Development of a PDMS removable enclosure for microchannels using ultra-thick SU-8 process on silicon and glass substrates

by

Takaya Ueda
B.A.Sc., Simon Fraser University, 2004

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

In the School of Engineering Science

© Takaya Ueda 2008

SIMON FRASER UNIVERSITY

Summer 2008

All rights reserved. This work may not be reproduced in whole or in part, by photocopy or other means, without permission of the author.
APPROVAL

Name: Takaya Ueda
Degree: Master of Applied Science
Title of Thesis: Development of a PDMS removable enclosure for microchannels using ultra-thick SU-8 process on silicon and glass substrates

Examining Committee:

Chair: Dr. Lesley Shannon
Assistant Professor
School of Engineering Science, Simon Fraser University

Dr. Bonnie L. Gray
Senior Supervisor
Assistant Professor
School of Engineering Science, Simon Fraser University

Dr. Ash M. Parameswaran
Supervisor
Professor
School of Engineering Science, Simon Fraser University

Dr. Albert Leung
Internal Examiner
Professor
School of Engineering Science, Simon Fraser University

Date Defended/Approved: June 17, 2008
ABSTRACT

In this thesis, the design, fabrication, and testing results of a flexible enclosure for sealing microfluidic systems are presented. Unlike conventional fluidic sealing methods, this enclosure seals microfluidic channels without using any adhesives or substrate bonding, yet remains liquid tight. Thus, cell and biological samples can be cultured in uncovered microchannels, promoting medium and waste exchange, yet sealed for flow testing. Using a newly developed process, channels were fabricated on silicon and glass substrates with 1mm high ridges and 300 μm grooves using SU-8 photopolymer. These geometric features fasten the flexible PDMS enclosure by aligning it to seal the top of the microchannel. Flow simulation using COMSOL was conducted for each channel width. A syringe pump was connected to the chip, and water flow rate and pressure were monitored until leakage occurred. Finally, cells were introduced into channels for 24 hour culturing to demonstrate cell viability in the system environment.

**Keywords:** microfabrication, microfluidics, SU-8, PDMS, microchannels, packaging
DEDICATION

To my mother, Shizuko Ueda, who has always believed in me.

To my grandfather, Yonezou Ueda, who I respect the most.
ACKNOWLEDGEMENTS

First, thanks to my supervisor, Dr. Bonnie Gray for accepting me as her graduate student and for all the guidance and the support she has given me. She has given me the freedom to try out any ideas I had. Some ideas I proposed did not work, but the chance to try was the best learning method I could had. Thanks to Dr. Parameswaran and members of his lab group for many advice and support. Thank you to Dr. Leung and Dr. Shannon for generously agreeing to be on my committee.

Thanks to Seema, Stephanie, Jasbir, and Ajit of Microinstrumentation lab for their support in research and fun times. Thanks to Yuchun Chen and Dr. Li in Chemistry for their help in testing the microchannels with cells. Thanks to Bill Woods and Eva Czyzewskia for their help in the clean room. Many thanks to Mr. & Mrs. Kiyota and members of Vancouver Shomonkai Aikido for years of aikido practice to keep me healthy and sane during my school. Thanks to Emiko for encouraging me always.

Most importantly, thank you to my mother for allowing me to pursue further studying. Without your support, I would not be here today.
# TABLE OF CONTENTS

APPROVAL ..................................................................................................................... II  
ABSTRACT ..................................................................................................................... III  
DEDICATION .................................................................................................................. IV  
ACKNOWLEDGEMENTS .............................................................................................. V  
TABLE OF CONTENTS ............................................................................................... VI  
LIST OF FIGURES ........................................................................................................ VIII  
LIST OF TABLES .......................................................................................................... X  
LIST OF EQUATIONS ................................................................................................... XI  
LIST OF ACRONYMS .................................................................................................... XII  

1   INTRODUCTION ..................................................................................................... 1  
    1.1   Objectives ...................................................................................................... 3  
    1.2   Chapter outline .............................................................................................. 4  

2   BACKGROUND AND MOTIVATION .................................................................... 5  
    2.1   Microfluidics ................................................................................................... 5  
    2.2   Material Background .................................................................................... 6  
          2.2.1   SU-8 ............................................................................................................ 6  
          2.2.2   PDMS .......................................................................................................... 8  
    2.3   Existing thick film process ............................................................................ 8  
    2.4   Existing microchannels for applications in cell study .................................... 9  
    2.5   Benefits of removable lid for cell testing...................................................... 10  

3   ULTRA THICK SU-8 PROCESS ........................................................................... 12  
    3.1   Thick SU-8 fabrication method ...................................................................... 12  
    3.2   Mass to thickness characterization .................................................................. 13  
    3.3   Uniformity ....................................................................................................... 15  

4   DESIGN AND FABRICATION OF FLEXIBLE ENCLOSURE ON A SILICON SUBSTRATE .......................................................................................... 17  
    4.1   General Design ............................................................................................... 17  
    4.2   Fabrication of SU-8 channels and PDMS lids on silicon .............................. 19  
          4.2.1   SU-8 channel fabrication ........................................................................ 19  
          4.2.2   Flexible enclosure fabrication and assembly ......................................... 21  
    4.3   Fluidic analysis and simulation ..................................................................... 23  
    4.3.1   Theoretical calculation .............................................................................. 24
4.3.2 Simulation..................................................................................................................26
4.4 Fluidic Testing..................................................................................................................29
  4.4.1 Evaporation Test.......................................................................................................29
  4.4.2 Pressure gauge calibration........................................................................................31
  4.4.3 Flow Rate Test.........................................................................................................33
  4.4.4 Comparing results....................................................................................................37
4.5 Cell viability testing.......................................................................................................38

5 DESIGN PARAMETER CHARACTERIZATION AND FABRICATION
OF CHANNELS ON GLASS SUBSTRATE...........................................................................40
  5.1 Reasons to fabricate on glass.......................................................................................40
  5.2 Fabrication..................................................................................................................40
    5.2.1 Adhesion and alignment layer...............................................................................41
    5.2.2 Fabrication sequence of channels and enclosure mould on glass.......................42
  5.3 Fluidic analysis and simulation....................................................................................45
    5.3.1 Theoretical calculation...........................................................................................45
    5.3.2 Simulation...............................................................................................................47
  5.4 Fluidic Testing.............................................................................................................48
  5.5 Preliminary cell testing...............................................................................................53

6 APPLICATIONS AND FUTURE WORK ...........................................................................55
  6.1 Connectors...................................................................................................................55
    6.1.1 Design....................................................................................................................55
    6.1.2 Fabrication and Results........................................................................................56
  6.2 Cell control chip............................................................................................................58
    6.2.1 Design....................................................................................................................58
    6.2.2 Fabrication and results..........................................................................................58

7 DISCUSSIONS AND CONCLUSIONS..............................................................................60

APPENDICES.........................................................................................................................64

APPENDIX A: LIST OF PUBLICATIONS............................................................................65

APPENDIX B: EQUIPMENT AND MATERIALS.................................................................66
  B.1 SFU Engineering clean room.....................................................................................66
  B.2 SFU Microinstrumentation Laboratory.......................................................................66
  B.3 SFU Chemistry Li Lab.................................................................................................67
  B.3 Material list..................................................................................................................67

APPENDIX C: FABRICATION RUN SHEET.........................................................................68

REFERENCE LIST................................................................................................................72
**LIST OF FIGURES**

Figure 3-1: Ultra thick SU-8 fabrication process ............................................... 13
Figure 3-2: SU-8 100 Thickness versus mass on 4 inch silicon wafer .............. 14
Figure 3-3: 62 5x5 mm squares on 4 inch silicon wafer for uniformity test ...... 15
Figure 3-4: Average height and standard deviation of each quadrant of a 4 inch silicon wafer ......................................................................................... 16
Figure 4-1: Cross-section of the SU-8 channel with PDMS enclosure.......... 18
Figure 4-2: Top-view of SU-8 channel chip with single straight microchannel .................................................................................................................. 18
Figure 4-3: SU-8 microchannel fabrication sequence on silicon.................... 20
Figure 4-4: Flexible enclosure fabrication sequence ........................................ 20
Figure 4-5: PDMS enclosures assembled on SU-8 channels......................... 22
Figure 4-6: Piece-wise linear model of the microchannel with inlet/outlet..... 24
Figure 4-7: Calculated flow rate versus pressure drop for 416 and 528 μm wide channels .............................................................. 26
Figure 4-8: Velocity output of 416 μm width channel at 0.5 ml/min input rate ....................................................................................................................... 27
Figure 4-9: Pressure profile (in Pascals) of 528 μm width channel at 0.5 ml/min input rate ....................................................................................................... 28
Figure 4-10: Simulation pressure versus flow rate for 416 and 528 μm wide channels ........................................................................................................... 29
Figure 4-11: Evaporation of liquid inside the channel at t= 0 (a), 1 hr (b), 2 hr (c), 3 hr (d), 4 hr (e) ......................................................................................... 30
Figure 4-12: Calibration data for PX26-005GV pressure sensor ................. 33
Figure 4-13: Flow test setup .............................................................................. 34
Figure 4-14: Photograph of 416 μm wide channel under flow ..................... 35
Figure 4-15: Experimental flow-rate versus flow for 416 μm wide channel.... 36
Figure 4-16: Pressure values for 416 μm channel by calculation, simulation, and experimentation .............................................................. 37
Figure 4-17: CEM-wt cells inside the channel through PDMS lid ............... 38
Figure 4-18: Leukaemia cells dyed with Typan blue. Dead cell shown as blue dot and live cells remain as clear circles ................................ 39
Figure 5-1: Alignment and adhesion layers process steps ............................... 42
Figure 5-2: Fabricated channels and moulds on 3 x 3 in glass......................... 44
Figure 5-3: Calculated flow rate versus pressure for 360 and 477 μm wide channel ................................................................. 46
Figure 5-4: Simulation of 360 μm channel under flow at 1ml/min. Maximum pressure: 1331 Pa ......................................................... 47
Figure 5-5: Simulated pressure versus flow rate .............................................. 48
Figure 5-6: 360 μm wide microchannel tested in submersion in water .......... 50
Figure 5-7: Experimental flow rate versus pressure for 360 μm wide channel.................................................................................. 51
Figure 5-8: Pressure values for 360μm channel by calculation, simulation, and experimentation......................................................... 52
Figure 5-9: Leukaemia cells in SU-8 microchannel at (a) x4, (b) x10, (c) x40 magnifications.......................................................................... 53
Figure 5-10: Leukaemia cells in SU-8 microchannel with assembled PDMS enclosure at x40 magnification........................................... 54
Figure 6-1: Connector diagram ..................................................................... 56
Figure 6-2: Single (left) and double-joint (right) connectors ................................. 56
Figure 6-3: Single (right) and double-joint (left) connectors ................................. 57
Figure 6-4: Three way cell channel [6] ................................................................ 58
LIST OF TABLES

