The Fabrication & Characterization of an Electrokinetic Microfluidic Pump

from SU-8, a Negative Epoxy-Based Photoresist

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Engineering

By

Nash Anderson

June, 2013

© 2013

Nash Anderson

ALL RIGHTS RESERVED

COMMITTEE MEMBERSHIP

TITLE:	The Fabrication & Characterization of an Electrokinetic Microfluidic Pump from SU-8, a Negative Epoxy-Based Photoresist
AUTHOR:	Nash Anderson
DATE SUBMITTED:	June, 2013
COMMITTEE CHAIR:	Dr. Richard Savage, Professor Materials Engineering Department Cal Poly, San Luis Obispo
COMMITTEE MEMBER:	Dr. Thomas Mackin Mechanical Engineering Department Cal Poly, San Luis Obispo
COMMITTEE MEMBER:	Dr. Philip Costanzo Chemistry Department Cal Poly, San Luis Obispo

Abstract

The Fabrication & Characterization of an Electrokinetic Microfluidic Pump

from SU-8, a Negative Epoxy-Based Photoresist

Nash Anderson

Microfluidics refers to manipulation, precise control, and behavior of fluids at the micro and nanoliter scales. It has entered the realm of science as a way to precisely measure or mix small amounts of fluid to perform highly controlled reactions. Glass and polydimethylsiloxane (PDMS) are common materials used to create microfluidic devices; however, glass is difficult to process and PDMS is relatively hydrophobic. In this study, SU-8, an epoxy based (negative) photoresist was used to create various electrokinetic microfluidic chips. SU-8 is commonly used in microelectromechanical design. Spin coating of various SU-8 formulations allows for 1 μm to 100 μm thick layers with aspect ratios reportedly as high as 50:1. Case studies were performed to understand the curing/crosslinking process of SU-8 by differential scanning calorimetry. Supplier (MicroChem) recommended parameters were then altered to allow for adequate development of microfluidic channels, while maintaining enough molecular mobility to subsequently bond the SU-8 to a secondary substrate. Three SU-8 layers were used to create fully (SU-8) enclosed microfluidic channels. An (1) SU-8 2050 fully cured base layer was used as a platform on silicon to build from, (2) an SU-8 2050 partially cured layer for developing microfluidic channels, and (3) an SU-8 2007 uncured layer for bonding a secondary substrate to enclose the microfluidic channels. Bond quality was verified by optical and scanning electron microscopy, which resulted in a nearly 100% bond with little to no reflow of SU-8 into channels. Working pressures (ΔP across the capillary) of 15.57 lb/in^2 (max detection) were obtained with no fluid leaks. Electroosmotic flow and streaming potential measurements failed. Electrophoretic behavior of glass particles was observed and particle velocities were compared by the application of 200 volts and 300 volts, across a channel length of 2 cm. Particle velocities obtained ranged from 100 $\mu\text{m/s}$ to 1500 $\mu\text{m/s}$.

ACKNOWLEDGMENTS

There are many people that have helped me grow tremendously during my stay here at Cal Poly.

I would like to acknowledge my advisor, Dr. Richard Savage for all the help and support he has provided throughout this thesis project. It has been a pleasure being one of your research students and thank you for all your support, inside and outside the clean room.

I would also like to thank the chemistry department and specifically the Polymers and Coatings program for their support and access to the chemistry facilities. Dr. Raymond Fernando and Dr. Philip Costanzo have helped me greatly with a better understanding of the chemistry and background for this project and my upcoming career.

I would like to thank my teacher and friend Dr. Thomas Mackin. I really enjoyed the work we did together on our research paper, as well as all the other smaller projects. It was always fun being part of the team.

