

Fabrication and Function of Microfluidic Devices for
Monitoring of In-Vitro Fertilization Processes

By

Jin Xu

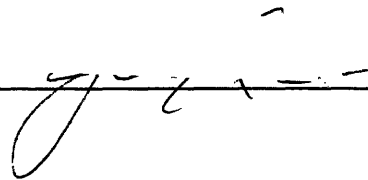
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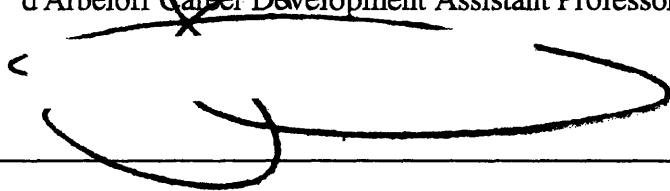
Department of Mechanical Engineering
May 11, 2007

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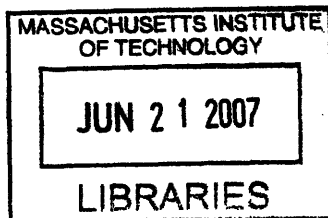
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Fabrication and Function of Microfluidic Devices for
Monitoring of the In-Vitro Fertilization Process

By

Jin Xu

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Degree of Bachelor of Science in Mechanical Engineering

ABSTRACT

The process of assistive reproduction is often a headache and heartache for those who choose to go through it. The field currently relies heavily on morphological characteristics to determine embryo health and development success, a highly unreliable method. While they appear healthy at implantation, many embryos, in reality, have poor development potential and fail to survive within the womb. Therefore, to offset the high chances of miscarriage, multiple eggs are implanted in the uterus. This has occasionally lead to multi-fetal pregnancies, which have a higher maternal mortality risk, and, in general, is more physically demanding.

This thesis researches a microfluidic device that aids in the crucial stages of in-vitro-fertilization. The device allows for a fertilized egg to be cultured within, and provides the ability to carefully monitor its health through a series of metabolic assays, a better indication of embryo health.

This microfluidic embryo health monitoring device is comprised of two layers of channel networks. It works through passing fluids along flow channels that are driven by control channels. The control layer, when pressurized with gas, operates as valves and peristaltic pumps along the flow layer to pump and transport fluids through the flow channels. As embryonic fluids are passed through the channels, the status of the fertilized egg can be monitored with metabolic assays taken of the embryo at various detection sites.

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1. Introduction

The recent evolution of microfluidics since the early 1990s has led to numerous application developments since. Microfluidics aims to deal with the precise control and manipulation of nanoliter and microliters of fluid. At such small scales in which fluid channel diameters measure on the order of μm , fluid dynamics dictate that flow is always conveniently laminar due to the low Reynolds number. In addition, fluid mixing is dictated by diffusion alone and turbulent effects are non-existent. While branches of this technology range from micro-propulsion to micro-thermal technologies, one of the most exciting is the so-called “lab on a chip” on which one chip performs various analyses of chemical or biological interactions.

One such application involving molecular biological fluids, and the topic of this discussion, is a microfluidic chip monitoring device for in-vitro fertilization. The impetus behind this device comes from a proposal by Mark J. and David Gardner from the Colorado Center for Reproductive Medicine. Their proposition is a new microfluidic system for analyzing and culturing eggs and embryos.

According to Mark and David, in the field of assisted reproduction, the ability to precisely identify embryos with good development potential from embryos with poor development potential is currently somewhat lacking. Morphologic characteristics, a current method of detection, are poor identification factors. An accurate and powerful ability to identify embryos with good development potential will allow a greater success rate of in-vitro fertilization, and reduce the possibility of multi-fetal pregnancies, as fewer embryos are implanted to ensure possibility of success.

David describes that “over the last 2 decades, a major focus of their laboratory has been on understanding the normal physiology of the egg and embryo, with an emphasis on metabolism, and more recently, gene and protein expression.” Studies through their laboratory have identified several key metabolites and proteins that are good candidates to indicate the health status of eggs or embryo when cultured [1].

Current sampling and analysis at their lab is done manually; it is labor intensive and extremely time intensive if each embryo must be measured at multiple points during development and hundreds of embryos are to be monitored. Manual sampling is also prone to technical problems that can greatly increase the amount of time needed to test each embryo. Such technical challenges are burdensome to the current embryo analysis process. The ultimate goal is to automate the identification and measurement process without affecting or harming the egg or embryo, so that more rigorous testing and monitoring of the health status of the embryo can be achieved

Under this proposal, there are currently two microfluidic chip devices under development: a device for metabolite detection, and a cell trapping and culturing device. The two devices are parts of the same process, the ultimate goal being one large device that houses two processes on the same chip. These two device schematics are shown below in figure 1.

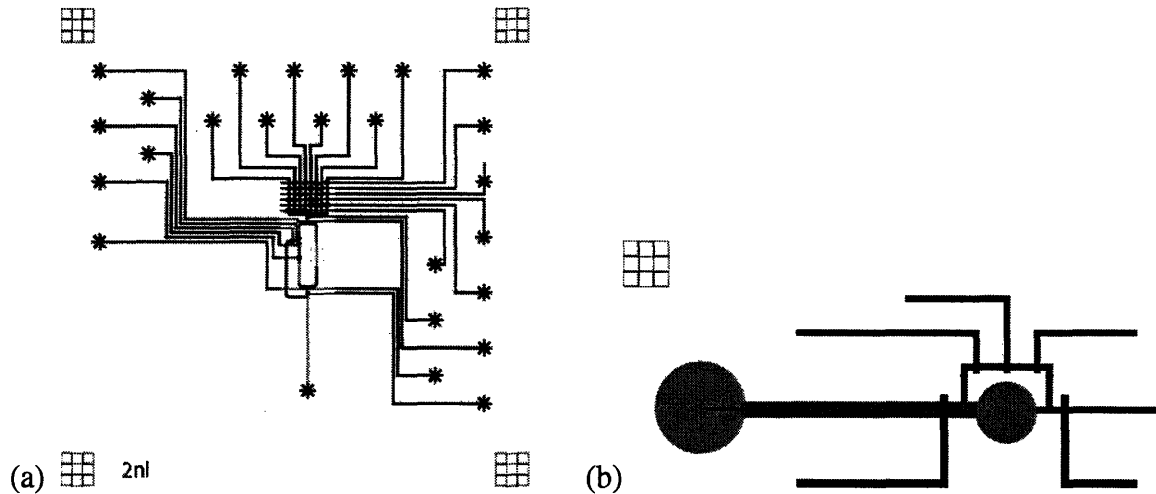


Figure 1 Chip configurations for the two part microfluidic device (a) Metabolite detection device (b) Cell trapping and culturing device.

Figure 1(a) illustrates a metabolite detection device that will enable cell samples to be measured. Cell culture media will be injected into this device and mixed with cocktail solutions. Through metabolic assays, concentration measurements will be obtained automatically and the health of the embryo can be determined. Before each measurement, fluorescence must be calibrated to ensure accuracy.

The cell trapping and culturing device shown in figure 1(b) is the chamber in which actual embryonic cells are stored. Various fluidic media may be agitated or recirculated over these embryonic cells, and the resulting samples may be collected for measurement in the metabolite detection device previously mentioned.

While the current implementation of this microfluidic monitoring device consists of two separate chips, the ultimate goal will be to combine these two building blocks into one single storage and monitoring chip.

Fabrication of both metabolite detection and cell trapping chips follows approximately the same method. They are comprised of two layers of multi-channeled

Polydimethylsiloxane (PDMS). The multi-channelled control layer acts as a peristaltic pump to distribute fluid along overlying flow layer channels. Through the pumping and mixing various cocktail and samples, this microfluidic device allows for the monitoring of fertilized egg using various metabolic assays that indicate embryo health.

Such channels are created via soft-lithography over mid-grade silicon wafers. The process involves photo-lithography deposit of the channel schematics onto the silicon surface. Polydimethylsiloxane (PDMS) is subsequently poured onto silicon channel molds and cured. Halfway through the curing process, flow and control layers are superimposed and re-baked, allowing them to finish curing as the two layers become fused.

In operation, the control layer operates valves in the flow layer to pump and transport fluids in the flow channel. When pressurized, the lower channels deflect the PDMS surface enough that it blocks the channel passage in the control layer. Through use of a mixer apparatus within the flow channel, the status of the fertilized egg can be monitored with metabolic assays taken of the embryo.

Ultimately, the entire process will be automated through the use of a computer program that is comprised of various functions to inject, mix, and measure the key proteins and metabolites within excreted cellular fluid.

2. Device Fabrication through Soft-Lithography

Manufacture of microfluidic devices is a variable process that involves the bonding of two Polydimethylsiloxane layers that have been poured over a silicon wafer mold and partially cured. Once the two layers are attached, the device is hole-punched and fully cured before it is ready for operation.