Table 4-1: Measured widths of channels and enclosure moulds in μm .......... 23
Table 4-2: Pressure drop summery (theoretical calculations)...................... 25
Table 4-3: Model parameters and boundary conditions .............................. 27
Table 4-4: Fluidic resistance of tubing connected to the chip ...................... 35
Table 5-1: SU-8 channel and PDMS lid fabrication parameters on glass substrate ......................................................................................................................... 43
Table 5-2: Measured widths and depths in μm............................................ 44
Table 5-3: Effective diameters and fluidic resistances of channels on glass ................................................................................................................................. 46
Table 5-4: Designed and measured enclosure widths in μm....................... 49
LIST OF EQUATIONS

Equation 4-1 ........................................................................................................ 24
Equation 4-2 ........................................................................................................ 25
Equation 4-3 ........................................................................................................ 31
Equation 4-4 ........................................................................................................ 32
Equation 4-5 ........................................................................................................ 32
# LIST OF ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>Chromium</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl-alcohol</td>
</tr>
<tr>
<td>MEMS</td>
<td>Microelectromechanical system</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>PR</td>
<td>Photoresist</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

Microfluidics is study of fluidics at the microscale for applications such as cell analysis, drug delivery, and sample handling [1, 2]. As the prefix implies, microfluidic devices are fabricated with features in micrometer scale that can handle volume of fluids dispensed in nanolitres. In order to fabricate these devices, traditional microelectronics processes such as deposition, etching, and photolithography are used on silicon or glass substrates. However, the material of choice for microfluidics has shifted towards polymers due to their transparency, cost, biocompatibility, and ease of fabrication [3]. Like traditional materials such as silicon which is patterned through photolithography and etching, polymers can also be patterned through photolithography and development and simple micromoulding [4]. Thus, they were easily adapted to the existing fabrication technologies. Examples of popular polymer materials for microfluidics are SU-8 and polydimethylsiloxane (PDMS), which are used to fabricated the devices in this thesis.

One of the applications for polymer microfluidic device and systems is cell experimentation, where the microchannels and devices can be on the same order as the cells. Microfluidic devices for cell species can conduct stress tests [5], drug tests [6], sorting [7], culturing [8] and fluorescence detection [9]. For some cell experiments, cells need to be cultured first in open channels to increase their numbers, promote confluence of healthy cells, or plate them on the substrates to make them adhere so that they will not be washed away with the fluid flow. However, in order to perform experiments on the cells under flow, the microchannels must be subsequently enclosed after a period of
microchannel cell growth to guide a pressurized flow through the microchannel. Enclosed microchannels may make it difficult to insert certain types of cells and may cause contamination while inserting these cells into the channels. Ideally, such cells should be inserted into an open channel to which the enclosure can be freely attached later.

Towards the goal of providing more versatility in culturing and flow testing, fabrication of a flexible enclosure for sealing microfluidic components is proposed in this thesis. The flexible enclosure, fabricated from PDMS, can enclose microchannels without any adhesives. Such an enclosure will enable more versatile cell testing while avoiding possible contamination that can risk cell health. In addition, although this thesis focuses on providing a flexible enclosure for cell testing platforms, the enclosure could be utilized for other low temperature bonding applications where an enzyme or other biospecies must be immobilized in a microchannel prior to enclosure.

In order to achieve such enclosure of a microchannel, the enclosure and the microchannel must be fabricated with materials that form a leak-tight seal when mechanically assembled. Previous research [10] showed that chip-to-chip connector microstructures yield best results when the connection is made with a soft material, like PDMS, and a somewhat more rigid material, like SU-8, to conform the other’s geometry. Thus, in this thesis PDMS and SU-8 were also selected to fabricate the enclosure-microchannel system.

To securely fasten the enclosure to the microchannel without any adhesives, the PDMS enclosure and the SU-8 microchannel need interlocking geometric features to secure them in place. Without any adhesives, friction is the factor that will fasten the two materials together. An ultra-thick film process was characterized and employed to
increase the surface areas of the geometric features that come into contact with the other material. Unlike the traditional thick film process utilizing spin coating of SU-8, where the typical film thickness of structures and moulds is 100 to 200 μm [11], the ultra-thick process presented in this thesis enables fabrication of SU-8 and PDMS structures that are thicker than 1 mm.

1.1 Objectives

The main objective for this thesis is to develop an SU-8 and PDMS process for the fabrication of microfluidic structures with a flexible enclosure for cell testing. In order to achieve this objective, this thesis includes the following main goals:

- Development and characterization of an ultra-thick SU-8 process to fabricate structures and moulds higher than 1 mm
- Fabrication and fluidic testing of SU-8 channels and PDMS enclosure on a silicon substrate as proof of concept
- Adaptation of fabrication and fluidic testing of the channels and enclosure onto a glass substrate to add transparency for easier optical observation of cells
- Analysis and simulation of fluid flow to characterize the fluid flow pressure profile inside the microchannels and compare the values with those obtained through experiment
- Proposal of additional applications for the thick SU-8 process and flexible enclosure
To analyze the fluid flow inside the microchannel, the theoretical pressure drop was calculated using first order approximations based on simple Poiseuille flow. Then a simulation model was created in the COMSOL Microfluidics module to obtain expected velocity and pressure. Finally, the experimental values were obtained through flow test of microchannels using a digitally controlled syringe pump and pressure sensor.

1.2 Chapter outline

Chapter 2 gives general background for the thesis including material properties and prior art. Chapter 3 describes the ultra-thick SU-8 process that was developed and used extensively in this thesis to fabricate the microchannels and the enclosure. Chapter 4 explains the design and fabrication method for the SU-8 channel and the flexible PDMS enclosure on silicon. Chapter 5 proceeds to fabrication of the channels and the enclosures on glass substrates. Chapter 6 demonstrates potential applications for the ultra-thick SU-8 process and the flexible enclosure. Chapter 7 provides discussion and summary of results for the whole thesis.
2 BACKGROUND AND MOTIVATION

This chapter covers a brief background of microfluidics and its applications. It then proceeds to prior art concerning material properties. The chapter concludes with examples of existing thick-film methods and microfluidic devices for cell studies.

2.1 Microfluidics

Microfluidics is the study of systems of fluids at micro or nanolitre scales [12]. The main advantages of handling fluids in such a small scale are to reduce the material costs and sample volume, as well as provide portable and/or disposable testing systems [13]. Microfluidic systems can be comprised of systems involving only simple channels [14, 15], or very complex complete lab-on-a-chip systems containing pumps [16], mixers [17], valves [18], sensors [19], and optics [20]. Common applications include DNA analysis [21], cell testing [1, 5], and drug delivery [2]. Multiple microfluidic systems can be realized in a single substrate [22] or modularly connected using fluidic interconnects to form more complex systems [23-25].

Fabrication methods used to create microfluidics systems are similar those used to fabricate microelectronics and microelectromechanical systems (MEMS). Just like MEMS, microfluidic components have traditionally been fabricated using photolithography, etching, and deposition. In addition, other methods such as embossing or micromoulding allow rapid production of polymer-based devices [12]. Microfluidic devices can be patterned on variety of substrate materials. They can be patterned on or in
silicon [26] and glass [27]. Polymers like SU-8 [14, 15, 28], polyimide [29], PDMS [4] and polymethylmethacrylate (PMMA) [30] are popular materials due to their low-cost, chemical stability, biocompatibility, and simple processing methods.

2.2 Material Background

The two materials used for this thesis were SU-8 and PDMS. SU-8 is a photo-patternable polymer, and PDMS is a thermally cured elastomer, as discussed below.

2.2.1 SU-8

SU-8 is a negative photoresist that was originally developed by IBM [31]. A photoresist is called negative when the areas exposed to certain wavelengths of light are cross-linked due to energy imparted by the light and thus remain on the substrate during development while the unexposed areas are developed away. SU-8 is sensitive to wavelengths below 400 nm and will absorb light shorter than 360 nm [32]. Exposure to near UV-light causes photoinitiator in the SU-8 formula to become a strong acid, which starts the cross-linking of SU-8, and the cross-linking is thermally accelerated [33, 34]. The SU-8 used in this thesis is distributed by Microchem [35]. Microchem manufactures many formulations of SU-8, denoted by a numerical suffix ranging from 2 to 100, which denotes the estimated thickness of the resulting SU-8 film in micrometers when spun at 3000 RPM [32, 36]. SU-8 enables fabrication of high aspect ratio structures, with film thicknesses greater than 200 μm that can be applied in a single coating [32]. By choosing the correct SU-8 formulation and controlling the spin speed, various thickness of SU-8 can be spun onto the substrate from 2 μm to greater than 200 μm in a single coating [11].
To process SU-8, Microchem suggests the following basic fabrication sequence [32, 36]:

1. Substrate preparation
2. Spin coat SU-8
3. Soft (pre-exposure) bake at 65-95°C
4. Expose
5. Post exposure bake (PEB) at 65-95°C
6. Develop
7. Rinse and dry
8. Hard bake optional

The SU-8 process used in this thesis mostly followed the above sequence. However, SU-8 structures as thick as 1 mm were fabricated and the process was modified to adapt to the thick SU-8 process by replacing the spin coating with a casting method.