Table of Contents

List of Tables.....	xi
List of Equations.....	xii
List of Figures.....	xiii
Keywords.....	xx
1. Introduction.....	1
1.1. Project Influence & Problem Statement	1
1.2. Project Scope & Summary	1
1.3. Broader Impacts.....	3
1.3.1. Health and Safety	3
1.3.2. Environmental Factors.....	3
1.4. Background.....	4
1.4.1. Microfluidics	4
1.4.2. Lab on a Chip	6
1.4.3. Micropumps	6
1.4.4. Electrokinetics	8
1.4.5. Microfluidics Mixing	10
1.4.6. Electroosmotic Flow	11
1.4.7. Zeta Potential	13
1.4.8. Streaming Potential	14
1.4.9. Measuring Flow in a Microchannel.....	15
2. Materials Selection.....	16
2.1. Glass.....	16
2.2. Polydimethylsiloxane (PDMS)	16
2.3. SU-8 by MicroChem	19
2.3.1. SU-8 Processing.....	20
2.3.2. SU-8 Applications	22

3.	Microfabrication Procedures	23
3.1.	Microfabrication Bond Iterations	23
3.2.	Bonding Method 1	25
3.2.1.	Mechanical Drilling of Fluid Input/Output Holes Through Pyrex Wafer	26
3.2.2.	Silicon & Pyrex Wafer Clean and Dehydration	27
3.2.3.	Spin Coating of SU-8	27
3.2.4.	Edge Bead Removal	29
3.2.5.	Soft Bake	30
3.2.6.	Exposure	31
3.2.7.	Post Exposure Bake	33
3.2.8.	Development	34
3.2.9.	Pre-Bond	34
3.2.10.	Bond Bake	35
3.2.11.	PDMS Microfluidic Hookup	36
3.2.12.	Method 1 Silicon to Pyrex Wafer Bonding Results	37
3.2.13.	Method 1 Silicon to Pyrex Cross Sectional SEMs	39
3.2.14.	Method 1 Results & Conclusion	42
3.2.15.	Plan for Testing & Continuing to Method 2	43
3.3.	Investigation of SU-8 Crosslinking	43
3.3.1.	Description of Crosslinking Investigation	43
3.3.2.	Scope of Experiment	44
3.3.3.	Crosslinking	45
3.3.3.1.	Step 1: Initiation	45
3.3.3.2.	Step 2: Propagation	47
3.3.3.3.	Step 3: Termination	47
3.3.4.	Experimental Methods & Materials	47
3.3.4.1.	DSC Analysis	48
3.3.4.2.	TGA Analysis	49

3.3.5.	DSC & TGA Results & Discussion	49
3.3.5.1.	DSC Results	49
3.3.5.2.	TGA Results	55
3.3.5.3.	Weight Loss Experiment in Developer (PGMEA)	58
3.3.5.4.	Conclusions of SU-8 Crosslinking Experiment	60
3.4.	Method 2 Bond Processing	61
3.4.1.	Base Layer Processing Steps & New Mask Design	61
3.4.1.1.	Silicon Wafer Piranha clean/Dehydration	62
3.4.1.2.	Spin Coat	62
3.4.1.3.	Edge Bead Removal	63
3.4.1.4.	Soft Bake	63
3.4.1.5.	Exposure	63
3.4.1.6.	Post Exposure Bake	63
3.4.1.7.	Physical Vapor Deposition of Gold	63
3.4.1.8.	Positive Resist Spin Coating	64
3.4.1.9.	Positive Resist Soft Bake	64
3.4.1.10.	Positive Resist Exposure	64
3.4.1.11.	Positive Resist Develop	65
3.4.1.12.	Hard Bake	66
3.4.1.13.	Gold Wet Etching	66
3.4.1.14.	Positive Resist Strip	67
3.4.2.	“Functional” Microchannel Layer Processing Steps & Bond	67
3.4.2.1.	Wafer Clean	68
3.4.2.2.	Wafer Dehydration	70
3.4.2.3.	Functional Microchannel Layer Spin Coating	70
3.4.2.4.	Soft Bake	72
3.4.2.5.	Exposure of Functional Layer	72
3.4.2.6.	Inhibited Post Exposure Bake of Functional Layer	73