2.1 Channel Schematics

The cell culturing and monitoring devices each consists of two channel layers: a flow layer and a control layer. These channel paths within the chip are initially designed through an image manipulation computer program, then transferred onto a photolithography template. The flow layer of the monitoring device consists of narrow channels holding fluids essential to the monitoring and health upkeep of fertilized eggs, while the bottom control layer also consists of narrow channels, but instead acting as a pump and valve system to transport fluid along the upper channels. Figure 2 shows the schematics of both fluid flow and pumping systems on the metabolic detection device, while figure 3 details the same schematics on the cell trapping and culturing device. To ensure alignment accuracy, crosshairs are included in the schematic on both layers.

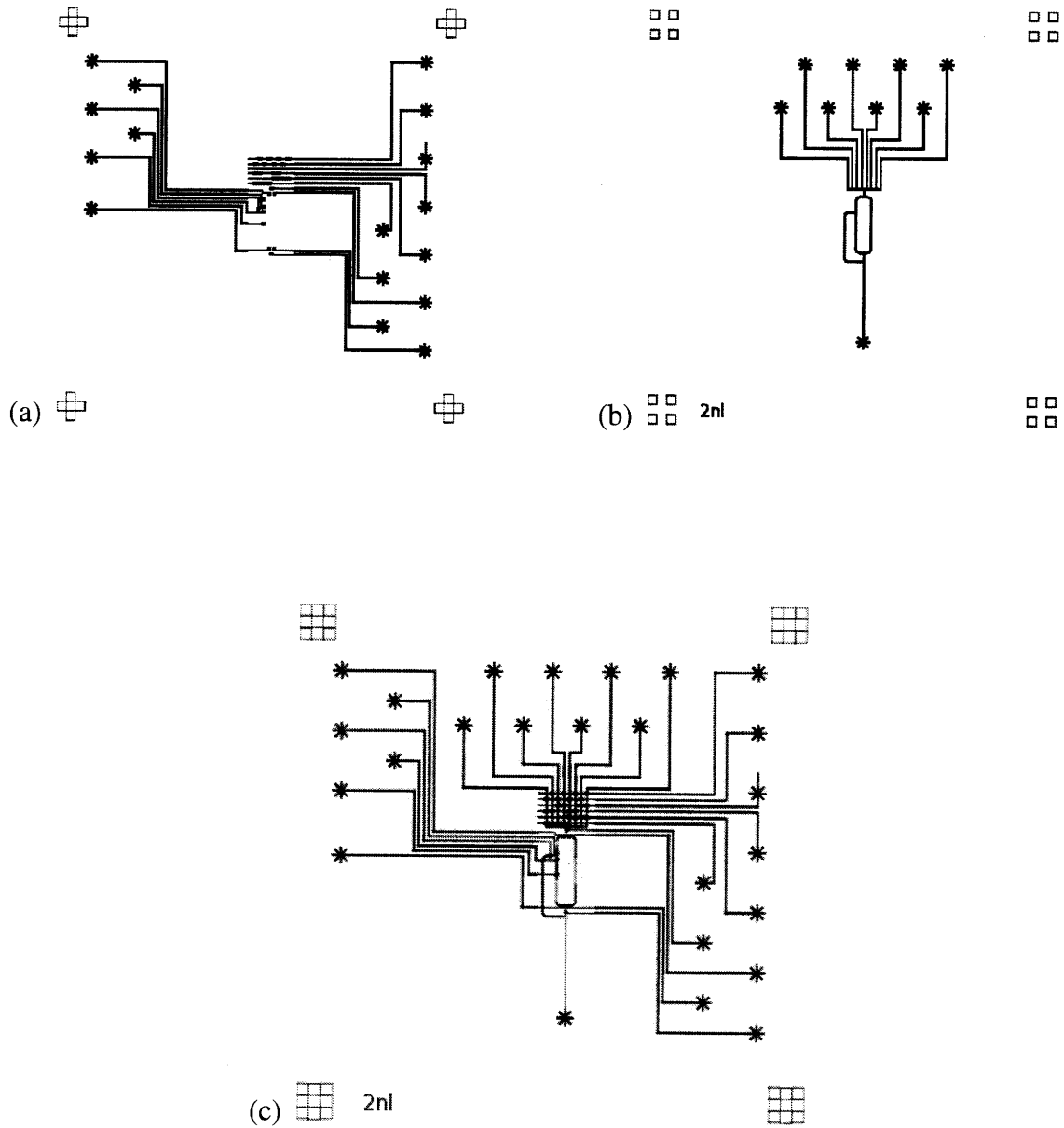


Figure 2 Metabolic detection device (a) control layer channels for fluid control, (b) flow layer channels for fluid transportation, (c) superimposed control and flow channels.

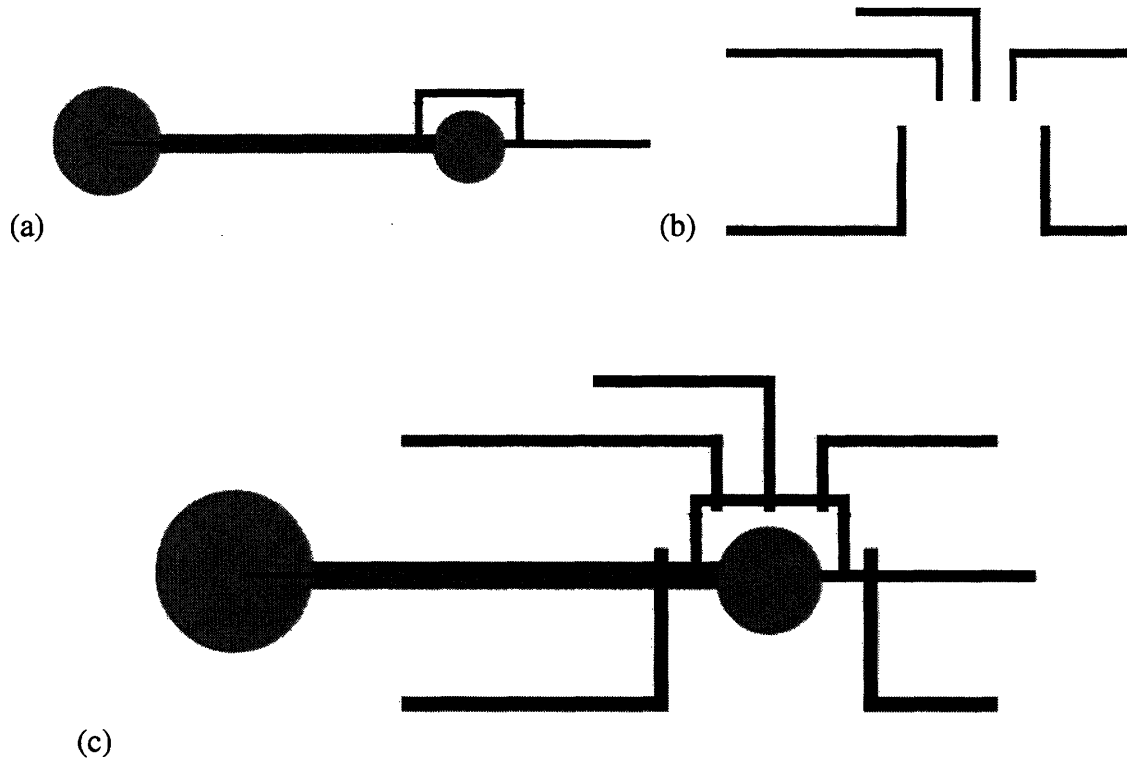


Figure 3 Cellular holding device (a) Flow channels including incubation chamber and embryo loading site (b) Control channels consisting of peristaltic pumps and valves (c) superimposed control and flow channels.

2.2 Photolithography of Silicon Wafer Surface

Channel molds are created on the surface of mid grade silicon through photolithography, a process deviating little from the general photolithography practice.

A recommended process of silicon preparation prior to the application of photoresist is the application of an adhesion promoter such as hexamethyldisilazane (HMDS) to the silicon surface. Once the surface is prepared, a spin coat of photoresist can applied to the silicon.

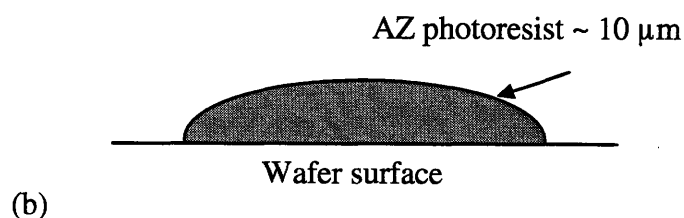
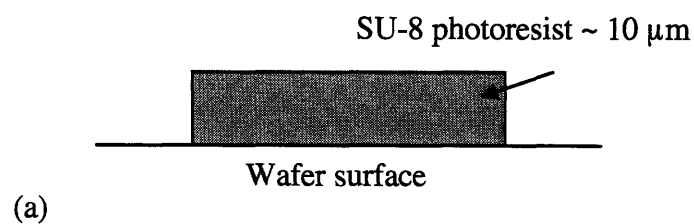
The photoresist process differs slightly depending on whether a control layer is manufactured, if a detection flow layer is manufactured, or if a culturing flow layer is manufactured.