SU-8 is used for a variety of structures and applications in MEMS and microfluidics. MEMS structures such as cantilevers [37] and out-of-plane structures [38] are just few examples of structures that can be fabricated. SU-8 suits microfluidic applications due to its transparency and biocompatibility [39]. Although the fabrication of enclosed microchannels presents some challenges [40], SU-8 can be used to fabricate microfluidic channels [1, 14], fluidic interconnects [24, 25], and other microfluidic structures [41, 42]. In addition, SU-8 is a popular mould for elastomers to rapidly fabricate PDMS devices using a process commonly called soft lithography [43].
2.2.2 PDMS

Polydimethylsiloxane (PDMS) is a flexible silicone elastomer that is often used for moulding microchannels [44] and microfluidic systems [45] due to its cost, biocompatibility, transparency and ease of fabrication [3, 4, 46]. Once cured, PDMS is chemically stable and can be used in wide range of temperature environments (-45 to 200°C) [47]. The micromould patterning using such material is known as “soft lithography” [4]. For this thesis, *Sylgard 184 Silicone Elastomer Kit* from Dow Corning was used. The kit contains two liquid parts: base and curing agent. The two are thoroughly mixed in 10:1 ratio. Then the mixture is poured onto the mould master, commonly a silicon wafer with SU-8 structures. The mixture, if left alone, will cure in 48 hours at room temperature [47]. For practical purposes, the temperature can be increased, for example, by a hotplate, for shorter curing time.

2.3 Existing thick film process

Fabricating structures using the SU-8 formulation with the highest viscosity (SU-8 100) will typically yield a film thickness of 100 to 200 μm with single spin [11]. It is difficult to coat a film thicker than 500 μm without multiple coatings [48]. Casting becomes more practical when fabricating structures near or over 1 mm in thickness because casting avoids chemicals wasted through spin coating [48, 49] and provides a more uniform film. Using thick films of SU-8 to fabricate ultra-thick structures without multiple spin coatings was extensively done by Lin et al. [49]. Lin proposed a method of creating ultra-thick (> 1mm) SU-8 films by simply casting SU-8 onto the substrate and reflowing it to form a uniform coating during pre-exposure (soft) baking. Conducting the pre-exposure bake at 120°C lowers the viscosity of the SU-8 formula enough to cause
reflow and planarization over the substrate surface. After thorough soft baking, ultraviolet (UV) exposure, post-exposure bake, and development, thick SU-8 structures can thus be fabricated. By using this method, structures as thick as 2 mm can be fabricated. One great advantage of Lin’s method is that only the needed amount of chemical can be dispensed, which means there is no wasted chemical such as the waste off the edges that occurs during spinning. This minimizes SU-8 waste, allowing a bottle of SU-8 to be used for the maximum number of fabrication runs, which is particularly critical when making very thick films as already a large amount of SU-8 is used. Lin’s work provides basis for the ultra-thick SU-8 process that is combined with normal (spin coat) SU-8 processing developed for this project.

2.4 Existing microchannels for applications in cell study

Microchannels are often utilized for cell studies because their dimensions are comparable to the actual size of the cells, which typically range from tens to hundreds of microns, thus facilitating experimentation that may be otherwise difficult or impossible. For example, endothelial cells (ECs), the cells which line the inner walls of arteries, typically range from 20-50 μm in diameter, which varies depending on whether they are free in solution or plated to the substrate. Their shape and elongation properties seem to relate to the preferential development of atherosclerosis [50]. One key method to elongate ECs is to apply shear stress onto the cells by placing stationary substrate-plated cells under flow such as that developed in a pressurized microchannel [51]. Another method, facilitated by microfluidics, is to physically alter their shape by growing them to confluence in a constricted microchannel channel [1, 40]. Another type of cell, free-floating cells like leukaemia cells which are about 10 μm in diameter, are easily affected
by the carrier fluid flow. A microfluidic channel can be designed so that the cells can be
individually manipulated and trapped for individual drug testing [6].

In the above examples and many others, SU-8, PDMS, and glass have proven to be good materials for fabricating cell study platforms for their biocompatibility, transparency, and ease of plating cells on the substrate surface.

2.5 Benefits of removable lid for cell testing

In many studies, it is desirable to test cells that have been plated on the substrate surface and allowed to grow to a level of confluence [5], which is when the few initially plated cells have multiplied to form an entire colony (often a monolayer on the substrate surface). In order to easily plate cells onto the substrate in large numbers, and to ensure cell confluence of healthy cell colonies, plating needs to be done in an environment where waste and cell medium (solution composed of nutrients, antibiotics, buffer, and a pH indicator [5]) exchange can easily take place, often over a period of hours or days. Therefore, the interior of an enclosed microchannel is not an optimal environment because nutrients and waste cannot be exchanged as freely as in an open channel system with the microchannels sitting in a Petri dish. However, in order to carry out a cell experiment involving flow, such as sheer stress testing of ECs [5] or individual leukaemia cell drug testing [6], fluid flow over and around the cell surfaces must also be easily predicted and controlled, which is not the case in an open channel system. Thus, the optimal situation is to culture cells in an open microchannel and then seal them in an enclosed microchannel for further testing. In prior research on ECs [51], sealing of the microchannel was done manually by removing the microchannel from the culturing medium, placing a simple flat gasketed enclosure on the channel, and manually applying
a sealant (glue) to secure the lid in place. Clearly, this process is cumbersome and more importantly can compromise cell health as the sealant outgases during curing. Ideally, the microchannel and the enclosure should be both introduced into the culturing environment without compromising cell health, yet result in a sealed (enclosed) microchannel where flow can be easily predicted using simplified Navier-Stokes equations and simulation. Therefore, a removable enclosure that can be applied onto the microchannel without bonding or clamping the lid to minimize assembly steps after cell plating, and in the presence of cell medium, provides the best solution. Enclosure without clamping or bonding will allow the placing and/or removing of the lid while the microchannel is immersed in the cell medium. This allows multiple testing events with easy immersion and incubation between testing. Thus, the motivation behind this thesis is to develop a microchannel-enclosure system that can be applied that is less risky to incomplete plating, cell contamination, and cell death.

Although the enclosure and channel system developed in this thesis is primarily to support research on ECs and leukaemia cells by providing flow-controlled cell chamber, the concept can be applied to the development of other microfluidic devices, e.g., sensors requiring an enzyme or biospecies for fabrication. A number of researchers have investigated low-temperature bonding for the packaging of such sensors in microfluidic systems [52, 53]. The proposed device may have further applications in the low temperature packaging of sensitive devices.
3 ULTRA THICK SU-8 PROCESS

The original ultra thick SU-8 process was introduced by Lin et al [49]. They proposed a method of creating thick (> 1mm) SU-8 structures without spin coating the SU-8. Instead of multiple spins, SU-8 was cast onto the substrate and reflowed at high temperature for planarization. In this method, a specific volume of SU-8 is poured onto the substrate, with the high temperature soft-bake at 120°C causing the SU-8 to reflow. SU-8 can thus coat the whole wafer by manually flattening the surface with a glass slide. The surface tension between the SU-8 and the substrate prevents the SU-8 from flowing off the substrate. Major advantages to this method are reduction of edge bead due to self-planarization, and saving of SU-8 solution because all of the SU-8 poured onto the substrate remains on the substrate, as opposed to spin coating where a large portion is wasted. Lin controlled the SU-8 thickness by using a syringe to dispense a specific volume. However, while trying to repeat this method, many air bubbles were created with the use of the syringe. Not all of the bubbles disappeared through the long baking process. To circumvent this problem with the bubbles, I experimented with dispensing a specific mass of SU-8 (as opposed to volume) to control the film thickness. The method presented below presents the resulting SU-8 thickness to mass relationship.

3.1 Thick SU-8 fabrication method

The general process flow for the fabrication of ultra-thick structures is outlined in Figure 3-1. This process is to be integrated into a more complex three-level SU-8 process in subsequent chapters.
SU-8 100 was then poured directly onto the centre of the wafer while monitoring its mass (a). After obtaining the target mass, the wafer was placed on a 120°C hot plate (Torrey Pines Scientific A130) and the SU-8 was spread to the edge of wafer using a glass slide. SU-8 was soft-baked for 3, 5, and 7 hours for 1 mm, 1.5 mm, and 2 mm thick SU-8 respectively, and cooled slowly down to the room temperature at natural rate before it was removed from the hot plate. Then the SU-8 was exposed (b). Post exposure bake at 65 °C for 30 min was done to complete the cross linking of the SU-8. Then the SU-8 was developed in SU-8 developer to remove the uncross-linked (unexposed) SU-8 (c).

### 3.2 Mass to thickness characterization

The ultra thick SU-8 process was characterized to determine the relationship between the thickness and mass. Figure 3-2 below shows the graph of the mass versus the resulting thickness of SU-8.
Figure 3-2: SU-8 100 Thickness versus mass on 4 inch silicon wafer

The error bars in the graph indicates the standard deviation over the average thickness of four points on a wafer. The above figure shows an approximately linear relationship between the mass of SU-8 and the thickness. From this graph, one can estimate the mass of SU-8 needed to create desired thickness. The slope of the graph is 102 μm/g of SU-8 100. This translates to approximately 1mm of SU-8 for every 10 grams of SU-8 100 on a 4-inch wafer. At some point, the graph may become non-linear as SU-8 may spill over the edge of the substrate, but this was outside of the range of desired thickness for the fabricated structures.
3.3 Uniformity

To test the uniformity of the ultra-thick SU-8 process, 62 1 mm thick, 5x5 mm blocks were patterned onto a silicon wafer using the process described in Section 3.1, and the thickness of each block was measured using a micrometer (Fowler IP54). Figure 3-3 shows 62 SU-8 blocks on a silicon wafer.

![62 SU-8 blocks on a silicon wafer for uniformity test](image)

Figure 3-3: 62 5x5 mm squares on 4 inch silicon wafer for uniformity test

The height of each square was measured and the average height of the whole wafer was 1110 μm with standard deviation of 91 μm. In addition, the height of each quarter of the wafer was calculated. Each quarter of a wafer consists of 13 5x5 mm SU-8 squares. The average heights and standard deviations for each quarter are shown below in Figure 3-4.
As shown in Figure 3-4, the film is thicker toward bottom left area of the wafer. This variation in thickness likely indicates that hotplate was slightly tilted toward the bottom left corner. The slightest tilt can cause variations in thickness across the wafer. In this project, variation in thickness resulted in areas that were not exposed with optimal dosage, resulting in structures that were wider or narrower than the fields on the mask. Although with process optimization this effect can be minimized, the effect of non-uniform thickness must be considered in structure design.
4 DESIGN AND FABRICATION OF FLEXIBLE ENCLOSURE ON A SILICON SUBSTRATE

A flexible enclosure for fluidic sealing of SU-8 microcomponents was developed using PDMS. The flexible enclosure can be mechanically assembled to SU-8 microchannels even in the presence of liquid without bonding or with aid of external clamping device, thus minimizing the assembly process. This section describes the design, simulation, fabrication, and testing results of the enclosure and the channels on a silicon substrate.