3.4.2.7.	Development of Functional Layer	73
3.4.2.8.	Drilling Pyrex Wafer Input/Output Fluid Holes	74
3.4.2.9.	Cleaning Pyrex Slide/Dehydration	74
3.4.2.10.	Bond Method 2	74
3.4.3.	Method 2 Bond Results	75
3.4.4.	Method 2 Conclusions & Hypothesis Revision 1	79
3.5.	Method 3 Processing Steps.....	80
3.5.1.	Pyrex Slide Spin Coating.....	80
3.5.2.	Soft Bake of Pyrex.....	81
3.5.2.1.	Method 3 Bond	81
3.5.3.	Method 3 Bond Results	81
3.5.4.	Method 3 Conclusions	86
3.6.	Method 4 Final Bond	87
3.6.1.	Method 4 Processing.....	87
3.6.2.	Flexible Polymer Substrate (PET) Selection	89
3.6.3.	Fluid Input/Output Hole Punching & cutting	90
3.6.4.	Spin Coating Polymer Substrate	90
3.6.5.	Soft Bake 91	
3.6.6.	Method 4 Bond	92
3.6.7.	Method 4 Bond Results	95
3.6.8.	Method 4 Conclusions	102
4.	Testing.....	103
4.1.	I dex Nanopore Attachment.....	103
4.2.	Fluid Leak Syringe Pump Tests	107
4.2.1.	Fluid Leak Test Setup.....	107
4.2.2.	Results of Fluid Leak Test	109
4.2.3.	Conclusions of Fluid Leak Test	113
4.3.	Syringe Pump Repeatability & Variability Test.....	113

4.3.1.	Syringe Pump Repeatability & Variability Test Results	114
4.3.2.	Syringe Pump Repeatability & Variability Test Conclusions	117
4.4.	Electroosmotic Flow Test	117
4.4.1.	Electroosmotic Flow Results	119
4.4.2.	Electroosmotic flow Conclusions	119
4.5.	Streaming Potential Test	119
4.5.1.	Streaming potential results	120
4.5.2.	Streaming Potential Conclusions	120
4.6.	Electrophoresis of Glass Particle Test	121
4.6.1.	Electrophoresis of Glass Particles Results.....	123
4.6.2.	Electrophoresis Conclusions	127
5.	Conclusions and Continuation of Project	128
5.1.	Recommendations.....	128
5.1.1.	Dissolution of Gold	128
5.1.2.	Future Testing for Streaming Potential & Electroosmotic Flow Chips	129
References.....		140

List of Tables

Table I. <i>Reported electroosmotic mobilities in microchannels made from various materials</i>	17
Table II. <i>Four films were deposited, exposed, post exposure heat treated, and developed for DSC and TGA testing</i>	48
Table III. <i>Amount of heat released from each film for the first and second exotherm. Data correlates to the DSC scans</i>	54
Table IV. <i>Weight loss of each film was tracked to determine percent weight loss due to development</i>	59
Table V. <i>Resistance measurements for various electrode locations</i>	66
Table VI. <i>Profilometer scans measuring SU-8 2050 microchannel layer thickness of two wafers, at various points on the wafers</i>	72
Table VII. <i>Measured flow rate calculated compared to each syringe pump setting</i>	113
Table VIII. <i>Flow rate measurements (fit from data) for four trials at 10 $\mu\text{L}/\text{min}$ after pressure equalization</i>	114
Table IX. <i>1-sample T-test statistical results from Minitab. (SE Mean = standard error of the mean)</i>	116
Table X. <i>Streaming potential readings taken at 10 $\mu\text{L}/\text{min}$</i>	120
Table XI. <i>2-Sample T-test comparing the mean particle velocities at 200 V and 300 V</i>	123
Table XII. <i>P-values for the ANOVA significance of input variables</i>	125