For control channels, a single layer of SU-8 photoresist is applied to the surface. This will create a single channel molds with a rectangular shape, as shown in figure 4(a).

For fluid flow channels in the metabolic detection device, a single layer of a softer AZ photoresist is applied, resulting in a rounded shape when complete. This is seen in figure 4(b).

The cell culturing flow channels require two layers of photoresist to be applied to the wafer surface. Because a tall rounded channel form is desired, a strong base photoresist is first applied, developed, and then subsequently covered with a softer photoresist layer on top. Figure 4(c) illustrates the result of dual photo-resist layers. The base photoresist used is SU-8, a strong, negative, epoxy based, near-UV (365 nm) photoresist, while the material on the top layer is AZ photoresist, a positive resist that slightly melts when soft-baked, forming the rounded channel shape [2].

Specifically for the cell trapping and culturing device, a base SU-8 photoresist is applied to a thickness of 100 μm and pre-baked to discharges excess solvent.



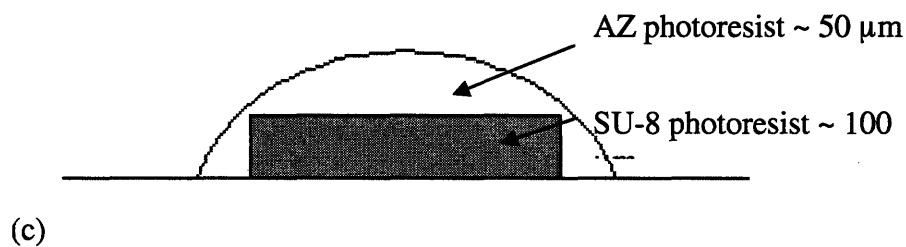


Figure 4 Photoresist applications (a) Single layer SU-8 application for control channels, (b) Single layer AZ application for flow channels of metabolic detection device, (c) Double layer application for fluid flow channel mold in cell culturing device.

Following the coating process, the desired channel schematic is recreated on a high resolution transparency mask and applied over the photoresist. The transparency mask and photoresist combination is then exposed to ultraviolet light and undergoes a post exposure bake at a typical temperature of 80° C for 20 minutes. After immersing the photoresist in a developer named SU-8 developer, the wafer is hard-baked to solidify the remaining photoresist. The same process is repeated with the second resist application, this time using AZ photoresist at a thickness of 50 μm .

2.3 Manufacture of Polydimethylsiloxane (PDMS) Layers

To fabricate the Polydimethylsiloxane (PDMS) layers, liquid silicone elastomer is used at two different concentrations for the flow layer and the control layer. The specific elastomer brand is Dow Corning's *Sylgard 184 Silicone Elastomer Kit*. The kit contains two separate liquids: a base labeled A and a hardener labeled B that are mixed at different concentrations to achieve the desired PDMS layer properties. The relatively flexible flow layer requires a ratio of 5:1, material A to material B. The more rigid control layer along the bottom of the device requires a ratio of 20:1, material A to material B [2].

After the PDMS is thoroughly mixed, it is cast over the channeled silicon wafer surface. The flow and control layers require different thicknesses of PDMS. The control layer is optimal at an exact thickness of 20 μm , while the flow layer is effective at a thickness of approximately five mm. To achieve an even coating of the control layer PDMS on the silicon, it is spun coated at a rate of 3000 revolutions per minute, for one minute. The flow layer PDMS is simply poured over the silicon wafer to the approximate thickness. A vacuum is applied to the combined flow layer PDMS and wafer for approximately three minutes to ensure removal of trapped air bubbles within the material.

In the curing process, a careful timing procedure is followed. The flow layer is partially cured at 80 degrees C for 15 minutes. Eight minutes after the flow layer was set to cure, the control layer is subsequently placed in the oven at the same temperature to partially cure for 13 minutes. At the end of the first 15 minute partial cure, the flow layer is carefully extracted from its silicon base using a sharp razor. At this point the flow layer PDMS has achieved a solid, yet still highly adhesive and flexible structure. Quickly using a needle punch, interconnecting holes are punched from the top surface to the flow channels, finishing just as the 13 minute control layer partial cure is completed.

After removal of the control layer PDMS, the previously extracted flow layers are carefully superimposed onto the control layers, using the crosshairs for alignment and ensuring that no foreign air or substance is trapped between the layers. The combined flow and control layers are returned to the oven and baked for about two hours until completely cured.

The entire PDMS layer fabrication process is illustrated in figure 5.

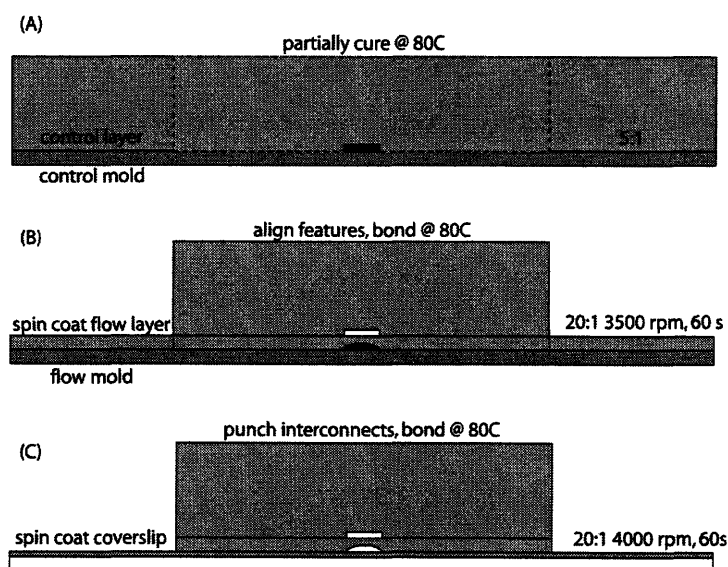


Figure 5 Summary of PDMS layer fabrication. a) PDMS is poured and over the control mold, b) Alignment of extracted control layer PDMS over spin-coated flow layer, c) Vertical interconnecting holes are punched out, and the entire device is allowed to bond.

2.4 Bottom Plasma Bonded Glass Surface Support

In practical operation and testing, the microfluidic device functions better and is more stable when permanently applied to a support surface. Thin glass microscopic slides serve this purpose when plasma bonded to the bottom surface of the microfluidic device. However, it is necessary to clean the surface carefully and place the chip on the slide such that foreign substances are not trapped within.

3. Metabolite Detection Device Operation

3.1 Channel Differentiation

Referring back to figure 2(a), there are a total of 16 control layer channels that serve as peristaltic pumps for the above flow channels. Notice that this layer of channels lies along the bottom glass surface of the microfluidic chip device.

The flow layers channels in the microfluidic device can be further differentiated into multiple purposes. A total of nine flow channels are present, with eight ports reserved for cocktail or sample fluids and one waste port. These input may used for multiple assays against various cocktail solutions, or used to generate several standard curves against one cocktail solution.

3.2 Valve Operation

The microfluidic device operates by pressurizing bottom control channels to obstruct the upper flow channels. As shown in figure 6 below, the un-pressurized control channel state allows fluid to flow freely through the upper channel. When a control channel is pressurized to about five psi, the PDMS membrane comprising the control channel roof deflects upwards into the rounded flow layer channel. Essentially, the lower membrane acts as a valve on the flow layer channels, blocking flow or functioning as a peristaltic pump in the upper channel when used with a series of other control valves.

The typical flow channel depth is between 10 – 50 μm deep.

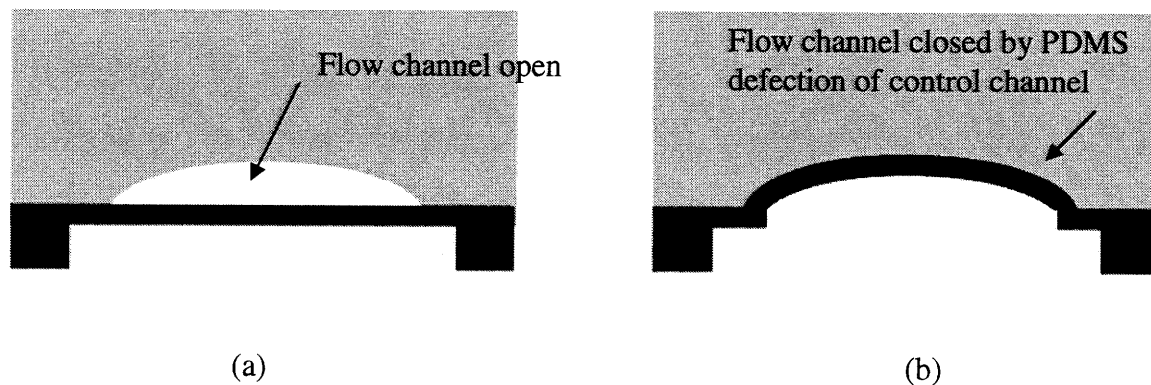


Figure 6 Valve operation schematic (a) Valve off position; the lower control channel is un-pressurized, (b) Valve on position; the lower control channel is pressurized and the PDMS material stretches to seal off the upper flow layer.

