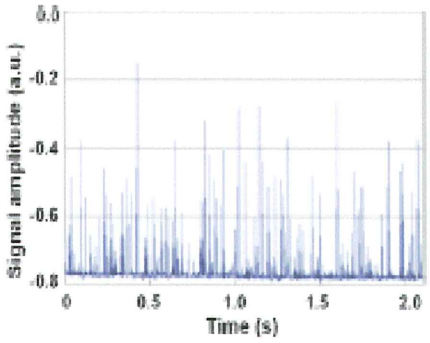


Figure 6. Graph of signal amplitude vs. time for dilute whole blood sample testing (Lee 2003)

In planning for optical detection, the specific range of absorption wavelengths for the cells must be considered. When photons of certain wavelengths and intensity encounter a cell, only the light within that range will be absorbed, while the remaining light is scattered at different angles (Sheetz 2002, Drezek 1999). These events lead to a lesser intensity of light traveling through the cell; these changes of light intensity can be measured, recorded, characterized, and analyzed to determine the presence of a cell passing in between a light source and detector (Lee 2003, Sheetz 2002, Drezek 1999). The counter was designed specifically for HeLa cells. HeLa cells are an immortalized cervical tumor cell line and are readily available, easy to culture, and exhibit characteristics similar to those of blood cells (Klebanov 2004).

The optical detection equipment was chosen for one of the high absorbance peaks of HeLa cells of 809 nanometers (Klebanov 2004). This will allow for maximum light absorption by the cells and for a maximum drop in light intensity to be detected by the photodiode. The wavelength will also dictate the properties of the light source (LED), detector (PD), and other optical equipment. The microspheres used in testing were chosen to mimic the optical properties of HeLa cells.

The microfluidics of the cells flowing through the microchannel also needed to be considered in the design and is the principal behind lab-on-a-chip systems. The cells need to be in laminar flow and in single file through the microchannel. Laminar flow ensures that all of the fluid in the channel is flowing with the same velocity and that no mixing of the fluid is occurring. Laminar flow occurs naturally in capillary tubes, similar to that of the designed microchannel (Gravesen 1993). Microscale fluid flows have very low Reynolds numbers due to the small value of length (L) in the equation $\mathrm{Re} = \frac{\rho \mu L}{\chi}$.

To assure that cells are flowing in single file in the channel, normally hydrodynamic focusing, adjusting the width of the sample by flowing sheath liquids on either side of the sample, is used. However, for the scope of the senior design, this component was not considered. As the counter was designed for HeLa cells and the average diameter of HeLa cells ranges from 20 – 25 microns, the diameter of the flow channel was designed as 30 microns by 30 microns. This should allow for single file cell flow without clogging of the flow channel.

It was decided to have ten events for every peak in amplitude, and the PD will have a one-megahertz sampling rate. Therefore, one peak with be ten microseconds long. Based on this and the channel size, the flow rate was calculated to 720 μL/sec. This gave

a linear velocity of 4000 mm/s. For visual assessment, this would be too fast, so the minimum flow rate of a 1 mL syringe was used of 0.73 μL/hr, which gave a linear velocity of 0.22 mm/s. This flow would be easier to manually observe on the microscope. In case microspheres or cells passed through the detection region in clumps, the time for one cell to pass would be determined, and the time for the large clump to pass would be divided by the time for a single cell. This would allow for determining the number of cells in the clump. This calculation could be visually tested to determine its validity and accuracy.

Objectives of the Design Project

The goals of this project were design and fabricate an on-chip cell counter, using MicroElectroMechanical Systems (MEMS) and microfluidics technologies, to provide an inexpensive and convenient way to obtain accurate cell concentrations without damaging the viability of cells being counted. Comparing the results to those from a Coulter counter, the device will be tested for accuracy and any necessary corrections to achieve accurate cell counts will be made. A poly-(methylmethacrylate) (PMMA) housing for the light sensing equipment and a disposable snap-on poly-(dimethylsiloxane) (PDMS) flow chamber will be designed to save time during sterilization of the system. The final design will be an accurate, economical, portable, nondestructive, and easy-to-use method for determining cell concentrations in samples.