4.1 General Design

To obtain a reversible fluid tight enclosure, the PDMS and SU-8 substrates contained interlocking structures that facilitate assembly and sealing together of the two substrates. Previous work with other interlocking structures in our lab indicates such a material combination produces the best mechanical and fluid-tight interconnect [10]. Figure 4-1 shows a cross sectional illustration of the microchannel with the flexible enclosure. Although both the microchannel and enclosure are fabricated with polymers, the enclosure is fabricated in a more flexible polymer (PDMS) so that it can be pressed to fit onto the microchannel (using the “bump”) and attached using specially designed structures (ridges, grooves, and holes).
As shown in Figure 4-1, the PDMS enclosure is assembled onto the SU-8 microchannel via 1 mm high ridges and holes, as well as shorter 300 μm ridges and grooves. By having the ridges and holes in the PDMS, and grooves and ridges in the SU-8, the enclosure is securely aligned and assembled onto the microchannel. To further improve the sealing between the two materials, the enclosure was fabricated with 100 μm bump to act as a cork on top of the microchannel that fits into the channel along the entire channel length to prevent leakage. Thus, the size of the microchannel is 300 μm wide by 10 mm long by 300 μm deep. Figure 4-2 shows the top view of the channels.
As shown in Figure 4-2, the chip has area of 16 x 8 mm and contains a 9 mm long channel with 500 μm diameter inlet/outlet holes on either end. The microchannel is surrounded by 300 μm deep groove and 1mm high ridges, where the PDMS enclosure locks. The widths of the channel were varied from 100 μm to 500 μm at 100 μm increments, which is in the main range of interest for testing ECs and other cells [1, 6]

4.2 Fabrication of SU-8 channels and PDMS lids on silicon

This section describes the fabrication sequence for SU-8 channels and the PDMS enclosure. The temperature and baking values for the SU-8 are much different from the values suggested by Microchem [32] because of the ultra-thick 1 mm high structures.

4.2.1 SU-8 channel fabrication

Before coating the substrate with SU-8, the substrate was cleaned to ensure good adhesion to the SU-8. Both silicon and glass substrates (process described in the following chapter) were cleaned in RCA 1 (NH₄OH:H₂O₂:de-ionized water in 1:1:5 ratio) at 80°C for 10 minutes to remove any organics on the surface. Silicon wafers were dipped in 5% hydrofluoric (HF) solution for 30 seconds to remove any native oxide.

Figure 4-3 shows the fabrication process for SU-8 channels.
a) spin SU-8 100 at 1500 rpm for 40 s

b) soft-bake and expose

c) spin SU-8 100 at 3000 rpm for 30 s

d) soft-bake, expose and post exposure bake

e) develop two layers

f) cast 10g of SU-8 100

g) soft-bake, expose and post-exposure bake

h) develop the SU-8 layer

Figure 4-3: SU-8 microchannel fabrication sequence on silicon
As shown in Figure 4-3, SU-100 was spun on the wafer at 1500 rpm for 40 seconds for 400 μm thickness (a). After soft-bake at 120°C for 10 minutes and 90°C for 25 minutes, the SU-8 was exposed for 3 minutes at 10 mW/cm² (b). The second structural layer was fabricated by spinning SU-8 100 at 3000 rpm for 30 seconds for a 100 μm thick film (c). After the soft-bake at 120°C for 2 minutes and 90°C for 10 minutes, the second layer was exposed for 2 minutes at 10 mW/cm², and post-exposure baked for 30 minutes at 65°C for cross-linking (d). After the cross-linking of the second structural layer, the two layers of SU-8 were developed using the SU-8 Developer for 10 minutes. Any residual SU-8 was washed away by spray a cleaning with SU-8 Developer and isopropylalcohol (IPA) (e). For the final structural layer, 10 g of SU-8 was poured onto the wafer. The wafer was soft-baked at 120°C for 20 minutes and 90°C for 3 hours (f). Then the SU-8 was exposed for 16 minutes and post-exposure baked at 30 minutes at 65°C (g). Following the post-exposure bake, the SU-8 was developed to remove the uncross-linked SU-8 for approximately 20 minutes (h).

4.2.2 Flexible enclosure fabrication and assembly

To fabricate the PDMS flexible enclosure, an SU-8 mould master was fabricated using the same fabrication steps as outlined in Section 4.2.1. The fabrication sequence of the flexible enclosure using the SU-8 mould master is outlined in Figure 4-4.
a) fabricate SU-8 enclosure mould using steps outlined in section 4.2.1

b) pour PDMS onto the mould, flatten the top surface, and cure

c) remove PDMS from the mould

Figure 4-4: Flexible enclosure fabrication sequence

PDMS was thoroughly mixed with the curing agent in 1 to 10 ratio, and the mixture was first placed in a vacuum oven for 20 minutes to remove air bubbles. Then the mixture was poured onto the mould master (a) and the top surface was flattened using a glass slide. The wafer was placed in a vacuum for additional 20 minutes to eliminate the bubbles introduced during pouring. Then the wafer was placed onto an 85°C hotplate for 90 minutes to cure the PDMS (b). Finally, the cured PDMS was detached from the substrate to be used as the flexible enclosure (c).

To assemble the enclosure to the channel, the holes in the enclosure were aligned with the ridges on the channels and pressed down firmly using a pair of tweezers, either in the absence or presence of fluid. Figure 4-5 shows several assembled device.
Figure 4-5: PDMS enclosures assembled on SU-8 channels

For the 400 and 500 μm channels fabricated on silicon, the measured widths of the channels and mould are summarized in Table 4-1.

<table>
<thead>
<tr>
<th>Mask width</th>
<th>Channel width</th>
<th>Channel depth</th>
<th>Mould width</th>
<th>Mould depth</th>
<th>ΔWidth</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>416</td>
<td>295</td>
<td>424</td>
<td>98</td>
<td>8 (2%)</td>
</tr>
<tr>
<td>500</td>
<td>528</td>
<td>298</td>
<td>545</td>
<td>101</td>
<td>17 (3%)</td>
</tr>
</tbody>
</table>

The right most column, ΔWidth, is simply the channel width minus the mould width; the percentage indicates relative difference. Note that for both channels, the lid mould width is wider than the channel width. This difference in width proved to be necessary to obtain the fluid tight seal between the resulting channel and lid, which will be described in later sections.

4.3 Fluidic analysis and simulation

In order to utilize the channel/lid system for cell experimentation, the fluid flow profile and pressure drop along the microchannel must be predictable and controllable. A first order analysis was done to calculate the approximate pressure drop along the channel
from inlet to outlet. Then the calculated values were contrasted with the results from simulation obtained from the COMSOL multi-physics program.

4.3.1 Theoretical calculation

To simplify the theoretical calculation, a piece-wise linear model as shown in Figure 4-6 was employed.

![Figure 4-6: Piece-wise linear model of the microchannel with inlet/outlet](image)

The model in Figure 4-6 assumes the inlet/outlet sections to be a square channel with constant width and length of 500 μm. The depth of the microchannel is assumed to be 200 μm with length of 9 mm. Pressure drop \( P \) in Pascals, along a channel length \( L \) in meters can be calculated using the Hagen-Poiseuille equation[54]:

\[
P = R Q = \frac{128 \mu L}{\pi D_{eff}^4} Q.
\]

In the above equation, \( R \) is the fluidic resistance in kg/m^4 s and \( Q \) is the flow rate in m^3/s, and the equation is analogous to Ohm’s law with current and resistance. The viscosity is denoted as \( \mu \) (0.00103 kg/m s for water), \( Q \) is the flow rate in m^3/s, and \( D_{eff} \) is the effective hydraulic diameter in meters, which is given by [54]
\[ D_{\text{eff}} = \frac{64}{K} \frac{2wh}{w+h}, \]

Where \( K \) is the correction factor and \( w \) and \( h \) are the width and height (depth) of the channel. Employing equation 4-2 onto a rectangular channel will give the equivalent circular channel with diameter of \( D_{\text{eff}} \), thus allowing the Hagen-Poiseuille equation to be utilized unchanged. Using the two equations, the correction factors, effective diameters, and fluidic resistances of the channels are summarized in Table 4-2. The depth of the channel, \( h \), was assumed to be nominally 200 \( \mu \)m. The correction factor and the effective diameter for the inlet/outlet parts of the channel are the same for inlet and outlet channel widths, so the resulting fluidic resistance for the inlet/outlet, \( R_{\text{in/out}} \), is \( 3.45 \times 10^9 \) kg/m\(^4\) s.

<table>
<thead>
<tr>
<th>Width (( \mu )m)</th>
<th>( K_{\text{channel}} )</th>
<th>( D_{\text{eff, channel}} ) (m)</th>
<th>( R_{\text{channel}} ) (kg/m(^4) s)</th>
<th>( R_{\text{total}} ) (kg/m(^4) s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>416</td>
<td>62.19</td>
<td>2.78( \times )10(^{-4})</td>
<td>6.32( \times )10(^{10})</td>
<td>6.67 ( \times )10(^{10})</td>
</tr>
<tr>
<td>528</td>
<td>65.47</td>
<td>2.84( \times )10(^{-4})</td>
<td>5.84( \times )10(^{10})</td>
<td>6.18 ( \times )10(^{10})</td>
</tr>
</tbody>
</table>

The total fluidic resistance, \( R_{\text{total}} \), is given by summing twice the \( R_{\text{in/out}} \) value with \( R_{\text{channel}} \). The pressure drop of the microchannel can be calculated by multiplying \( R_{\text{total}} \) by the flow rate. Figure 4-7 shows the graph of flow rate versus pressure drop for the two channels of different widths over a range of 0 to 1mL/min, which is the range of flow expected for cell testing, e.g., ECs [40].
As shown in Figure 4-7 the pressure increases linearly with flow rate, and the narrower 416 μm wide channel has higher fluidic resistance, thus resulting in higher pressure drop. As the flow rate increases, the difference in the widths of the channels has more significant effect in the resulting pressure drop difference.