3.3 Metabolite Device Mixing Ring

Figure 7 details the mixer component of the flow channel layer. Mixing and analysis occurs within this ring component. The mixing ring is driven by seven peristaltic pumps around the perimeter, and access into the ring is controlled by three valves, one covering each of the connecting inputs/outputs. When the three valves close off all outlets to the mixing ring, two fluids may be mixed within the ring as the seven peristaltic pumps around the ring function in succession.

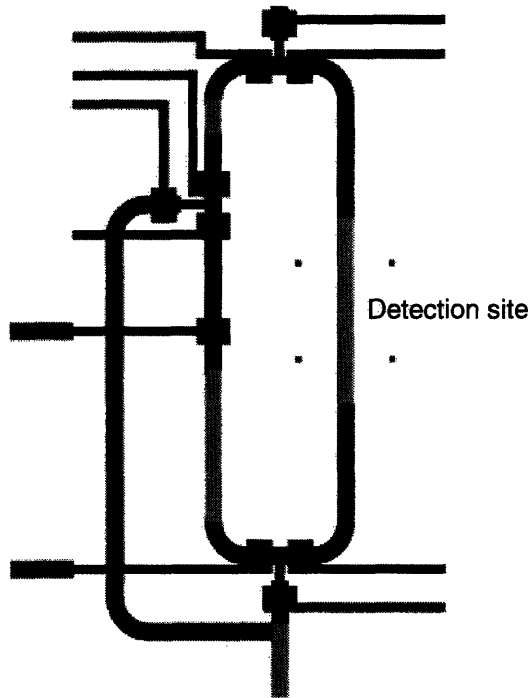


Figure 7 Mixer apparatus within the flow channel layer.

3.4 Metering and Measurement Strategy

3.4.1 Device Calibration

Device calibration is performed by generating calibration curves. Prior to any sample analysis, the mixing ring must first be completely loaded with cocktail, and the initial background fluorescence measurement must be taken. Calibration curves are then generated by diluting standard fluids stored in the 1:1 mixing ring, with the cocktail in a 1:10 ratio. Sample calibration curves involving NADH are available in the appendix [3].

The device performs automatic calibrations to generate standard concentration curves for each metabolite. A stock solution containing 0.1 mM each of Glucose, Pyruvate, and Lactate is mixed, in the 1:1 mixing ring, with Phosphate Buffer Solution (PBS) to generate a range of samples between 0 mM and 0.1 mM. Following its creation,

the calibration sample is stored within the top left corner of the mixing ring as excess fluids are purged out of the exit at the bottom of the mixing ring, and time the detection chamber is washed clean with PBS and emptied using an air purge.

Fresh cocktail is then directed to the remainder of the mixing ring, as excess cocktail flows out the side port. The cocktail and sample are thoroughly mixed, using peristaltic pumping, for an approximate three minutes. The cocktail and sample are subsequently propelled into the detection chamber, in which the measurement should be performed immediately, and identification is assigned to the measurement. After measurement, the channels are cleansed with PBS and the next measurement is performed. The resulting measurement and concentration data is collected and used to create calibration charts [3].

3.4.2 Sample Analysis

An unknown sample is similarly analyzed by injecting it into the mixer by displacement through the valves, and diluting it in a 1:10 ratio with the cocktail in the mixing ring. The fluids are thoroughly mixed through the use of peristaltic pumps, which distribute the fluids around the ring for 5 – 10 seconds, and the sample is allowed to incubate for several minutes. Once the sample is locked down at the detection site with a set of valves, a fluorescent measurement of the combined mixture is then taken, and recorded under a DAPI filter set. A simplified diagram of the measurement process is found below in figure 8.

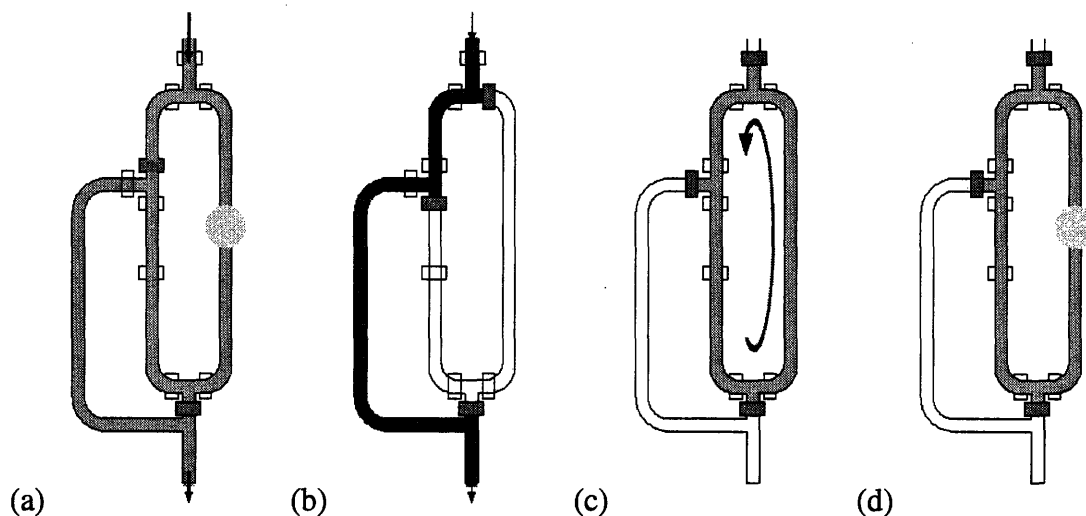


Figure 8 Sample measurement process (a) Load cocktail, take initial background fluorescence measurement, (b) Inject 1/10 volume sample into mixer by displacement, (c) Mix contents of ring using peristaltic pumps (d) take sample measurement.

The detection site is a chamber with significantly deeper channels (100 μm) and a rectangular cross section. The presence of such a chamber allows for a fluorescent signal sufficient enough to be measured. During a measurement operation, the detection site must remain stationary under a microscope, with the objective focused upon it.

After measurement, the diluted test sample is pumped from the mixing ring and the channels are cleaned before the next analysis occurs.

3.4.3 Culture Media Fluid Analysis

In the media fluid measurement process, a modified loading step is performed so that sample waste is reduced. The sample metering part of the mixing ring must first be cleansed with PBS and an air purge. The subsequent metering and mixing with appropriate cocktail is the same as previously described. Measurement on culture media samples should be performed multiple times for each of the three metabolites.

3.5 Practical Microfluidic Device Operation

As seen in the figure below. Reservoirs of sample are connected to the flow layer of the microfluidic device via tygon tubing and pins. Gas reservoirs, pressurized between one and five psi, are connected to the control layers of the microfluidic device. Computer controlled micro-solenoids trigger valves on the reservoir, allowing the compressed air to travel through the control layer, effectively pumping fluids in the flow layer [2].

The device can be placed underneath a microscope for visual analysis of fluid flow. The picture below shows a microfluidic chip in action as fluorescent beads are pumped into the chip.



Figure 9 A single microfluidic device with inputs and outputs attached.

3.5 Proposed Software Commands and General User Interface Controls

Full automation of the microfluidic device can be achieved with the creation of software that controls all aspects of the device. Several possible functions are suggested

as subroutines of the software [4]; these functions are not exhaustive and alternatives are always possible.

- **InputToMixer(left/right)** - purges mixer ring, through the bottom waste channel, for a given amount of time.
- **CocktailToMix** - for Glucose, Pyruvate, and Lactate cocktail, this function locks a sample in the top left area of the mixing ring and fills the rest of the ring with cocktail, allowing the excess to flow through to the side waste channel.
- **Mix** - mixes contents of ring with peristaltic pumps.
- **ChamberWash** – initiates PBS rinse and air purge of the bottom channel and detection bin, through the right side of the mixer.
- **Measure** - sends fluid contents of mixer to an empty detection chamber, using an air pump. The valve at the end of the detection chamber is closed, and as the sample moves into place, the valve at the top of the detection chamber also closes.
- **SampleLoad** – because the volumes of real fluid sample are limited, this function closes the valve at end of sample section of the mixing ring, and delivers a slug of air at the opposite end when metering culture samples such that the pressurized sample does not accidentally shoot into the waste. This function should only be used following a ChamberWash operation.
- **OpenAll** – opens all valves in the mixer apparatus.
- **CloseAll** – closes all valves in the mixer apparatus.