Detailed Design of the Cell Counter

Our design will consist of a PMMA wafer, a PDMS wafer, microneedles, microtubing, computer-controlled syringe pumps, a light emitting diode (LED), a photodiode (PD), and optical fibers. The chip housing for the optical equipment will consist of a micromilled PMMA wafer and the PDMS wafer housing the flow channel will "snap-on" to the PMMA wafer. The cell sample will be injected into the PDMS wafer into a reservoir narrowing into the microchannel that ends in another reservoir. Each reservoir is connected to a computer controlled syringe pump via microneedles and tubing. One syringe pump infuses the cells into a reservoir, and the other pump creates a suction to withdraw the cells from the channel and into the reservoir at the opposite end of the channel. The hardware needed to detect cells in the channel will be an LED, a photodiode, and optical fibers. We will use an LED to deliver a steady light source. This light will be transmitted by a fifty-micron multimode optical fiber that is partially housed in the PMMA wafer. A nine-micron single mode optical fiber located on the opposite side of the channel will receive the light and transmit it to the photodiode. The photodiode will be connected to a computer where the data will be collected and analyzed

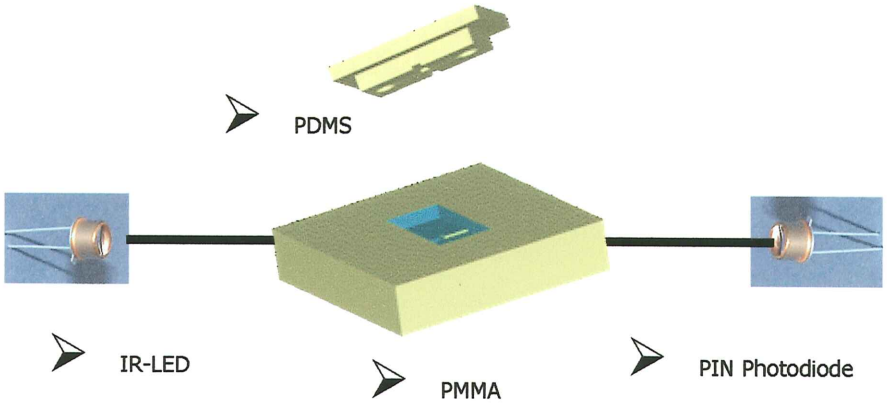


Figure 7. System Integration

with the use of LabVIEW software. We will also use an input/output card to control voltages going to our sensing equipment and to receive voltages coming from our photodiode.

The PMMA wafer will serve as a protective casing for the optical fibers. These fibers will be inserted into the wafer using the micromachining technique of alignment (Brunnschweiler 1999, Cui 2002). The PDMS wafer will be a disposable layer that will house the microchannel. The chemical properties of PDMS allow it to bind to glass and PMMA; this will secure the snap-on wafer and enclose the microchannel to prevent leakage (Cui 2002). As the PDMS wafer is disposable, the size of the microchannel will be adjustable to allow different cell sizes to be counted.

Thesis Specific Aims

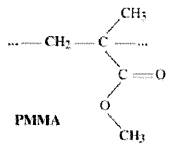
One of the main components of the cell counter is the microfluidic channel through which the cells will flow. The fabrication of a microchannel is a delicate and exact process. This thesis will elaborate the design and fabrication of the microchannel used in our design project and the testing of the fluidics. It is important to maintain a consistent velocity of the particles in solution. The velocity of the fluid flow will be tested by using syringe pumps to infuse a microsphere solution through the microchannel, to apply suction to the opposite end of the microchannel, and to apply both infusion and suction forces to flow the microsphere solution. The base of the microchannel, which will house the optical sensing equipment, will be constructed from poly-(methamethacrylate). The actual microchannel will be constructed out of poly-

(dimethylsiloxane). The reasons for choosing such materials and the fabrications procedures and explanations will be discussed further.