List of Equations

Equation 1. Equation to determine Reynolds number for laminar vs. turbulent flow [12].....	5
Equation 2. Hagen-Poiseuille equations are used to predict required pressure to obtain a particular flow rate in a microchannel.	9
Equation 3. Empirical equation on the mixing in a microfluidic T-mixer [27]	10
Equation 4. Used to calculate electro-osmotic mobility [19]	13
Equation 5. Equation from streaming potential which relates zeta potential to a measured potential difference across a microchannel.	15

List of Figures

Figure 1. Typical microfluidic mixer (top) in which is shown to have chemical surface modification (bottom left) and topological changes (right). These types of techniques can be combined to obtain three dimensional flows, and potential mixing [20].	6
Figure 2. Various classifications of micropumps by a Stanford University research group [21].	7
Figure 3. Velocity profile inside a microfluidic channel [24].	9
Figure 4. A representative microchannel displaying diffusion dominated mixing.	10
Figure 5. Diagram of electric double layer formed between a solid and liquid interface with substantial electroosmotic effect taking place [31].	11
Figure 6. SiO ₂ surface chemistries at a) untreated b) treatment with sodium hydroxide (aq.) c) treatment with HCl (aq.) and air (oxygen) plasma treatment d) after interaction with water at neutral pH [31].	12
Figure 7. Electroosmotic flow obtained due to the electric double layer formation of the representative material [33].	12
Figure 8. (Not to scale) Depiction of a SiO ₂ /aqueous electrolyte interface. An electro-osmotic velocity profile is also included (right). The shear plane is where hydrodynamic motion becomes possible and ζ is the potential at this plane [34].	14
Figure 9. Tri-Star recommended setup for O ₂ gas addition to Ar [41].	18
Figure 10. Showing the high aspect ratio of SU-8 2000 series. Features are 50 μ m tall [43].	19
Figure 11. MicroChem suggested process flow chart [43].	20
Figure 12. SU-8 monomer which has on average 8 epoxy groups [42].	21
Figure 13. Chemical reaction that takes place during photolithography exposure [42].	21
Figure 14. Crosslinking process which starts during the Post Exposure Bake (PEB) [42].	21
Figure 15. Major SU-8 processing steps. (1) spin coating, (2) exposure, (3), post exposure bake, and (4) development.	22
Figure 16. Bond method 1 cross sectional view.	23
Figure 17. Bond method 2 cross sectional view.	24

Figure 18. Bond method 3 cross sectional view.	24
Figure 19. Bond method 4 cross sectional view.	25
Figure 20. Method 1 silicon and glass wafer processing steps used.	26
Figure 21. Glass wafer/slide drill set up.	27
Figure 22. Model WS-400-8N/L spin coater was programmed to complete the spin cycles and obtain a 100-120 μm thick SU-8 layer.	28
Figure 23. Edge bead removal of various substrates [50].	29
Figure 24. Micro cracking in an SU-8 layer due to rapid thermal expansion during a wafer bake step.	30
Figure 25. Hot plates used for dehydration at 200°C, and step baking at 65°C and 95°C are monitored with a thermocouple. The hot plates are located in a fume hood to prevent contamination during baking procedures.	30
Figure 26. Mask used during photolithography of silicon wafer to produce microchannels within SU-8.	32
Figure 27. Manual alignment set up to expose SU-8 layer. Dummy wafer was sent through the aligner to initiate exposure.	33
Figure 28. SU-8 2050 that was exposed with “spacers” between the polymer film and transparency mask causing detrimental effects upon development.	33
Figure 29. Wafer clamping setup. Wafers are clamped in between the top and bottom plate. Plates previously heated to 85°C.	35
Figure 30. Bond bake furnace that would contain entire bond apparatus. Temperature was monitored by a thermocouple.	36
Figure 31. Silicon-SU-8-Pyrex microfluidic chip with PDMS fluid hookups.	37
Figure 32. Cross section SEM image (30x magnification) of SU-8 microchannels and reservoir. Image shows channel integrity withheld at bond temperature 170°C.	38
Figure 33. SEM high magnification cross section image of microchannels. Image shows channel integrity withheld at bond temperature 170°C.	39