4. Cellular Holding Device Operation

4.1 Channel Differentiation and Function

Figure 10 shows the two channel layers of this device. In this case, the flow channels are much wider and deeper than their counterparts in the metabolite device. Practically, because this device is the site of embryo culture and incubation, the channels must be wide enough to accommodate a typical fertilized egg (~ 150 μm). The flow layer consists of an incubation chamber connected to a loading entrance through a wide channel, and two thin cocktail or sample flow lines. The control layer consists of five channels that are of the typical thin and narrow type. As seen in the figure, two valves located at the left and right of the culturing chamber open and close the chamber as needed. Three other control channels along a bypass around the culturing chamber are designated as peristaltic pumps. When the chamber is closed these pumps serve to circulate fluid around the chamber. Alternatively, when the left and right chamber valves are open, the peristaltic pumps allow fluids and eggs to be moved into or out of the cell culturing chamber.

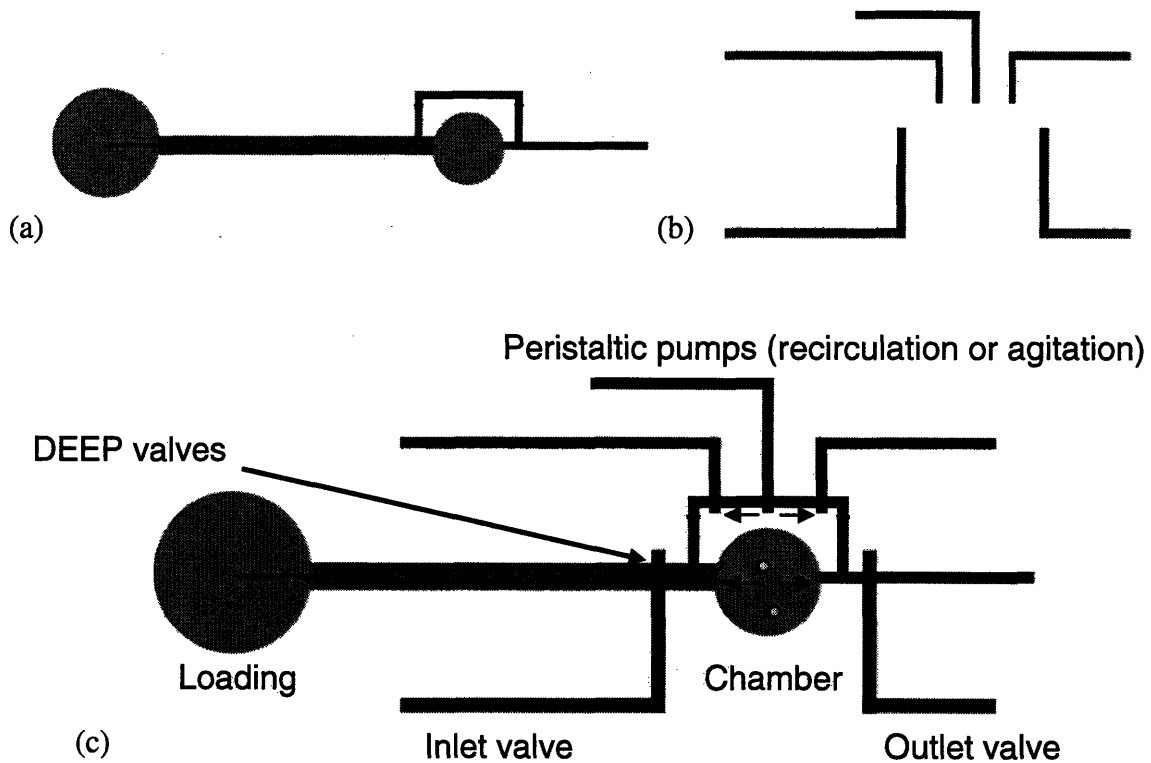


Figure 10 Cellular Holding Device a) Flow channels including incubation chamber and embryo loading site, b) Control channels consisting of peristaltic pumps and valves, c) combined device.

Ideally, the culture chamber is integrated with a cell trap, such that the cell does not travel beyond the desired area and traps itself in the outlet valve. The cell is loaded and unloaded via the large opening on the left, and travels into the culturing chamber via main inlet channel; the process is powered by the pumping network.

To accommodate different culturing needs, four chamber volumes are created in this design: $.5 \mu\text{l}$, $1 \mu\text{l}$, $2.5 \mu\text{l}$, and $5 \mu\text{l}$. The large geometry of the loading channels as well as the chamber volume is not necessarily optimal for all chamber volumes. An experimental report in the following pages will discuss the effectiveness and practicality of these different chamber volumes, in relation to valve ability and function.

5. Utility for Monitoring In-Vitro Fertilization

5.1 Automation of a Difficult and Time Consuming Process

The need to create an automated system arises from the desire to develop a repeatable and explicit format for embryo and egg analysis. Currently, most labs that focus on reproductive medicine use visual assessment under a microscope to speculate the health of an embryo. While there is massive documentation regarding visual deformations that correspond to certain abnormalities, the reverse does not hold true. For example: asymmetric cell divisions, cell fragmentation, and dark cells are certain indicators of poor cell health. However, a cell without any of these visual characteristics is not necessarily guaranteed healthy [1]. It is well accepted within the assisted reproductive field that morphologic characteristics are not a good indicators of cell health.

David Gardner and his lab for reproductive medicine have identified several key metabolites and proteins that are secreted into or consumed from the culture media which are good indicators of embryonic cell health. Measurements of such key metabolites are labor and time intensive. Currently, sampling and analysis is done manually. One single analysis takes 10 minutes, more if technical problems occur. To monitor a single embryo for various different metabolites at frequent periods in the development stage is daunting operation. In order to perform more rigorous analysis of embryos, an automated device would be ideal. In addition to providing more accurate test results, a microfluidic device will allow for the development of a set of standardized clinical tests that may be used on embryos.

The first step, the current version of this microfluidic device under development, allows for 3 different metabolites from 8 samples to be assayed automatically. Details of these metabolic assays are discussed in the following section.

5.2 Metabolic Assays Indicate Embryo Health

Non-invasive assays, as proposed by the *Colorado Center for Reproductive Medicine*, can only measure the rate of nutrient consumption and metabolite release. For example, to determine the activity of glycolysis in a cell, one would quantitate glucose consumption together with lactate production. The proposed procedures for metabolic assays within the microfluidic device are as follows.

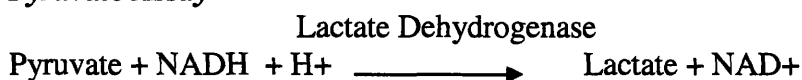
For each assay, cocktail solutions containing all the elements needed for the reaction (a buffer, co-factors, and enzymes) are prepared. First, the background fluorescence of the cocktail is typically measured when the pyridine nucleotides in the cocktail is exposed to a UV light source. Only a small amount of cocktail (1-20 nl) is required, the exact volume depending on the substrate to be analyzed and the size of the embryonic cell in question. A UV routine exposure lasts for up to 0.5 seconds.

Following the initial determination of background fluorescence, a small sample (1-5 nl, depending on the substrate) of fluid from the embryonic culture media is added to the reagent cocktail drop. The reaction is initiated when a substrate is added to the mix, and left until the reaction is fully completed. This can take between 3 minutes to 1 hour, depending on the substrate. It is worthwhile to take readings at set intervals so that one is aware of when the reaction has completed.

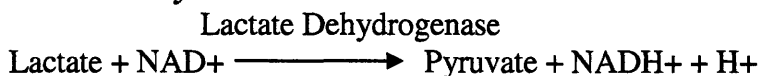
After reaction completion, the fluorescence of the drops is again determined. The amount of fluorescence in the reagent cocktail and its corresponding concentration should have a linear relationship with the before and after addition of the sample values. A new set of standards should be run on each day of an experiment to determine whether the fluorescence range is linear within the concentration of the substrate. The linear regression value should typically exceed .99.

Once a linear standard curve has been achieved for the day, the concentration of substrates from samples can be calculated from this standard. The reactions and assay conditions for four substances pyruvate, lactate, glucose, and glutamine are detailed as follows [1]:

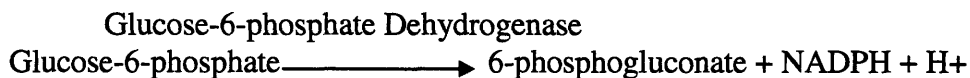
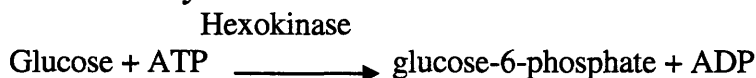
Pyruvate Assay



Lactate Assay

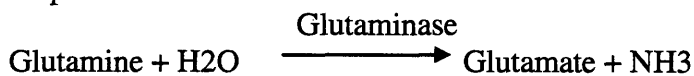


Glucose Assay

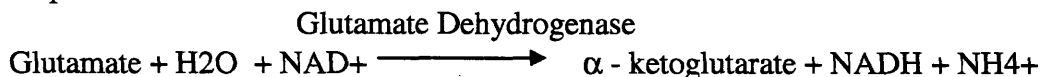


Glutamine Assay

Step 1.