Materials Choice

Poly-(methylmethacrylate)

PMMA was chosen to house our optical fibers because it is a durable and hard material with a high Young's Modulus of 6.2 GPa and will therefore be protective of the fibers. It is a synthetic polymer of methyl methacrylate and is a transparent thermoplastic. It is does not filter ultraviolet light but does allow infrared light to pass, which is ideal for the design needs of 809 nm light being used with the HeLa cells (Tangram 2004).

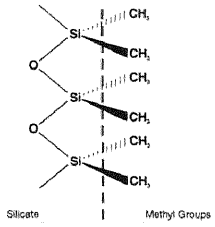


Holmes, 2004.

Figure 8. Chemical structured of PMMA

Poly-(dimethylsiloxane)

Poly-(dimethylsiloxane) is an organo-silicone polymer composed of repeating units of siloxy (Si-O) monomers that form the backbone of the polymer. The two methyl groups bonded to the silicon atoms are chemically "inert and hydrophobic with low intemolecular forces and surface energies" (Abrutyn 1997). The bond length between the silicon and oxygen is relatively long with a flatter bond angle, which allows for flexibility in the molecule and for easier diffusion of gas molecules. Compared to three other silicone polymers, He, O_2, N_2, CO_2, and CH_4 gases diffused through PDMS significantly faster than the other polymers. The surface energy is about 20 erg/cm^2 (McDonald 2002). Due to their low



McDonald, 2002.

Figure 9. Chemical structured of PDMS

molecular interaction, the methyl groups are also "highly surface active, can reduce adhesion and produce barrier effects" (Abrutyn 1997). The reduction in adhesion is important so that the microspheres and cells are not attracted to the walls of the microchannel, which could allow for clogging. The liquid pre-polymer of PDMS replicates the features of the master mold is poured on with high fidelity (McDonald 2002).

PDMS is transparent, electrically insulating, and exhibits elastomeric and viscoelastic behavior. These elastomeric and viscoelastic properties allow it to conform to various surfaces and allows for reversible deformation. Important in its biomedical usage is that PDMS is nontoxic. The chemically stable and unreactive siloxy backbone and the unreactive methyl groups make PDMS suitable for use in and with biological materials (McDonald 2002).

PMMA and PDMS components

When using cells and biological materials, sterile procedures must be carefully practiced. Therefore, we incorporated a snap-on disposable wafer, which will contain the flow channel for the cells and allows for fast and easy clean up and does not require expensive and time-consuming sterilization techniques. Having disposable flow channels also allows for the dimensions of the flow channel to be changed depending on the type of cells in the sample.

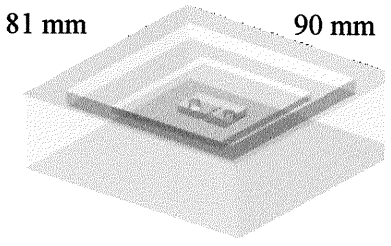


Figure 10. PMMA mold for PDMS

Employing this method permits our cell counter to be universal for many different cell types. For the snap-on chip, we chose PDMS because its properties allow it to bind to glass and because it is a flexible material.

Dimensions for these wafers were determined using prior research and considering ease of use. The dimensions chosen for the combined wafers were 45mm long, 46 mm wide, and 10 mm tall. These dimensions were determined after consulting literature and considering ease of use of the counter. Considering the PDMS wafer must be inserted and removed from the PMMA wafer, the wafers must be large enough to be manipulated by human hands. The flow channel dimensions in the PDMS wafer were determined considering the average diameter of HeLa cells, twenty micrometers. The 30μm x 30μm channel size will allow cells to flow in single file without clogging the flow chamber and insuring accurate single cell detection.

The PMMA wafers were fabricated in two halves, each 45 mm long, 23 mm wide and 10 mm tall. On the side of each piece, half of the optical fiber channels were milled, each being 150 microns wide. The inner diameters of the optical fibers are 50 microns on the side transmitting the light and 9 microns on the end receiving the light. The fibers are jacketed and cladded for protection and to prevent interference from the environmental light. The two fibers will then be inserted into these channels, aligned under a microscope, and glued together using a UV resistant epoxy.