Figure 34. Silicon/SU-8/Pyrex wafer post method 1 bond. Red circles indicate fully bonded areas.	40
Figure 35. Complete bond of SU-8 to SU-8 without void space. This is the dark region marked by red circles in Figure 33. Bond temperature 160°C.....	41
Figure 36. Partially bonded SU-8 to SU-8 with void space allowing for fluid passageways. This is the light region marked by blue circles in Figure 34. Bond temperature 160°C....	42
Figure 37. UV cleavage of a sulfonium salt that becomes a sulfur centered cationic species that reacts with SU-8 monomers [55].....	46
Figure 38. Initiation of the photoinitiator and representative SU-8 monomer reaction with the cationic species [55]. H+ represents the cationic species.	46
Figure 39. Chain growth and the chain transfer of the epoxide polymerization [55].	47
Figure 40. (PEB: 55°C, 3 min) Film 1 DSC scan. The scan shows the initial heating of the sample with a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.	50
Figure 41. DSC scan of film 2 (PEB: 65°C, 3 min). The scan shows the initial heating of the sample with again a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.	51
Figure 42. DSC scan of film 3 (PEB: 75°C, 3 min). The scan shows the initial heating of the sample with again a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.	52
Figure 43. the DSC scan of film 4, the MicroChem recommended baking procedure (PEB: 65°C 3 min, 95°C 8 min).....	53
Figure 44. Overlay of DSC scans for films 1-4. The DSC scan of film 4 only shows the second step of the reaction.....	54
Figure 45. The TGA graph for PEB of 65°C and 3 minutes. The amount of weight loss from ambient to >300°C is ~6.4%.	56
Figure 46. The TGA graph for PEB of 75°C and 3 minutes. The amount of weight loss from ambient to >300°C is ~7.9%.	57

Figure 47. The TGA data overlay for PEB 55°C 3 min and 65°C 3 min.	57
Figure 48. The TGA data overlay for PEB 55°C 3 min and 65°C 3 min from ambient temperature to ~300°C.....	58
Figure 49. SU-8 2050 showing surface pin hole features with reduced post exposure bake (55°C, 3 min).	60
Figure 50. Bond method 2 cross section.	61
Figure 51. Processing flow chart for SU-8 2050 base layer and gold electrode patterning.	62
Figure 52. Transparency mask CAD drawing of electrode placement which will align to reservoirs of fluid channels.	65
Figure 53. Wafer with SU-8 base layer and Au patterned electrodes.	67
Figure 54. Process flow for the functional microchannel layer and bond procedure.	68
Figure 55. Discoloration of SU-8 2050 base layer due to Piranha exposure.....	69
Figure 56. Discoloration of Piranha solution after removal of wafer (wafer shown in Figure 55). .	69
Figure 57. Discoloration of SU-8 due to prolonged Au etchant exposure. Picture of embedded mask design (not discussed in this report).	70
Figure 58. Silicon wafer with SU-8 base layer and gold electrodes previously patterned and the SU-8 2050 material for the microchannel layer ready to be spin coated.....	71
Figure 59. Severe adhesion failure occurring upon microchannel layer development. Root cause unknown (worst case observed).	74
Figure 60. Method 2 bond set up.	75
Figure 61. Insufficient bond result of bond method 2 (1).	76
Figure 62. Insufficient bond result of bond method 2 (2). Clamp method: c-clamp.....	76
Figure 63. Pyrex cracking as a result of bond method 2 (1). Clamp method: c-clamp.....	77
Figure 64. Pyrex cracking as a result of bond method 2 (2). Clamp method: c-clamp.....	77
Figure 65. Pyrex cracking as a result of bond method 2 (3). Clamp method: c-clamp.....	78
Figure 66. Pyrex cracking as a result of bond method 2 (4).	78
Figure 67. Bond method 3.	80
Figure 68. Method 3 bond sequence (1).....	82