### 4.3.2 Simulation

Pressure drop in the channel was simulated using the COMSOL Multi-physics fluidics package. The channel models were drawn using the parameters and boundary conditions summarized in Table 4-3.
Table 4-3: Model parameters and boundary conditions

<table>
<thead>
<tr>
<th>Width (μm)</th>
<th>Length (μm)</th>
<th>Inlet/outlet diameter (μm)</th>
<th>Depth (μm)</th>
<th>Inlet boundary condition</th>
<th>Outlet boundary condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>416</td>
<td>9000</td>
<td>500</td>
<td>200</td>
<td>0.1-1 ml/min flow rate</td>
<td>Pressure = 0</td>
</tr>
<tr>
<td>528</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All other surfaces were set to the no-slip condition. For each channel width, the simulations were done for input velocities ranging from 0.1 ml/min to 1.0 ml/min. Simulation convergence and mesh independence were assured in all simulations.

Figure 4-8 shows an example flow velocity profile at an input maximum flow rate of 0.5 ml/min for the 416 μm width channel. Arrows represent the direction of the flow and their size is proportional to the maximum flow velocity.

Figure 4-8: Velocity output of 416 μm width channel at 0.5 ml/min input rate
The maximum velocity at 0.5 ml/min input flow rate was $4.24 \times 10^{-2}$ m/s. The pressure drop across the entire channel length for the same conditions is shown in Figure 4-9.

![Figure 4-9: Pressure profile (in Pascals) of 528 μm width channel at 0.5 ml/min input rate](image)

Figure 4-9 shows that the maximum pressure, 379 Pa, occurs near the inlet of the channel (left side of the figure) and decreases as it nears the outlet (right side of the figure). The pressure drops across the 416 μm and 528 μm wide channels for flow rates from 0 to 1.0 ml/min are summarized in Figure 4-10.
Figure 4-10: Simulation pressure versus flow rate for 416 and 528 μm wide channels

Figure 4-10 shows a linear relationship between pressure and flow rate and a higher pressure drop for the narrower channel which is consistent with calculated results in Figure 4-7. Both simulated and first order calculated results will be compared with experimental data in Section 4.4.4.

4.4 Fluidic Testing

To test the fluidic performance of the microchannels and the PDMS flexible enclosure, two tests were conducted: evaporation test and flow rate test.

4.4.1 Evaporation Test

An evaporation test was conducted to observe how effectively the flexible enclosure seals the microchannel to prevent the liquid from evaporation. A 416 μm wide
channel was filled with dyed water and subsequently sealed with the flexible enclosure. The inlet and outlet ports were also sealed. The chip was placed at room temperature (22°C) and observed every hour until all the liquid in the channel was evaporated. Figure 5 shows the progress of the evaporation test.

As shown in Figure 4-11, the channel is completely filled in the beginning (a) and the liquid gradually evaporates as the hours go by (b – e). The channel mostly emptied (less than 50% remaining liquid) after 4 hours. The liquid was completely gone before 5 hours had elapsed.

The above results may not seem encouraging for a fluid tight microchannel. However, the results must be placed in the context of the system goals. One goal of the system is to fabricate an open channel system for cell culturing/enzyme immobilization, etc., that can be closed for flow experiments. In the open channel system, the channel can be immersed in a cell medium for cell culturing and the abundance of cell medium will
prevent dehydration of the microchannel and the cells. After enclosure, the microchannel-enclosure system will be used for flow experiments: thus the microchannel will constantly receive a fresh supply of cell medium through the inlet to prevent the microchannel from drying. Even if the enclosed system is used in air without constant flow input, many tests only take few minutes and the microchannel may be returned to the culturing medium well before the microchannel dries. Therefore, the microchannel-enclosure should seal liquid inside the microchannel enough for the goals of this project. Furthermore, provided that there is flow and/or no-flow testing takes a short amount of time, the microchannel-enclosure system is also employable in many other situations, as well.

4.4.2 Pressure gauge calibration

Before conducting the flow rate test, the pressure gauge (Omega PX26-005GV) was calibrated to obtain its voltage-to-pressure relationship. The PX26-005GV pressure gauge has the capacity to detect up to 5 psig (34 kPa) [55]. Amplification circuit created by Jaffer [10] removes the DC bias and provides higher accuracy at mV range measurements. The ideal gas law was applied for calibration.

\[ PV = nRT, \]

4-3

By keeping the moles of gas \( n \), gas constant \( R \), and temperature \( T \) constant, change in pressure from change in volume can be calculated by
Therefore, to calibrate the sensor, the input end of the sensor was attached to a 1 ml syringe filled with air and the output end was plugged with silicone. As the syringe was pushed to decrease the volume, the pressure inside syringe, as measured by the pressure gauge, increased. With the change in volume, the pressure inside the syringe can be calculated using equation 4-4. The pressure-to-voltage relationship was then graphed as shown in Figure 4-12. The inverse equation of the graph,

\[ y = 38.294x - 0.9942, \]

was used to convert a voltage reading obtained from the pressure gauge to pressure in kilo-Pascals. The error bars indicate the possible range of values if the syringe reading was off by ±0.01 ml.
4.4.3 Flow Rate Test

The 416 and 528 μm width channels were connected to an external syringe pump and pressure sensor to test the maximum flow rate and pressure that the flexible enclosure can withstand without leakage. As shown in Figure 4-13, the experiment was setup as follows: Steel hypodermic tubing was attached to Tygon tubing. Then the steel tubing was attached to the inlet hole of the flexible enclosure. The opposite end was attached to a pressure sensor. Another length of tubing was used to connect from the pressure sensor to a syringe (Hamilton 1002 TLL, 2.5ml), which was digitally controlled for fluid dispensed mechanically by a syringe pump (Harvard 11 Plus). Finally, another steel hypodermic and Tygon tube were attached to the outlet hole of the enclosure for waste removal.
In the above setup, the pressure was measured between the syringe and the chip. Hence, the pressure measured with the pressure gauge also includes pressure drops from the tubes connecting the pressure gauge to the inlet of the chip and the outlet of the chip to waste collection. Therefore, the fluidic resistances of the tubes were calculated using equations 4-1 and 4-2, and are summarized in Table 4-4 with tube lengths and inner diameters. Note that the gauge-to-chip tube has two parts because tubing with a larger inner diameter was connected to the pressure gauge and the other end of it was connected to a tube with a smaller diameter, which connected to the chip. In order to obtain only the pressure drop across the chip microchannel, the pressure drop from the tubes must be subtracted from the pressure value obtained from the pressure gauge.
Table 4-4: Fluidic resistance of tubing connected to the chip

<table>
<thead>
<tr>
<th>Tube Section</th>
<th>Inner Diameter (in)</th>
<th>Length (cm)</th>
<th>Fluidic resistance (kg/m^4 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gauge to chip (wide)</td>
<td>.03</td>
<td>13.5</td>
<td>$1.68 \times 10^{10}$</td>
</tr>
<tr>
<td>Gauge to chip (narrow)</td>
<td>.02</td>
<td>26.5</td>
<td>$1.67 \times 10^{11}$</td>
</tr>
<tr>
<td>Chip to out</td>
<td>.02</td>
<td>11.0</td>
<td>$6.93 \times 10^{10}$</td>
</tr>
<tr>
<td>Total resistance</td>
<td></td>
<td></td>
<td>$2.53 \times 10^{11}$</td>
</tr>
</tbody>
</table>

To test the chip, the syringe was filled with dyed water to facilitate the visual observation of the flow through the chip. First, the chip and the tubing were primed at 0.01 ml/min flow rate. This slow flow rate was necessary to not rupture the flexible enclosure with airflow pressure during priming. After the chip and tubing were completely filled with water, as shown in Figure 4-14, the flow rate was gradually increased. At each flow rate, the voltage reading from the pressure sensor was recorded, and the voltage was converted to pressure based upon calibration curves obtained prior to testing.

Figure 4-14: Photograph of 416 μm wide channel under flow
Figure 4-15 shows the graph of flow rate versus pressure for the 416 μm wide enclosed microchannel. The maximum operating flow rate for the flexible enclosure was 0.9 ml/min at 1029 Pa and 0.9 ml/min at 1360 Pa for the 416 μm and 528 μm wide channels, respectively. The ±0.001 V (±38.3 Pa) voltage fluctuation was taken into account for error. The experiment was repeated using the same enclosure-microchannel, and the maximum operating flow rate was 0.8 ml/min, thus showing that the enclosure is removable and can be used multiple times. This was also repeated with previously fabricated enclosure with equal results with 0.9 ml/min flow rate.

**Figure 4-15: Experimental flow-rate versus flow for 416 μm wide channel.**
4.4.4 Comparing results

From sections 4.3.1, 4.3.2, and 4.4.3, the pressure across the chip was obtained by calculation, simulation, and experimentation. To compare the three values at each flow rate, the pressure values versus flow rate from each method are shown in Figure 4-16.

By looking at Figure 4-16, the simulated values have slightly higher values than the calculated values. The maximum error between simulation/theory and experiment was 80 Pa at 1029 Pa, which is less than 8% error. These results show that the microfluidic system behaves in a reasonably controllable and predictable manner from 0.1 ml/min to 1 ml/min, and may thus be employed for flow testing over this flow range.
4.5 Cell viability testing

A preliminary cell viability testing was conducted with the channels using leukaemia cells (CEM-wt) [6]. Cells cultured in cell medium were inserted into the channel and immediately sealed with the PDMS enclosure in order to provide the most challenging situation to cell viability as possible. The whole chip was placed in a pool of cell medium (α-MEM medium, Gibco; 10% fetal bovine serum, ATCC; 50 U/ml penicillin, Sigma Aldrich) to prevent the channel from drying out due to evaporation. The chip was placed in an incubator and cultured for 24 hours. After 24 hours, the chip was observed under a microscope. Figure 4-17 shows the cells inside a microchannel.

![Figure 4-17: CEM-wt cells inside the channel through PDMS lid](image)

To determine if cells were actually still alive, the PDMS lid was removed to insert Typan Blue (0.4%, Sigma Aldrich) solution to dye the cells. The solution dyes the dead cells blue but leaves live cells untouched. Figure 4-18 shows leukaemia cells dyed with Typan Blue. As seen in the figure, some dead cells are present amidst the more numerous live cells.
The results shown in Figure 4-18 show that SU-8 channels with PDMS enclosures are compatible with leukaemia cells. More importantly, the majority of the cells survived for 24 hours in the isolated (enclosed) silicon/PDMS/SU-8 environment. Thus, this result shows the potential for developing the channel/lid system for cell testing, at least from the standpoint of cell culturing and biocompatibility.
5 DESIGN PARAMETER CHARACTERIZATION AND FABRICATION OF CHANNELS ON GLASS SUBSTRATE

After the channels and enclosures were fabricated and tested on silicon wafer, the channels were then fabricated on glass. With this change in the substrate material, two major changes in fabrication process were implemented: 1) addition of adhesive and alignment layers and 2) change in exposure dosage.