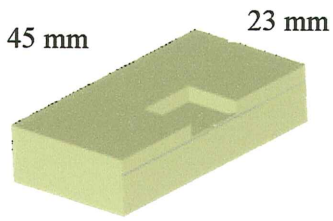


Figure 11. Half of PMMA wafer

A PMMA mold will be milled in which to make the PDMS microchannel wafer. A block of PMMA, 24.1 mm tall will have milled into it a relief of the PDMS microchannel. The final PDMS wafer will be 15 mm tall, 11 mm wide, and 20 mm long.

Materials for Wafer Fabrication

- Polymethylmethacrylate: 2 blocks: 10 mm tall and 24.1 mm tall (McMaster Carr)
- Polydimethylsiloxane curing agent and prepolymer (Dow Corning Sylgard 184)
- Degasser connected to hood (Gast)
- Incubator
- Weight boat
- Spatula
- Micromilling equipment
- Microscope connected to computer with MetaVue viewing program

Wafer Fabrication

The 10 mm tall PMMA block was cut using a bandsaw into small blocks with dimensions roughly 5 mm larger than those in the design were. The pieces were then brought to the Biological Engineering machine shop technician for finishing to obtain smooth and exact cuts close to those in the design. The design did need to be redrawn to match the exact dimensions of the finished blocks. The finished blocks were then brought to the micromill in Atkinson

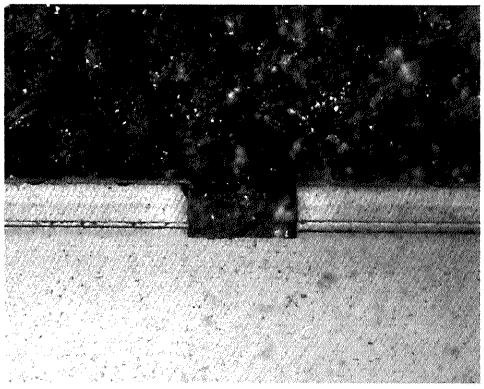


Figure 12. Flow channel at 2x magnification

Hall for Jason Guy in the Chemistry Department to mill our flow and fiber microchannels. The finished blocks were then viewed under the microscope on the computer using the MetaVue program. The pieces were inspected for clean, smooth lines and for dimensioning of the channels. Pictures of the finished channels were captured at 10x and 40x magnifications.

The 10 mm tall block of PMMA was cut into a smaller block of rough dimensions on the band saw. This piece was then taken to Jason Guy for milling of the PDMS relief mold. The finished mold was also inspected on the microscope using MetaVue to ensure the edges of the channel and reservoirs are clean and smooth so that the flow channel and reservoirs are smooth and do not have intrusions or ridges that could interrupt the flow of particles. Pictures were taken on MetaVue.

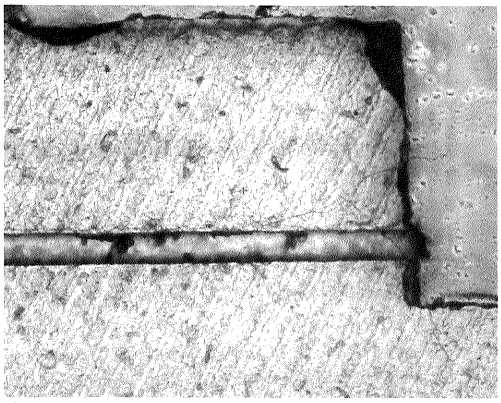


Figure 13. End of fiber channel at flow channel at 10x magnification

The PDMS wafer was made using a 1:10 mixture of curing agent to prepolymer. A weigh boat was weighed, and the scale tared. The 1 ml of curing agent was poured into the boat, and the scale tared. Then 10 ml of prepolymer were added to the curing agent, and the mixture was stirred using a spatula for about 3 minutes. The weight boat was placed in the hood and cyclically degassed at 25 atmospheres for 3 cycles until no air bubbles were observed. The prepolymer mixture was then slowly poured, to ensure no air bubbles formed, into the PMMA mold and cured in an incubator at 65°C overnight. The cured PDMS was then removed from the mold and sealed into the PMMA wafer in the channel.