Figure 69. Method 3 bond sequence (2).....	82
Figure 70. Method 3 bond sequence (3).....	83
Figure 71. Method 3 bond sequence (4).....	83
Figure 72. Method 3 bond sequence (5).....	84
Figure 73. Method 3 bond sequence (6).....	84
Figure 74. Method 3 bond sequence (7).....	85
Figure 75. Optical Microscopy (top down) of a 200 micron wide channel with significant SU-8 2007 reflow.....	86
Figure 76. Method 4 processing steps (1/2).	88
Figure 77. Method 4 processing steps (2/2).	89
Figure 78. Dummy silicon wafer set up for spin coating polymer substrates with SU-8 2007.....	91
Figure 79. Method 4 cross section of bond interfaces being brought together.....	92
Figure 80. Method 4 bond, initial contact of coated polymer substrates with silicon wafer microchannels. (1).....	93
Figure 81. Method 4 bond with wetting started. Temperature at time of photo 55°C. (2).....	94
Figure 82. Method 4 bond with wetting nearly complete. Temperature at time of photo 65°C.(3).....	94
Figure 83. Completed bond with Idex Nanopore connections made (discussed in Section 4.1). Left chip, particle shown trapped between a polymer substrate and silicon chip which resulted in an incomplete bond. (4).....	95
Figure 84. Top-down optical microscopy of 200 μm channel.	96
Figure 85. Top-down optical microscopy of 200 μm wide channel.....	96
Figure 86. Top-down optical microscopy of 500 μm wide channel.....	97
Figure 87. Top-down optical microscopy of 500 μm wide channel.....	97
Figure 88. Top-down optical microscopy image of a fluid mixing reservoir containing mixing posts (SU-8 2050 features) with 200 micron channels leading in and out of the reservoir.	98

Figure 89. Top-down optical microscopy image of a Z-shape channels (far right) entering S-shape channels to increase length fluid flow in chip. Channels are 200 microns in width.	99
Figure 90. Top-down optical microscopy image of a fluid reservoir etched in the SU-8 2050 microchannel layer with patterned gold electrode and coated polymer substrate bonded on top. (Polymer substrate misaligned with fluid reservoir).	100
Figure 91. Top-down optical microscopy image of a fluid reservoir etched in the SU-8 2050 microchannel layer with patterned gold electrode and coated polymer substrate bonded on top.	100
Figure 92. SEM cross section of a 500 μm channel that had been detached from the silicon wafer (should be on bottom). The SU-8 layers show signs of complete bonding; the polymer substrate/SU-8 2007 layer however shows signs of delamination.	101
Figure 93. Sample used for SEM cross section was taken from fractured wafer. Top-down view and side view can be compared with noted amount of bowing in SU-8.	102
Figure 94. Idex Nanoport cross section. [58]	103
Figure 95. Nanoport assembly 1 mm x 1 mm, part numbers N-121S [59].	104
Figure 96. Luer adapter assembly, part number P-622 [59].	104
Figure 97. Four Nanoports bonded to two microfluidic chips (one input, one output on each chip).....	105
Figure 98. Full Nanoport assembly.	106
Figure 99. Microfluidic Chip with Nanoport connection via PDMS reinforcement.	106
Figure 100. System setup used to track flow rate versus pressure.	108
Figure 101. Output tube shown running from microfluidic chip to scale.	108
Figure 102. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 5 $\mu\text{L}/\text{min}$	109
Figure 103. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 15 $\mu\text{L}/\text{min}$	110