5.1 Reasons to fabricate on glass

The main reason for moving to a glass substrate is to make the channel and enclosure structures more applicable to cell studies by providing a transparent substrate for an inverted microscope. Unlike the compound microscope, the inverted microscope requires the object under it to be transparent to obtain the magnified image. Since both structural materials, SU-8 and PDMS, are transparent materials, changing from a compound to inverted microscope only requires a change in the substrate material to result in an optically transparent chip.

5.2 Fabrication

This section describes the fabrication parameters of the microchannels and enclosures on a glass substrate. The fabrication method is similar to that on silicon, but the SU-8 has poor adhesion to glass: thus, an adhesion layer was added to the process. Also, SU-8 and glass are both optically transparent so a chromium layer was added to
ease alignment. Finally, baking and exposure times were adjusted for the lower thermal conductivity and transparency of glass.

5.2.1 Adhesion and alignment layer

In order to fabricate the channels and enclosures on glass, two layers were added to the process: adhesion and alignment layers. The main difficulty encountered when fabricating on glass was obtaining good adhesion between the SU-8 and the glass. SU-8 structures, especially structures that are larger than 1 cm, do not adhere well to glass without any adhesive layer between the glass and the SU-8. As options for the adhesive layer, OmniCoat (Mircochem) [35], a 50 nm thick chromium layer, and a 5 μm thick SU-8 2005 layer were tested. For both OmniCoat and chromium, structures sized hundreds of micrometers or less adhered well to the surface, but very large structures (larger than 1 cm in size) fell off the substrate due to large sheer stress induced during fabrication. The best adhesive that overcame this sheer stress problem was SU-8 2005. A 5 μm thick layer of SU-8 2005 was spun onto the substrate, and the film was cross-linked during the soft-bake of the first structural layer to adhere the first structural layer to the thin film by inducing wrinkles along the SU-2005 surface by placing it on a 120°C hot-plate without ramping, thus thermal shocking the film.

Many initial fabrication runs failed due to misalignment. Multiple SU-8 structural layers were very difficult to align, because the glass and the SU-8 are transparent and thus there is not enough contrast between them for alignment. To solve this problem, a 50 nm chromium layer was deposited and patterned on the glass as an alignment layer.
5.2.2 Fabrication sequence of channels and enclosure mould on glass

To fabricate SU-8 channels on glass, the alignment and adhesion layer must be processed first. This process is outlined in Figure 5-1.

First, 50 nm of chromium was deposited onto a 3x3 glass slide (Microscope Depot), and the SC1813 photoresist (PR) was spun on at 3000 rpm for 30 seconds (a). Then the PR was soft-baked at 100°C for 3 minutes and exposed for 18 seconds at 10 mW/cm². After hard bake at 100°C for 1 minute, photoresist was developed in MF319 to develop away the exposed areas (b). The glass was then dipped into a chromium etchant for 30 seconds to pattern the chromium (c). After a thorough rinse with deionized (DI) water, the remaining photoresist was stripped off in acetone and rinsed in DI water (d).
Finally, SU-8 2005 was spun on at 3000 rpm for 30 seconds, soft-baked at 95°C for 3 minutes, and exposed for 20 seconds at 10 mW/cm² (e).

After completing the steps outlined in Figure 5-1, structural layers were fabricated in the same sequence as shown in Figure 4-3 in the previous chapter. However, baking and exposure parameters were different, and theses parameters are summarized in Table 5-1.

Table 5-1: SU-8 channel and PDMS lid fabrication parameters on glass substrate

<table>
<thead>
<tr>
<th>Layer</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st structural layer</td>
<td>-spin SU-8 100 at 1500 rpm for 40s</td>
</tr>
<tr>
<td></td>
<td>-bake at 120°C for 35 min and cool to room temperature</td>
</tr>
<tr>
<td></td>
<td>-expose for 3 min at 10 mJ/cm² s</td>
</tr>
<tr>
<td>2nd structural layer</td>
<td>-spin SU-8 100 at 3000 rpm for 30s</td>
</tr>
<tr>
<td></td>
<td>-bake at 120°C for 15 min and cool to room temperature</td>
</tr>
<tr>
<td></td>
<td>-expose for 2 min 15 s at 10 mJ/cm² s</td>
</tr>
<tr>
<td></td>
<td>-post-exposure bake 15 min at 65°C</td>
</tr>
<tr>
<td></td>
<td>-develop in SU-8 Developer for approximately 4 min</td>
</tr>
<tr>
<td></td>
<td>-rinse with fresh SU-8 Developer and IPA and dry</td>
</tr>
<tr>
<td>3rd structural layer</td>
<td>-dispense 7.50 g of SU-8 100</td>
</tr>
<tr>
<td></td>
<td>-bake at 120°C 2 hours</td>
</tr>
<tr>
<td></td>
<td>-expose for 10 in at 10 mJ/cm² s</td>
</tr>
<tr>
<td></td>
<td>-post-exposure bake for 30 min at 65°C</td>
</tr>
<tr>
<td></td>
<td>-develop for approximately 20 min</td>
</tr>
<tr>
<td></td>
<td>-rinse and dry</td>
</tr>
<tr>
<td>PDMS enclosure</td>
<td>-mix base and curing agent of Sylgard 184 in 10:1 ratio</td>
</tr>
<tr>
<td></td>
<td>-place mixture in vacuum oven for 15 min</td>
</tr>
<tr>
<td></td>
<td>-pour mixture into the mould and use glass slide to flatten the top</td>
</tr>
<tr>
<td></td>
<td>-place in vacuum oven for 15 min</td>
</tr>
<tr>
<td></td>
<td>-bake at 85°C for 90 min</td>
</tr>
</tbody>
</table>

As shown in Table 5-1, the soft-baking temperature was done at 120°C constantly without lowering the temperature to 95°C because the thermal conductivity of glass is
lower than that of silicon, thus requiring a longer baking time to remove the solvents from SU-8. The moulds for the PDMS enclosure were fabricated on the same substrate as the microchannels using the same process as shown in Section 4.2.1. Figure 5-2 show the fabricated SU-8 channels and moulds on a glass substrate.

![Channels with lid and Lid moulds](image)

**Figure 5-2: Fabricated channels and moulds on 3 x 3 in glass**

Channel width and depth were measured using a microscope and a profilometer; the values are shown in Table 5-2. In this fabrication run, moulds with various channel widths were fabricated to determine the optimal channel to lid width. This will be discussed more in detail in the experimental section.

<table>
<thead>
<tr>
<th>Mask width</th>
<th>Channel width</th>
<th>Channel depth</th>
<th>Mould depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>360</td>
<td>295</td>
<td>93</td>
</tr>
<tr>
<td>500</td>
<td>477</td>
<td>298</td>
<td>91</td>
</tr>
</tbody>
</table>
5.3 Fluidic analysis and simulation

5.3.1 Theoretical calculation

The effective diameters and fluidic resistances of the channels were calculated using equations 4-1 and 4-2 and are summarized in Table 5-3. Once again, the depth of the channel was assumed to be nominally 200 μm. The dimensions of the inlet/outlet sections were same as those on silicon, so their fluidic resistance remains the same ($R_{\text{inlet/outlet}}=3.45\times10^9$ kg/m$^4$ s).
Table 5-3: Effective diameters and fluidic resistances of channels on glass

<table>
<thead>
<tr>
<th>Width (μm)</th>
<th>$K_{\text{channel}}$</th>
<th>$D_{\text{eff , channel}}$ (m)</th>
<th>$R_{\text{channel}}$ (kg/m$^4$ s)</th>
<th>$R_{\text{total}}$ (kg/m$^4$ s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>360</td>
<td>62.19</td>
<td>2.65x10^{-4}</td>
<td>7.70x10^{10}</td>
<td>8.39x10^{10}</td>
</tr>
<tr>
<td>477</td>
<td>65.47</td>
<td>2.76x10^{-4}</td>
<td>6.56x10^{10}</td>
<td>7.25x10^{10}</td>
</tr>
</tbody>
</table>

$R_{\text{total}}$ values of Table 5-3 are multiplied with flow rate, ranging form 0 to 1 ml/min, to obtain the flow rate versus pressure relationship which is shown in Figure 5-3.

![Figure 5-3: Calculated flow rate versus pressure for 360 and 477 μm wide channel](image)

As consistent with previously calculated pressure values on silicon, the pressure drop increases linearly with flow rate and higher pressure drop occurs for the narrower 360 μm wide microchannel.
5.3.2 Simulation

Using COMSOL, the pressure drop along the channel was simulated. Figure 5-4 show the simulated pressure of the 360μm wide channel at 1 ml/min flow rate.

Figure 5-4: Simulation of 360 μm channel under flow at 1 ml/min.
Maximum pressure: 1331 Pa

The maximum pressure of 1331 Pa was predicted near the inlet. Just as in Section 4.3.2, results of simulation are graphed as pressure versus flow rate in Figure 5-5.
By contrasting Figure 5-3 and Figure 5-5, the simulated pressure values are higher than that of the calculated values, and this trend is more prominent for the narrower channel. Both calculated and simulated values will be further compared with experimental values in Section 5.4.