Materials for Fluidics Testing

- PDMS wafer
- Stainless steel tubing, 17 gauge, 0.058 inches outer diameter (OD) and 0.048 inches inner diameter (ID) (McMaster Carr)
- Tygon tubing, 2 sizes: 0.06 in OD and 0.02 in ID, 0.1 mm OD and 0.062 mm ID
- Luer-loc syringe and adaptors, 23 gauge (BD Scientific)
- Heat shrink tubing, 0.0975 in preshrunk, 0.045 in shrunk (McMaster Carr)
- Soldering iron
- Dremel® drill and cut-off tool #402
- Microscope with connection to computer with MetaVue viewing program
- Hemacytometer
- Computer controlled syringe pumps connected to computer (New Era 500 OEM)

Testing Procedure

The stainless steel tubing was cut into 1-2 inch segments using the 402 cut-off wheel on the Dremel drill. One end of the segment was then sharpened using various sharpening and polishing bits in the Dremel drill. The sharpened end then served as a punch to puncture a hole into the PDMS wafer into each of the reservoirs. An unsharpened piece of stainless steel was then was connected to the 0.02 in ID size Tygon tubing. A small

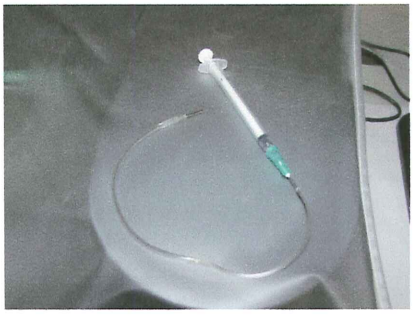


Figure 14. Tubing connections to syringe

section of heat shrink tubing was connected around the junction of the steel and Tygon and sealed by touching the tubing with the soldering iron so that the heat shrink tubing fit snugly around the junction. The smaller size of Tygon tubing was connected to the larger size and sealed with the heat shrink tubing at the junction. The end of this piece of Tygon connected to the needle on the luer-stub adapter, which was then fitted on the 5 ml syringe. The syringe was loaded into syringe pump. This tubing and syringe setup was performed twice, one for the infusion pump and for the suction pump. The unconnected ends of the stainless steel tubing were then inserted into the holes in the PDMS wafer, and epoxy was used to secure around the edge where the steel tubing met the wafer.

For testing, first the 25 micron polystyrene beads and then HeLa cells will flow through the cell counter. The PDMS wafer will be inserted into the PMMA base wafer. The wafers will then be placed on the microscope connected to the computer with MetaVue viewing program. A hemacytometer will be placed under the wafers. With the optical detection equipment operating, the syringe pump creating a suction will turned on first. Then the infusion syringe pump will be turned on to start the flow of microspheres. As the beads flow in the channel, multiple pictures will be acquired to time how long it takes a microsphere to move between two marks on the hemacytometer. Also, the timing will be assessed visually without the use of pictures. This will be used to determine the velocity of the microspheres in the flow channel, as the distances are known between markings on the hemacytometer. The

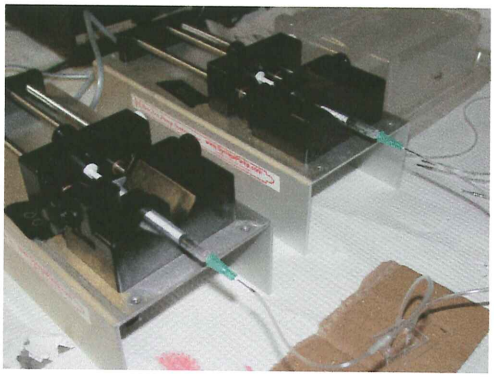


Figure 15. Testing setup :syrgine pumps connected to wafer

velocity will be tested using only infusion, only suction, and using both infusion and suction in combination.