Figure 104. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 25 $\mu\text{L}/\text{min}$	111
Figure 105. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 50 $\mu\text{L}/\text{min}$	111
Figure 106. Empirical vs. theoretical flow rate values for a given pressure difference across the microchannel.....	112
Figure 107. Representative trial (1) for syringe pump reliability and variability test.	115
Figure 108. 1-sample T-test concluding a significant difference of measured flow rate vs. set point flow rate of syringe pump.	116
Figure 109. Probe tips making contact with electrodes.	118
Figure 110. Interaction of a glass particle submerged in an aqueous medium (pH 7) with an applied electric field.....	122
Figure 111. Velocity measurements taken from potential differences of 200 volts and 300 volts.	123
Figure 112. Results for ANOVA test to analyze input variable effect on particle speed.	124
Figure 113. Residuals plot that was obtained with ANOVA analysis.....	125
Figure 114. SEM of glass particles used for electrophoresis tests.....	126
Figure 115. SEM of glass particles used for electrophoresis tests.....	126
Figure 116. Gold capped reservoir for a drug dispensing device.	128
Figure 117. Dissolved gold electrodes (Nanoports removed). Left electrode completely dissolved, right is partially dissolved.	129
Figure 118. Design for testing of a new electrokinetic chip that allows for more room due to moving the Nanoport connections.	130
Figure 119. Microchip design for polymer substrate dissolution for full SU-8 chip.	131
Figure 120. Additional electrodes for electrochemical detection for electrophoresis and streaming potential measurements.....	132

Keywords [1]

Bond strength - In chemistry, bond strength is measured between two atoms joined in a chemical bond. It is the degree to which each atom linked to another atom contributes to the valency of this other atom. Bond strength is intimately linked to bond order.

Crosslink - When polymers are produced in which the polymer molecules are linked to each other at points other than their ends, known as a side bond that links two adjacent chains of atoms in a complex molecule.

Electrokinetics – A branch of physics dealing with the motion of electric currents or charged particles.

Electroosmotic flow - The motion of liquid induced by an applied potential across a porous material, capillary tube, microchannel, or any other fluid conduit.

Electrophoresis - The movement of charged particles in a fluid or gel under the influence of an electric field.

Fracture - The cracking or breaking of an object or material.

Gel Point - Point at which an infinite polymer network first appears.

Microchannel - A fluid passageway with dimensions on the microscale.

Microfluidics – Deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale.

Polydimethylsiloxane (PDMS) - belongs to a group of polymeric organosilicon compounds that are commonly referred to as silicones.

Photolithography – a process involving the photographic transfer of a pattern to a surface for etching.

Polymer - A substance that has a molecular structure consisting chiefly or entirely of a large number of similar units bonded together, e.g., many synthetic organic materials used as plastics and resins.

SEM (scanning electron microscope) - An electron microscope in which the surface of a specimen is scanned by a beam of electrons that are reflected to form an image.

Streaming Potential - The build-up of a streaming potential happens when liquid is pressed through a capillary, or along a charged wall, and drags the charges of the electric double layer with it.

SU-8 - A commonly used epoxy-based negative photoresist. It is a viscous polymer that can be spun or spread over a thickness ranging from <1 micrometer up to >300 micrometer and still be processed with standard contact lithography.

Transdermal – Relating to, being, or supplying medication in a form for absorption through the skin into the bloodstream.

1. Introduction

Microfluidics refers to manipulation, precise control, and behavior of fluids at the micro and nanoliter scales. It has entered the realm of science as a way to precisely measure or mix small amounts of fluid to perform highly controlled reactions. The properties of microfluidics, compared to macrofluidics, are dramatically affected by size scaling. Due to the increase in surface area to volume ratio at the microscale, surface forces play a much larger role in fluid manipulation. Surface tension and diffusion become relevant, and eventually dominant, in microfluidic based systems. This means that microfluidic pumping is not as easily accomplished by traditional mechanical actuation. In fact, the surface forces can be great enough that it is near impossible to overcome with a mechanical force before a catastrophic pressure is obtained.