5.4 Fluidic Testing

Using the same setup as Section 4.4.3, the channels were tested under flow. In addition to fluidic testing, various sizes of PDMS enclosures ranging from 400 μm to 460 μm and 500 μm to 560 μm were tested to determine the optimal channel to enclosure width relationship. Table 5-4 summarizes the designed and measured widths of the enclosure mould.
Table 5-4: Designed and measured enclosure widths in μm

<table>
<thead>
<tr>
<th>Mask width</th>
<th>Measured width</th>
<th>Mask width</th>
<th>Measured width</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>347</td>
<td>500</td>
<td>503</td>
</tr>
<tr>
<td>410</td>
<td>382</td>
<td>510</td>
<td>517</td>
</tr>
<tr>
<td>420</td>
<td>417</td>
<td>520</td>
<td>527</td>
</tr>
<tr>
<td>430</td>
<td>433</td>
<td>530</td>
<td>532</td>
</tr>
<tr>
<td>440</td>
<td>452</td>
<td>540</td>
<td>524</td>
</tr>
<tr>
<td>450</td>
<td>431</td>
<td>550</td>
<td>563</td>
</tr>
<tr>
<td>460</td>
<td>464</td>
<td>560</td>
<td>553</td>
</tr>
</tbody>
</table>

The above enclosures were individually tested with the 360 and 477 μm wide microchannels. As a result, the 417 μm and 532 μm wide enclosures withstood the fluidic testing and were able to contain liquid under flow without leakage. The 360 μm wide channel with 417 μm wide enclosure withstood a flow rate of 0.3 ml/min, corresponding to a maximum pressure of 3857 Pa. The 477 μm wide channel with 532 μm wide enclosure had poorer performance with a maximum flow rate and pressure of only 0.15 ml/min and 1196 Pa, respectively. In order to better simulate the application environment for the channels, the entire chip was submerged in water to imitate wet cell culturing environment. Then the enclosure was placed on the channel and tested for pressure versus flow rate. Figure 5-6 shows a 360 μm wide channel submerged in water with green coloured water running through the channel and exiting at the outlet freely into the uncoloured water.
Figure 5-6: 360 μm wide microchannel tested in submersion in water

Figure 5-7 shows the flow rate versus pressure measured using the syringe pump and pressure gauge. The values shown in the figure take the pressure-drop across the external tubing into account.
Figure 5-7: Experimental flow rate versus pressure for 360 μm wide channel

Again, the error bars indicate error resulting from 0.01 V voltage fluctuation (± 366 Pa). Results of the calculated, simulated, and experimental flow rate versus pressure for the 360 μm wide channel are compared in Figure 5-8.
Figure 5-8: Pressure values for 360μm channel by calculation, simulation, and experimentation

Due to the scale of Figure 5-8, the calculated and simulated values are placed on top of each other. However, the actual values indicate that the simulated values are slightly larger than those of the calculated values. Unlike the results obtained for channels on silicon (Figure 4-15), the experimental results are much higher than both of simulated and calculated values. The most likely reason is that the experimental values are very sensitive to the voltage output: 0.01 V difference in the output results in 365 Pa difference after voltage to pressure conversion. Other reason may be due to slow leakage between the SU-8/PDMS interface that cannot be seen. Also in theory and calculation, the channel is surface is assumed to be uniform with no change in depth. However, according to the profilometer reading, the surface of the microchannel is not perfectly
This may result in a narrower channel in places leading to higher pressure drop along the microchannel. However, these results show that the glass/SU-8/PDMS channel and enclosure system can be utilized under flow and remain fluid tight, although the system is not as predictable as the silicon substrate system.

5.5 Preliminary cell testing

Leukaemia cells [6], CEM-wt previously shown in Section 4.5, were inserted into the fabricated channels on glass to observe if cells in the microchannel can be observed under the inverted microscope. This test determines if the glass substrate microchannel/enclosure system is fit for cell studies using an inverted microscope.

First, leukaemia cells were inserted into the microchannels using a pipette. The glass substrate with SU-8 channel was placed under an inverted microscope (Nikon Eclipse TE300). Figure 5-9 shows leukaemia cell inside the microchannel at various magnifications.

Figure 5-9 (c) shows the leukaemia cells at 40 times magnification, where each cell can be seen clearly. Next, the PDMS enclosure was placed on the channel to test if cell were still visible under the microscope through the PDMS layer. Figure 5-10 shows
the cells inside the microscope channel through the PDMS enclosure at 40 times magnification. A yellow filter was used to add more contrast between the PDMS and the cells.

![Image of cells in SU-8 microchannel with assembled PDMS enclosure at x40 magnification]

**Figure 5-10: Leukaemia cells in SU-8 microchannel with assembled PDMS enclosure at x40 magnification**

Observing cells through the PDMS enclosure is more difficult than without the enclosure. To enhance the visual quality, the PDMS enclosure needs to be fabricated very cleanly without air bubbles. In addition, with the utilization of optical filter to enhance cell image, the cells can very easily be seen. These preliminary cell viability tests show that the SU-8/PDMS/glass channel-enclosure system is fit for cell studies and observation under inverted microscope.
6 APPLICATIONS AND FUTURE WORK

This section discusses additional potential applications of the removable lid and the thick SU-8 process. One potential application is the development of PDMS connectors that utilize the thick SU-8 process to fabricate world-to-chip connectors that can be attached to existing microchannels. Another potential application is a cell manipulation chip that utilizes the removable enclosure by modifying the current channel design so that single cells can be manipulated with flow for cell drug testing. These applications are still in development, but the results obtained from this thesis provide a good foundation and show promising potential for these applications.

6.1 Connectors

As an application of the ultra-thick SU-8 process, a modular PDMS world-to-chip connector was developed. The connector can be applied to existing microfluidic channels to provide an easy and convenient world-to-chip interface.

6.1.1 Design

The connector was designed to interface with a microchannel. Connectors are 1mm thick PDMS cylinders with a 200 μm diameter hole in the centre and are fabricated by casting onto an ultra-thick SU-8 mould. These PDMS connectors, as shown in Figure 6-1, connect to enclosed microchannels to provide a world-to-chip interface.
Two types of connectors were designed and fabricated: single and double-joint connectors as shown in Figure 6-2.

Individual connectors were designed with single hole and double-joint connectors were designed with two holes with predetermined centre-to-centre length. The double-joint connectors have centre-to-centre lengths of 4200 and 9800 μm. The double-joint connectors were designed for compatibility with the enclosures from this thesis.

6.1.2 Fabrication and Results

The SU-8 mould master fabrication followed the exact same fabrication process as the ultra-thick SU-8 process outlined in Chapter 3. The liquid PDMS was then poured into the mould to form the connectors. Figure 6-3 shows the fabricated single and double PDMS connectors.
The connectors were then attached to the PDMS enclosure using uncured PDMS as an adhesive, and placed on a hot plate to cure. Although adhesive bonding was used, surface activation bonding [23] could easily be used instead. The enclosure with the connectors was placed on the SU-8 microchannel for testing. The fluid passed through the connector and the microchannel using a syringe pump at a flow rate up to 0.3 ml/min. The double connectors performed well and leakage occurred between the SU-8 and PDMS interface and not at the connector-enclosure interface at 0.3 ml/min for a 360 μm wide channel. On the other hand, the single connectors did not function at all because the connector hole was blocked by the uncured PDMS. The experiment showed that even though the single connectors might be more versatile to be used in any microchannel, its size makes gluing very difficult as it may easily block the connector hole. Even if surface activation bonding were used instead, the single connectors would be more difficult to handle for manual assembly. In contrast, the larger double connectors are easier to handle and bond without blocking the holes with adhesive, but must be fabricated for specific channel lengths.
6.2 Cell control chip

6.2.1 Design

The removable enclosure and channel designs may be altered to suit specific cell experiments. For example, for some cancer cell drug testing, cells need to be individually separated and controlled for individual drug tests. Li et al. [6], proposed microchannels fabricated in PDMS for cancer cell drug testing as shown in Figure 6-4. Dr. Li’s lab has been working with our group toward the development of a cell platform with removable enclosure using the technology outlined in this thesis.

![Three way cell channel](image)

**Figure 6-4: Three way cell channel [6]**

The platform features two inlet channels and one outlet channel. A cell can be retained using hydrodynamic force from inlet flow when testing. A cell can be stopped and retained inside a groove, situated in the center of channel, and drugs can be applied to it directly from the centre channel.

6.2.2 Fabrication and results

A prototype was fabricated on a glass substrate using the same fabrication sequence introduced in Section 5.2. However, the initial testing resulted in leakage from between the enclosure/channel interface at a low flow rate (0.01 ml/min). The most likely
factor for the low operating pressure is due to the complex shape of the channels. Hence, similar to Section 5.4 to optimize the channel widths, wide range of enclosures with differently sized features need to be tried in order to find the optimized sizes for each feature on the enclosure.
7 DISCUSSIONS AND CONCLUSIONS

The development of an adhesive-free PDMS enclosure for sealing SU-8 microchannels has been presented in this thesis. In order to fasten the PDMS enclosure to the SU-8, and to provide moulds for the PDMS enclosure, a thick SU-8 fabrication process was characterized so that film thickness can be predicted by mass. Using the thick SU-8 process newly combined with multiple layers of conventionally (spin coated) SU-8 layers, microchannels and moulds for the PDMS enclosure were fabricated with special interlocking structures. The resulting sealed systems on both silicon and glass substrates were tested under flow and compared with calculated and simulated values. The results showed that SU-8 channel with PDMS enclosures have great potential for use in cell testing. These results are further discussed below.

As a first step in fabricating the SU-8 channels and moulds, an ultra-thick SU-8 fabrication process was characterized based on dispensed mass for determining film thickness. Predicting SU-8 film thickness by mass improved upon previously published work that predicts film thickness by volume because volume dispensing by a syringe increases air bubbles content due to syringe dispense that cannot be removed during the soft-baking process. In contrast, it has been well known by other researchers that dispensing by pouring minimizes air bubbles. Thus, dispensing by mass minimizes the initial air bubbles in the SU-8 so that all bubbles can be removed during the soft-bake. It was found that 10.00 g and 7.50 g of SU-8 results in 1 mm high structures for 4 inch Si wafer and 3 x 3 inch glass substrate, respectively. Despite improvements in air bubble
content, during the characterization, the film thickness across the substrate differed more than 200 μm. Thus, levelling of the hot plate during soft-bake is critical for fabricating a uniform film, as other researchers have reported for thinner films.