Testing Modification

The milling of the mold for the PDMS wafer finished in time for only a couple PDMS wafers to be fabricated and for only preliminary testing to begin. The PDMS prepolymer mixture was poured into the mold and cured until it was solidified and no longer sticky to the touch. A razor blade was used to cut around the edges of the mold and release the wafer. Many attempts to seal the wafer to PMMA and to glass slides were attempted. However, when the wafer was connected to the syringe pumps and fluid infused, the fluid would leak. The PDMS would not form a seal that would resist the pressure forces from the suction and infusion from the syringe pumps. Dr. Maggie Witek in the Department of Chemistry was contacted. She suggested trying a plasma oxidizer in the Chemistry Department. The wafer and slide to be oxidized were placed in the round opening as seen in the Figure. The oxidizer, when turned on, should glow with a bright purple indicating that a strong supply of oxygen is in the chamber. The oxygen plasma would oxidize the surface of the PDMS and glass in order for them to form an irreversible bond when placed together. Catherine Situma, a graduate student in Dr. Soper's lab, demonstrated how to use the plasma oxidizer. No success was had in attempting to seal the PDMS to glass slides. When this was unsuccessful, PDMS layers

Figure 16. Plasma cleaner PDC – 32G (Harrick 2004)

were poured into 35 mm Petri dishes and cured. This PDMS layer was then oxidized with the PDMS wafer and were able to form a permanent seal.

When tested in the lab, the PDMS sealed to PDMS withstood the pressure from the syringe pumps in testing. However, after multiple trials with the syringe pumps and after multiple reinsertions of the stainless steel tubing, these wafers began to leak when infused with fluid.

In attempt to have better visualization of the microspheres flowing in the wafer, the microspheres were dyed using red food coloring. However, they still could not be viewed on the stereomicroscope.

The larger microscope connected to the computer with MetaVue was used to view the flowing beads. The syringe pumps were used to test the flow of fluid in the channel. The flow needed to be controlled by hand-pumping the syringes because when testing with the beads because the seal between the PDMS wafer and base began to degrade and leak with continuing use. Also, one the syringe pumps began to give error responses when prompted with the code line. Testing by hand was the easiest method.

Results

The milled PMMA wafers to house the optical fibers worked well. Inspected under the microscope on MetaVue, the milled lines were straight and clean. The diameters of the optical fiber channels were measured on MetaVue by setting the magnification of viewing before capturing the image. The diameter of the channel for the 9 micron optical fiber was 150 microns and of the 50 micron channels was 150 microns. These values are not quite exact but are close enough for this prototype design.

First, the flow of water dyed with red food coloring was tested in the microchannel to determine if the sealed PDMS was strong enough to withstand the pressure exerted by the syringe pumps. When only suction was applied to the sample injected into the reservoir, the fluid did not flow. This could be due to the high surface tension of the water that it resisted flowing down the channel and into the empty reservoir. Since PDMS is hydrophobic, this explains why the water would not be attracted to the walls of the microchannel and would not flow. However, Duffy (1998) found that plasma oxidization created silanol (SiOH) groups on the inside of PDMS microchannels, which helped with the electroosmotic flow of fluid. Though this application does not use electroosmotic flow, the presence of silanol groups would help make the walls of the microchannel more attractive for the water. Though this was initially thought to help with the flow, flow was not evident in testing with suction only. With infusing only, fluid did flow in the microchannel. However, the fluid began to overflow out of the reservoir where the stainless steel tube was inserted. The flow rate for the fluid flow from the syringe pump would need to be further tested to find the maximum rate that would not have any backflow. When the combination of the suction and infusion was applied, the fluid flowed smoothly through the channel. The suction helped reduce the inlet pressure and less backflow spilling around the stainless steel tube occurred. Fluid flow was consistent throughout the length of the channel.

Testing with microspheres began with a very dilute sample. In this very dilute sample, the images were captured in a "Stream Acquisition" of two beads flowing in the channel. However, the channel began to leak large amounts even when hand application

of the infusion was performed. When more concentrated samples of microspheres were tested, the channel clogged immediately. The wafer was then tested. However, the seal on this wafer was very weak and the microspheres leaked out of the reservoir. A couple microspheres did flow down the channel, and this was captured using stream acquisition. The images were compiled into a stack and made into a movie. The movie displayed the timing of the flowing spheres, 1.54 seconds. In MetaVue, calibrated calipers were used to measure the distance over which the spheres moved, 398.64 microns. The average linear velocity was calculated using this distance and the timing from the timestamps on the stack as 0.334 mm/s +/- the standard deviation of 0.348. However, this was hand-pumped flow, and therefore the flow was not consistent throughout the total pumping time. The outliers in this data are 0.0523 mm/s and 0.853 mm/s. This is relatively close to calculated linear velocity of 0.22 mm/s from the minimum flow rate from the syringe pump; however, more optimization of this value is needed.