Step 2.



6. Experimental Investigations

6.1 Analysis of Flow and Valve Leakage

The use of fluorescent beads allows analysis of fluid movement across flow channels. This is a method of testing the effectiveness of the control valves, and also determining the flow characteristics in the fluid.

As shown in the figures 11 and 12 below, a partial valve closure is a recurring problem within deeper flow channels. This occurs when the PDMS does not have enough flexibility under the given gas pressure to contour around the flow channel shape, resulting in flow leakage around the valve.

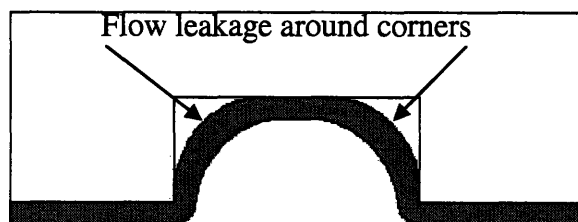


Figure 11 Control layer PDMS is unable to deflect the necessary amount to completely seal off upper flow channel.

While this issue has largely been solved in the metabolic detection device through the use of rounded fluid flow channels; it is a much harder issue in the cell culturing device because of the sheer volume in the cell culturing chamber and the wider channels that are used to transport embryonic cells.



Figure 12 Flow leakage around corners of the valve as shown in a fluorescence photograph.

6.2 Analysis of resulting channel shape as a result of photolithography with SU-8 and AZ

As previously mentioned in section 1, the cell trapping and culturing devices are created using a double photolithography process designed to produce molded channels that have gently rounded corners.

Because the culturing device was designed with four different volume capacities, it was necessary to look at all of them. In addition to volume capacities, two different control channel widths were also considered in this design: 1000 μm and 750 μm .

Cross-sectional photographs were taken of each chip size at three different positions, the entrance inlet, the middle culturing chamber, and the exit outlet. Figure 13 shows the cross section at each of the three locations in the 1000 μm channel device for each of the four volume capacities, while figure 14 shows the cross section at each of the three locations in the 750 μm channel device for each of the four volume capacities.

Examining the various inlet photographs in which both SU-8 and AZ photoresists were used, and comparing them to the mid-section photographs in which only SU-8 photoresist is applied, the effect of the softer resist on top is seen.

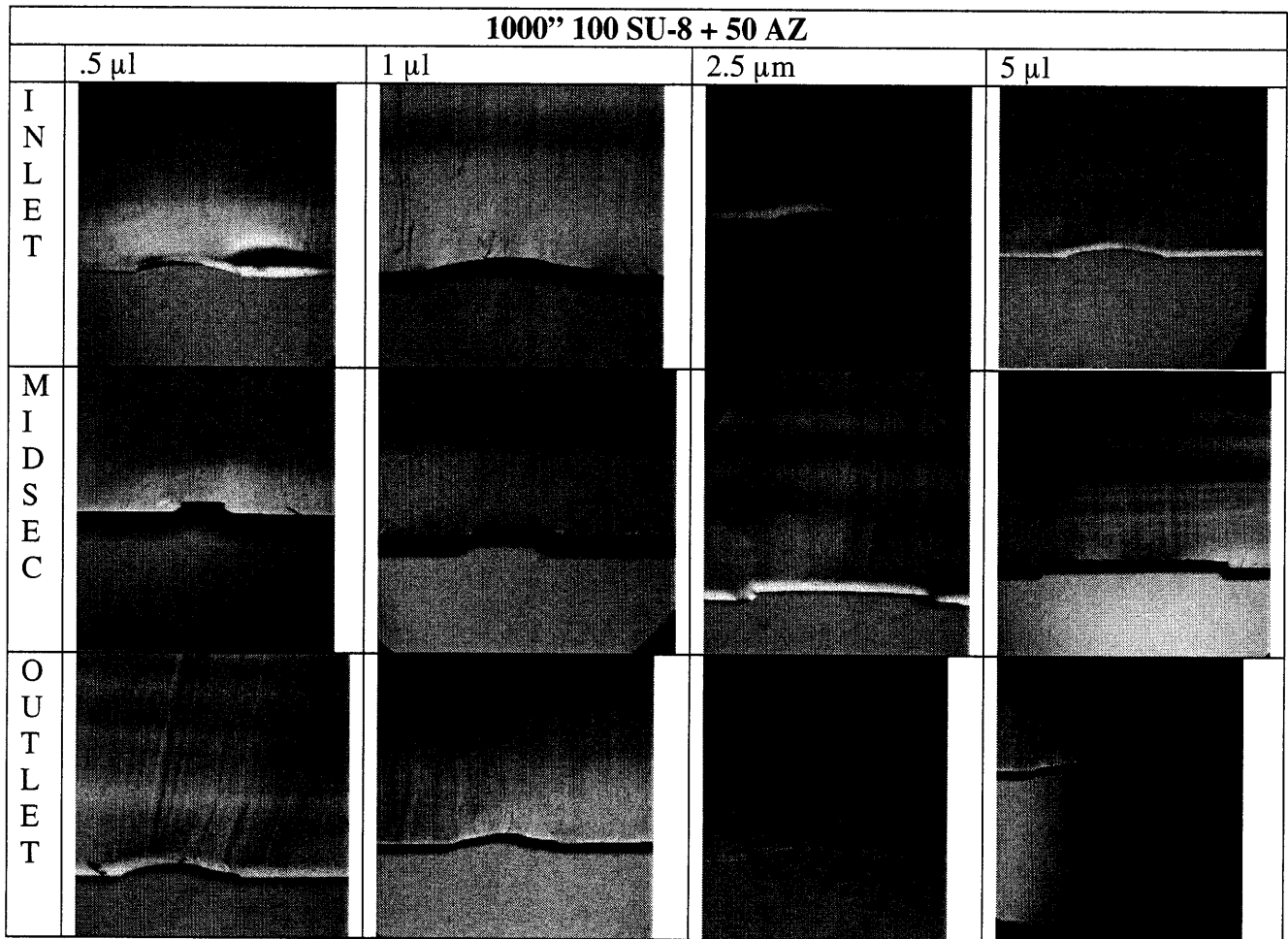


Figure 13 PDMS cross sections across the inlet, the middle chamber, and the outlet of the cell trapping culturing device at different device capacity volumes. Control channels of this device are 1000 μ m wide.