As a prototype for the removable enclosure, the channels and enclosure moulds were fabricated in silicon using a fabrication process combining the characterized thick film process with conventional (spin coated) SU-8 layers for the first time. Once the channel and enclosure were assembled, simple evaporation test showed that the enclosure-microchannel interface was not completely fluid tight, but for the intended cell application, channel drying can be eliminated or minimized using three methods: 1) constantly replenishing the system with new cell medium, even at a very low flow rate; 2) immersing the entire system in a pool of cell medium; 3) performing static flow tests with the system in air that require only a few minutes. Flow testing results show that the lids remained fluid tight under pressurized flow up to 0.9 ml/min flow rate for both 416 and 528 μm wide channels corresponding to 1029 Pa and 1326 Pa maximum pressures, respectively. The experimental pressure drops were within error percentage of the calculated and simulated values. In addition, cell viability tests were performed through successful culturing of cells in open channels with subsequent enclosure to isolate the channel system. Overall, the experimental results obtained from the microchannel-enclosure on silicon system showed the validity of the concept for cell study and experiment.

Then the same enclosure and channels were fabricated on glass substrates to better accommodate cell imaging and potential application involving fluorescence. Poor adhesion of SU-8 to glass prevented using the exact same fabrication sequence as that on
silicon, but testing showed that SU-8 2005 and chromium adhesion layers provided better adhesion and alignment of structural layers. Measuring the dimensions of channels and moulds showed the enclosure width must be wider than that of the channels to obtain fluid tight seal by at least 11 to 15% to result in optimally sealed systems. Channels on glass remained liquid tight up to 0.3 ml/min with 3857 Pa and 0.15 ml/min with 1562 Pa for 360 and 477 μm wide channels, respectively. Initial cell testing demonstrated that the channels are suitable for clearly observing cells under an inverted microscope, even with light scattering through the enclosure layer. Unfortunately, the maximum operating flow rate was lower than those on a silicon substrate, but the maximum pressures were higher, even considering the differences in channel size between the channels on silicon and glass. This indicates a high fluidic resistance terms likely due to uneven channel size caused by the more nonuniform SU-8 on glass process. Still, the pressures and flow rates were within a usable range, even though flow rate and pressure prediction (and hence shear stress prediction on cells adhered to the channel wall) may require a calibration step to accurately predict for each system.

In addition to the successful fabrication of simple channels for cell experiments, the ultra-thick SU-8 process has many other potential applications. The ultra-thick SU-8 process can be used to fabricate world-to-chip interconnect for existing microfluidic systems or for designs that are incompatible with the ultra-thick SU-8 process. Such connectors were successfully demonstrated. In addition, the SU-8 microchannel and PDMS enclosure designs can be modified to accommodate specific cell testing requirements, e.g., individually testing of cells with different pharmaceutical or other drugs. Furthermore, the process can be investigated for applications of low temperature
bonding for fluidic packaging of fragile sensors after enzyme immobilization. More work is needed for all of these applications, but the results presented in this thesis form a solid foundation from which these applications can be explored.

In conclusion, the research presented in this thesis successfully demonstrates the potential for a removable PDMS enclosure that can be used to seal SU-8 microfluidic components based solely on a mechanical fit.
APPENDIX A: LIST OF PUBLICATIONS

T. Ueda, B.L. Gray, Y. Chen, P. Li "Flexible Enclosure for Fluidic Sealing of Microcomponents," presented at Microfluidics, BioMEMS, and Medical Microsystems VI, San Jose, CA, USA, 2008.


APPENDIX B: EQUIPMENT AND MATERIALS

B.1 SFU Engineering clean room

1. Photoresist Spinner: Headway Research

2. Aligner: Quintel Q-2001CT

3. Hot plate: Torrey Pines Scientific A130

4. Oven: Fisher Scientific 825-F

5. Sputter: Corona Vacuum Coater

6. Ultrasonic cleaner: Branson 2210

7. Microscope (compound): Olympus BH2-UMA

8. Microscope measurement: Olympus OSM and OSM-D4

9. Profilometer: Tencor Instruments Alpha-Step 500

B.2 SFU Microinstrumentation Laboratory

1. Digital multimeter: Fluke 8010A

2. DC power supply: Circuit specialists CSI3005XII

3. Microscope: Motic SMZ-168

4. Camera: Canon Powershot S3-IS

5. Pressure sensor: Omega PX26-005GV

7. Micrometer: Fowler IP54

B.3 SFU Chemistry Li Lab

1. Microscope (inverted): Nikon Eclipse TE300

2. Cell medium: Gibco α-medium; ATCC fetal bovine serum; Sigma Aldrich penicillin

B.3 Material list

1. PDMS: Dow Corning Sylgard 184

2. SU-8: Microchem SU-8 100 and SU-8 2005

3. Photoresist: Shipley SC1813

4. Glass slides: Microscope Depot 3x3 in microscope slides

5. Silicon Wafers: Silicon Sense <100> single side polished silicon wafers
### APPENDIX C: FABRICATION RUN SHEET

<table>
<thead>
<tr>
<th>Process Step #</th>
<th>Process Conditions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cleaning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>__ 1</td>
<td>RCA 1</td>
<td>Volumes used and temperatures</td>
</tr>
<tr>
<td></td>
<td>-100:15:15 of water:NH₄OH: H₂O₂ at 80 (± 5 °C) for 10 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-DI rinse 3 min</td>
<td></td>
</tr>
<tr>
<td>__ 2</td>
<td>HF Dip</td>
<td>-do not do for glass substrate</td>
</tr>
<tr>
<td></td>
<td>-10:1 of water:HF at room temp for 30s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-DI rinse 5 min</td>
<td></td>
</tr>
<tr>
<td>__ 3</td>
<td>Spin Dry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use wafer spinner and N₂ gun.</td>
<td></td>
</tr>
<tr>
<td><strong>1st structure layer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>__ 1</td>
<td>Prebake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temp = 120°C, Time = 30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Let cool for 5 mins.</td>
<td></td>
</tr>
<tr>
<td>__ 2</td>
<td>Spin Photoresist</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use Microchem SU-8 Pour from bottle onto wafer, spiral like a snail around resist on surface. Leave a ~ 1” diameter blob on wafer. Spin promptly. Spread: Spin for ~ 1-2 seconds, repeat 3 times, to spread resist on wafer, near edge. Spin: At comments: see time, speed. (suggested time: 1-2min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SU-8 Type:_______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time: ____ seconds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Speed: ______ RPM</td>
<td></td>
</tr>
<tr>
<td>Process Step #</td>
<td>Process Conditions</td>
<td>Comments</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------</td>
<td>----------</td>
</tr>
</tbody>
</table>
| __ 3          | **Softbake (remove solvents)**<br>Use hotplate.<br>  
  - Silicon: start 120°C then decrease to 90°C  
  - Glass: start 120°C  
  - Cool to room temperature | Start: ____ C, for ____ min  
  Ramping: ____ C / hr ( __ min)  
  **Softbake:**<br>  
  Temp (C)  
  Time (min)  
  Cool: ____ C, for ____ min |
| __ 4          | **Align and Expose (Quintell: 410nm)**<br>Use attached sheet.<br>  
  (350nm and higher, optimally 365nm) | Time (in minutes) |
| **2nd t structure layer** | | |
| __ 1          | **Spin Photoresist**<br>Use Microchem SU-8 Pour from bottle onto wafer, spiral like a snail around resist on surface. Leave a ~ 1” diameter blob on wafer. Spin promptly.<br>  
  **Spread:** Spin for ~ 1-2 seconds, repeat 3 times, to spread resist on wafer, near edge. **Spin:** At comments: see time, speed. (suggested time: 1-2min) | SU-8 Type:______  
  Time: ____ seconds  
  Speed: ______ RPM |
| __ 2          | **Softbake (remove solvents)**<br>Use hotplate.<br>  
  - Silicon: start 120°C then decrease to 90°C  
  - Glass: start 120°C  
  - Cool to room temperature | Start: ____ C, for ____ min  
  Ramping: ____ C / hr ( __ min)  
  **Softbake:**<br>  
  Temp (C)  
  Time (min)  
  Cool: ____ C, for ____ min |
| __ 3          | **Align and Expose (Quintell: 410nm)**<br>Use attached sheet.<br>  
  (350nm and higher, optimally 365nm) | Time (in minutes) |
# Fabrication Run Sheet for 3-Layer SU-8 Structures and PDMS Run

<table>
<thead>
<tr>
<th>Process Step #</th>
<th>Process Conditions</th>
<th>Comments</th>
</tr>
</thead>
</table>
| __ 4           | Post-Exposure Bake (PEB) (for full cross-linking) | Start: _____ C, for _____ min  
Ramping: _____ C / hr ( ____ min)  
PEB 1 (sugg. 65C) |
| __ 5           | Develop | Time: ____ minutes |
| __ 6           | Rinse | -spray clean SU-8 developer  
-spray IPA |
| __ 7           | Spin Dry | Spin wafer dry, use nitrogen gun where applicable. |
| **3rd Structure layer** | **Cast Photoresist** | Use Microchem SU-8. Pour from bottle onto wafer. Use ~10g for 1mm thick on Si wafer. 7.5g for 1mm thick on glass.  
Mass of SU-8 _____ g |
| __ 2           | Softbake (remove solvents) | Start: _____ C, for _____ min  
Ramping: _____ C / hr ( ____ min)  
Softbake:  
Temp (C)  
Time (min) |
| __ 3           | Align and Expose (Quintell: 410nm) (350nm and higher, optimally 365nm) | Time (in minutes) |
| __ 4           | Post-Exposure Bake (PEB) | Start: _____ C, for _____ min |
## Fabrication run sheet for 3-layer SU-8 structures and PDMS run

<table>
<thead>
<tr>
<th>Process Step #</th>
<th>Process Conditions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>___ 5 ___</td>
<td>** DEVELOP **</td>
<td>Ramping: ____ °C / hr ( ____ min)</td>
</tr>
<tr>
<td>___ 5 ___</td>
<td>** DEVELOP **</td>
<td>** PEB1:**</td>
</tr>
</tbody>
</table>
| ___ 5 ___ | ** DEVELOP ** | ** Temp (°C)  
Time (min)** |
| ___ 6 ___ | ** Rinse ** | Time: ____ minutes |
| ___ 7 ___ | ** Spin Dry ** | |

### PDMS enclosures

| ___ 1 ___ | ** Prepare PDMS ** | Base mass ________g  
Curing agent mass _______g |
| ___ 2 ___ | ** Vacuum PDMS ** | Time: ________min |
| ___ 3 ___ | ** Cast PDMS ** | |
| ___ 4 ___ | ** Vacuum PDMS ** | Time: ________min |
| ___ 5 ___ | ** Cure PDMS ** | Temp: _______ °C  
Time: ________min |
REFERENCE LIST