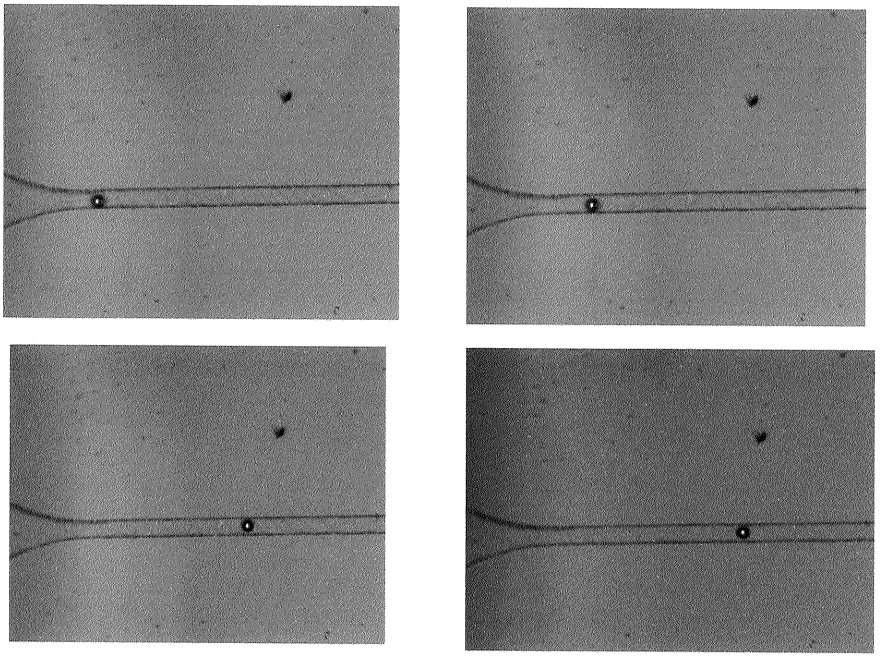


Figure 17. Progression of microspheres in flow channel

Discussion

Fabricating the base wafer for the optical fibers could be made more accurate by methods other than milling. In the scope of this project, micromilling was least expensive and fastest method. Other methods include solid-object printing and lithography (McDonald 2002). This technique involves uploading the design CAD file to the printer, and the printer will layer a thermoplastic material until the design in the CAD drawing has been formed. Lithography entails using a printer to produce a high-resolution transparency of a CAD file. The transparency is placed over a photoresist and irradiated with UV light to produce a relief of the transparency. The polymer can then poured over the resist to form the desired image (McDonald 2002). These alternate methods would produce the base wafer with higher accuracy of the channels.

Lithography would also not produce any mill marks on the PMMA wafer used for the mold for the PDMS. This would improve the quality of the PDMS wafer and could aid in producing a stronger, longer-lasting bond between the PDMS wafer and base layer. Also, alternate methods need to be investigated for sealing the PDMS to glass.

To avoid the microchannel clogging, the filleting from the reservoir into the channel could occur at a less steep incline. Also, the channel width could be increased to twice the diameter of the microspheres. This would allow for reduced pressure inside the channel. Also due to the pressure gradient from the velocity profile in the channel around the microsphere, beads would remain behind one another and would not flow through the channel side-by-side.

In the fluidics testing, error in measuring the velocity could occur from human use. The pictures acquired from MetaVue had to be taken by manually pressing the mouse on the "Acquire" button, so there is some lag time in taking the pictures relative to the flow of the cells. Also, the visual assessment of the velocity relies on human use to press the start and stop buttons on the timer, which could not always be exact to the moment when the microsphere passes the markings on the hemacytometer. Viewing when the microsphere passes also contains error, as timing for different tests could start or stop when the microsphere is in different distances on the hemacytometer.

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