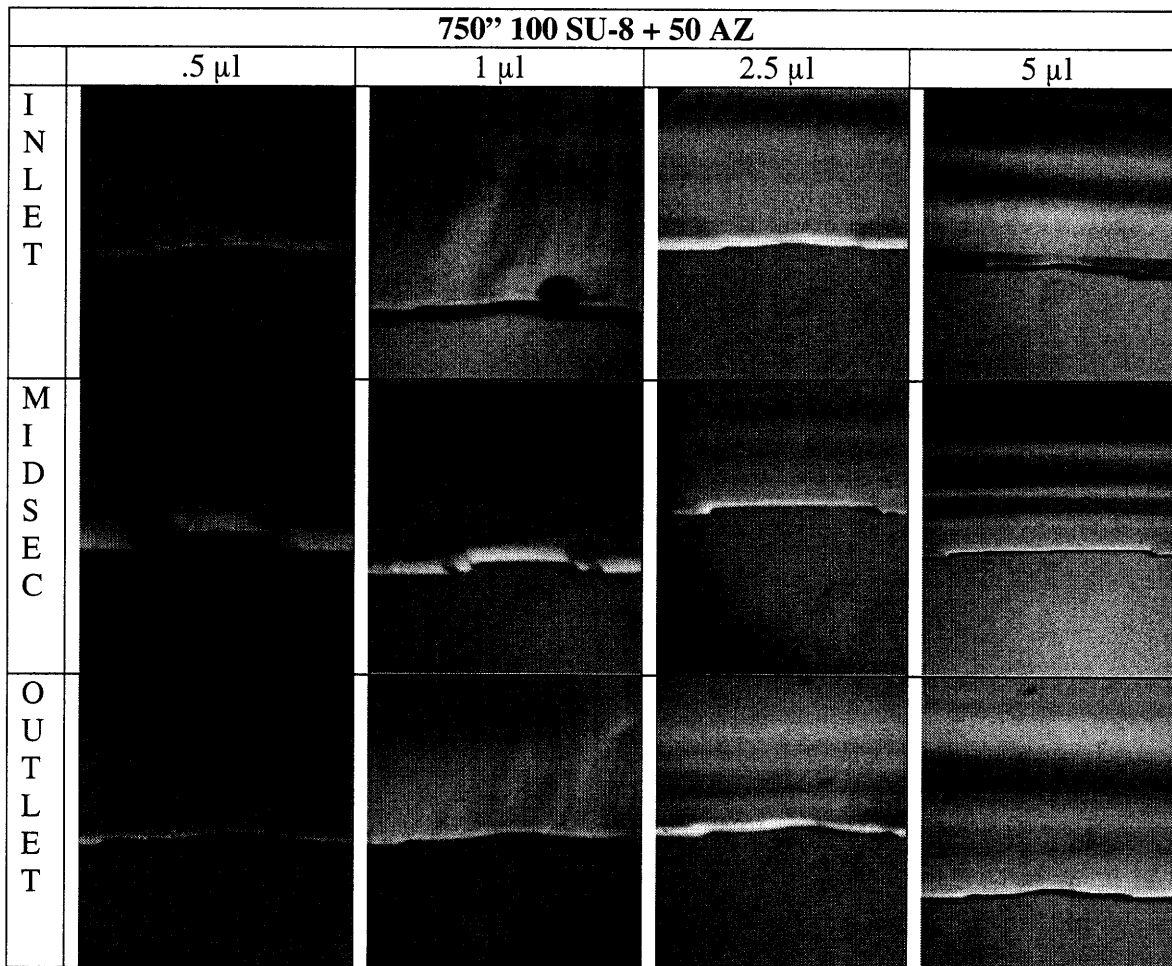


Figure 14 PDMS cross sections across the inlet, the middle chamber, and the outlet of the cell trapping culturing device at different device capacity volumes. Control channels of this device are 750 μ m wide.

Unfortunately, the result does not quite reflect the desired rounded shape. Rather, there are two shallow slopes at the sides, and one deeper hump in the middle. While theoretically, this shape should not have any adverse effect on valve closure, it is not practical to transport cells through channels of this shape. Because of the sheer size of a human embryo (at least 100 μ m), the egg will only fit through the deep indentation in the middle section of the channel.

It is suspected that the unusual shape of the channels is a result of over-heating of the AZ photoresist after the resist has been developed. The material achieved a low enough viscosity that the rounded form collapsed.

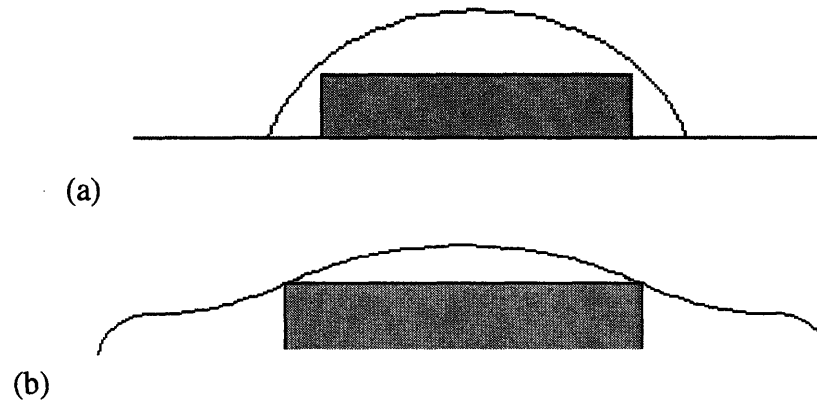


Figure 15 (a) Desired channel shape as a result of applying AZ photoresist, (b) Actual channel shape resulting from the suspected over-baking of AZ photoresist.

A recommendation for future fabrication would be to hard-bake the AZ photoresist at a lower temperature and shorter length of time. Given a higher viscosity, the AZ photoresist theoretically should not flatten across the SU-8 and silicon surface as much.

6.3 Proposition to Measure Ultraviolet Radiation Transfer Across PDMS Membranes

The current manifestation of the all-encompassing embryonic monitoring chip is comprised of two separate devices. Because the metabolite detection process requires exposure to an ultraviolet light source, the effects of which are possibly harmful to embryonic cells and other biological material, and because the propagation properties of ultraviolet radiation through PDMS material are still unknown, the choice is made to separate the metabolite detection device from the cell trapping/culturing device.

However, to reach the ultimate goal of one all-encompassing device for fertilization and incubation and monitoring, the effects of ultraviolet radiation transfer across the PDMS membrane must be determined.

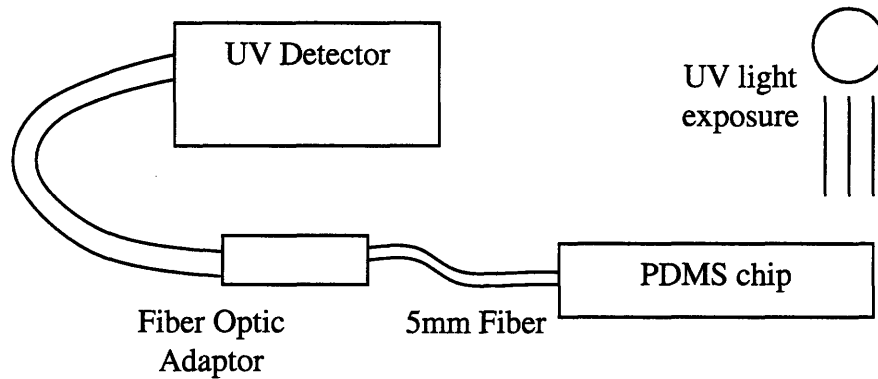


Figure 16 Proposed UV detection apparatus across PDMS membrane.

Figure 16 illustrates a proposed method of UV detection across the PDMS membrane through the use of fiber optic cables. A small 5mm optical fiber cable is directly inserted horizontally into the PDMS membrane [6]. The cable is customized such that the end connecting into the PDMS is a bare optical fiber end, while the adaptor end is a standard SMA connector. A custom adaptor is designed and machined to couple the optical fiber cable at one end, and the much bigger detection chord of an ultraviolet detector at the other end. As seen in the drawing below, the adaptor is simply a small 1 inch cube with carefully sized holes, closely fitting the fiber optic cable connectors, at either end.

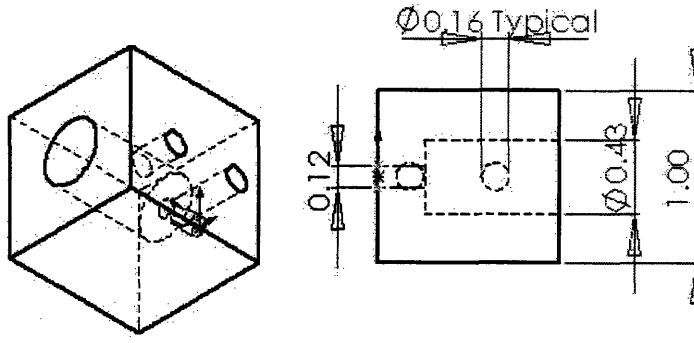


Figure 17 Schematic Drawing of Fiber Optic Adaptor.

Figure 17 is a detailed machine drawing of the fiber optic adaptor. The adaptor features two set screw holes to securely hold the two fiber optic cables in place. In addition, the fitted ends should be snug against the fiber optic cable ends as to prevent extraneous light from passing through.

While extensive tests have not been performed using this apparatus due to a lack of time, it seems to be a fairly robust and accurate method of determining the amount of ultraviolet radiation that is transmitted through the PDMS material. The results of these tests will determine whether or not protective measures must be included in the final combined cell culturing and metabolic detection device.

7. Conclusions

The microfluidic device detailed in this thesis has the potential to become a useful tool that aids in the in-vitro fertilization process. The current manifestation is in two separate chips, one chip for the purpose of cell trapping and culturing, while the other chip enables monitoring of embryo health through analysis of fluid excretions from the cultivated embryo. The ultimate goal will be to combine these two separate chips into one device, once ultraviolet propagation effects through PDMS are accurately determined and safety measures are enacted, if necessary

These microfluidic chips are easily manufactured from Polydimethylsiloxane (PDMS) and features two close-lying layers of channel networks. These channel imprints are created through molding liquid elastomer on top of photo-lithographed silicon wafers. A top layer functions as a passageway for fluid transport, while an underlying layer controls the upper channels through pressurizing and releasing various control channels. Effectively, this process allows the control layer to serve as valves and peristaltic pumps on the flow layer.

With further research and refinement, the device will be automated and easily controlled through computer programs. Some current areas for improvement include finding a more optimal way to form channel shape through perhaps a different photo-lithography process, controlling leakage around the corners of the valves, and fully examining whether there is a possibility of ultraviolet radiation across the PDMS material and channels.

Once completed, this device will be an essential part of the in-vitro fertilization process, not just by allowing more accurate identification of embryo health and

development potential, but ultimately allowing fewer embryos to be implanted, saving maternal carriers from the dangers from multi-fetal pregnancies.

References:

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5. Thorsen, Todd. (2002) Microfluidic Large-Scale Integration. *Science*. October 18, 2002, 580-584.
6. Wu, Min-Hsien, (2005) A SU-8/PDMS Hybrid Microfluidic Device with Integrated Optical Fibers for Online Monitoring of Lactate. *Biomedical Microdevices*. 7:4, 323–329.

Appendix:

PDMS Spin Coat Thickness vs. Spin RPM*

PDMS Spin Coat Thickness

Estimate wafer membrane thicknesses for ACEO cases

JPU EMPIRICAL DATA

21.0297 52.73557
29.4117 100
-17.244

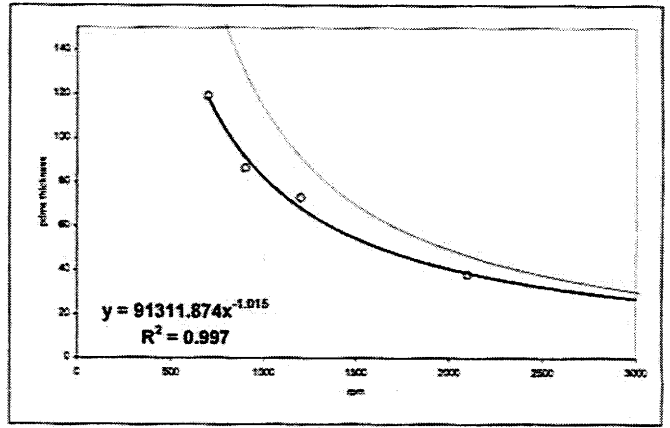
OLD DATA

exp=zygo	700	119
exp=zygo	900	66
exp	1000	73
exp=zygo	2100	38
exp	5000	15

8.1788 78.5
4.2099 40.40656

5.6017 50
2.6486 23.64104

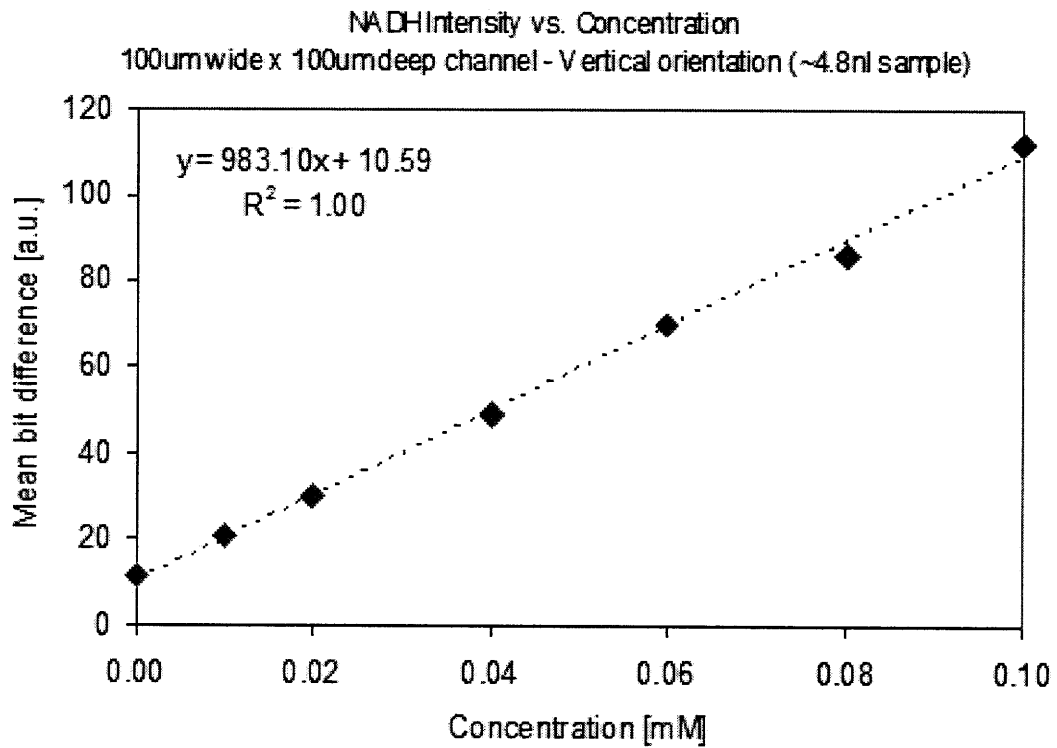
rpm	thickness	features	membrane	rpm	thickness	features	membrane
200	800.3	50	750.3	200	422	10	412
300	490.0	50	440.0	300	279	10	269
400	348.9	50	295.9	400	209	10	199
500	284.1	50	214.1	500	166	10	156
600	211.8	50	151.8	600	133	10	128
700	179.8	50	129.8	700	115	10	108
800	149.5	50	99.5	800	103	10	93
900	129.7	50	79.7	900	92	10	82
1000	114.2	50	64.2	1000	82	10	72
1100	101.7	50	51.7	1100	75	10	65
1200	91.6	50	41.6	1200	68	10	58
1300	83.1	50	33.1	1300	63	10	53
1400	76.0	50	26.0	1400	59	10	49
1500	69.9	50	19.9	1500	55	10	45
1600	64.6	50	14.6	1600	51	10	41
1700	60.1	50	10.1	1700	48	10	38
1800	56.1	50	6.1	1800	45	10	35
1900	52.5	50	2.5	1900	43	10	33
2000	49.3	50	-0.7	2000	41	10	31
2100	46.5	50	-3.5	2100	39	10	29
2200	44.0	50	-6.0	2200	37	10	27
2300	41.7	50	-8.2	2300	35	10	25
2400	39.6	50	-10.4	2400	34	10	24
2500	37.7	50	-12.3	2500	32	10	22
2600	36.9	50	-14.1	2600	31	10	21
2700	34.3	50	-15.7	2700	30	10	20
2800	32.8	50	-17.2	2800	29	10	19
2900	31.5	50	-18.5	2900	28	10	18
3000	30.2	50	-19.8	3000	27	10	17
3100	29.6	50	-21.0	3100	26	10	16
3200	27.9	50	-22.1	3200	25	10	15
3300	26.9	50	-23.1	3300	25	10	15
3400	26.0	50	-24.0	3400	24	10	14
3500	25.1	50	-24.9	3500	23	10	13
3600	24.2	50	-25.6	3600	22	10	12
3700	23.4	50	-26.6	3700	22	10	12
3800	22.7	50	-27.3	3800	21	10	11
3900	22.0	50	-28.0	3900	21	10	11
4000	21.3	50	-28.7	4000	20	10	10
4100	20.7	50	-29.3	4100	20	10	10
4200	20.1	50	-29.9	4200	19	10	9
4300	19.5	50	-30.5	4300	19	10	9
4400	19.0	50	-31.0	4400	18	10	8
4500	18.5	50	-31.5	4500	18	10	8
4600	18.0	50	-32.0	4600	17	10	7
4700	17.5	50	-32.5	4700	17	10	7
4800	17.1	50	-32.9	4800	17	10	7
4900	16.7	50	-33.3	4900	16	10	6
5000	16.3	50	-33.7	5000	16	10	6
5100	15.9	50	-34.1	5100	16	10	6
5200	15.5	50	-34.5	5200	15	10	5
5300	15.2	50	-34.8	5300	15	10	5
5400	14.8	50	-35.2	5400	15	10	5
5500	14.5	50	-35.5	5500	15	10	5
5600	14.2	50	-35.8	5600	14	10	4
5700	13.9	50	-36.1	5700	14	10	4
5800	13.6	50	-36.4	5800	14	10	4
5900	13.3	50	-36.7	5900	14	10	4
6000	13.1	50	-36.9	6000	13	10	3
6100	12.8	50	-37.2	6100	13	10	3
6200	12.6	50	-37.4	6200	13	10	3
6300	12.3	50	-37.7	6300	13	10	3
6400	12.1	50	-37.9	6400	13	10	3
6500	11.9	50	-38.1	6500	12	10	2
6600	11.6	50	-38.4	6600	12	10	2
6700	11.4	50	-38.6	6700	12	10	2
6800	11.2	50	-38.8	6800	12	10	2
6900	11.0	50	-39.0	6900	12	10	2
7000	10.8	50	-39.2	7000	11	10	1



* Data provided by J. P. Urbanski

NADH Calibration for Metabolic Analysis Vertical Orientation [2]*

Vertical Orientation (~4.8nl)



NADH Calibration for Metabolic Analysis Horizontal Orientation [2]*

Horizontal Orientation (~6.4nl)