1.1. Project Influence & Problem Statement

People with Type I diabetes and various other disorders may rely on multiple injections a day to survive. Syringe injection of a drug causes a large increase of concentration in the blood stream. This sudden spike in concentration is hard on the body, and if not calculated correctly can be detrimental. Transdermal delivery of smaller, but more frequent doses of the drug would be much less invasive to the individual. The influence of this project is to develop microfluidic research that aims to create precisely controlled systems allowing for control of minute amounts of molecules. This will be aimed to mimic cell activity in the body, and specifically, the cells release of insulin to the body.

1.2. Project Scope & Summary

This project was aimed to advance (Cal Poly) research and development on microfluidics. This was done by creating a new method of micro-scale fabrication techniques that can be implemented for future projects involving microfluidic cells, electroosmotic flow, and electrophoresis based technology. Previously, all (Cal Poly) fabrication techniques involved materials such as glass and polydimethylsiloxane (PDMS). Glass requires difficult processing by

wet etching which results in isotropic channel profiles or by deep reactive ion etching which is slow and limited in depth. PDMS is relatively hydrophobic and will adsorb many hydrophobic compounds and biomolecules [2]. There are surface treatments that will temporarily increase the hydrophilicity of PMDS, but none are permanent. These materials are described in more detail in Section 2. The goal of this project is to fabricate an electrokinetic microfluidic pump by constructing channels out of SU-8 2050 due to the material's desirable electroosmotic properties, compared to that of PDMS [3]. SU-8 2050 was originally studied for the ability to form channels via photolithography, and subsequently bond it to a Corning 7740 Pyrex substrate with predrilled fluid input/output holes. With MicroChem suggested processing, this resulted in <10% bonded area. Case studies were performed to better understand the molecular changes taking place during each processing step. Differential scanning calorimetry was used to track the crosslinking reaction after exposure of the film. These results showed two exothermic reactions taking place upon baking subsequent the exposure step. The process was then altered to inhibit crosslinking to allow for increased molecular mobility to obtain a complete bond. With multiple trials the SU-8 would either partially bond to the Pyrex (~70%) or the Pyrex would crack due to thermal expansion of the bond stack. Due to the immediate limitations of a sufficient bond apparatus, the processing shifted to developing channels in SU-8 2050 and using an SU-8 2007 uncured adhesion layer to ensure full wetting (bonding) to the surface. Additionally, a flexible polymer substrate (thought to be polyethylene terephthalate) second substrate was used instead of Pyrex. This allowed for nearly 100% bonded area and a robust bonding procedure to create encapsulated microfluidic channels of various sizes. The original goal for testing was to create an electrokinetic pump by inducing electroosmotic flow with an applied electric field to sputtered gold electrodes. Flow rate measurements of electroosmotic flow from various buffer solutions and electric field strengths would have allowed for the zeta potential to be deduced; however, realistic flow was not obtained. This was most likely attributed to:

1. The resistance of fluid movement in the required input and output tubing of the chip was greater than the electroosmotic strength induced by the electric field. The electrodes were designed at either end of the fluid chip, which is actually a small fraction of total fluid

volume that would require movement in the system. Greater length of the chip would help.

2. Gold electrodes exposure to chloride ions, which will react to form gold (III) chloride complexes which are soluble in aqueous electrolyte at voltages as low as 1.04 volts (discussed more in future work section) [4] , [5].
3. Relatively small voltages were used (< 300 V).

Due to this failure, the pump was tested in a different manner. The pump was tested as an electrophoretic cell to observe the behavior of glass particles in an aqueous solution, under the influence of an electric field. The pump was characterized by glass particle velocities with respect to field strength. Future implementation of an SU-8 based electroosmotic flow driven pump is discussed in the future work/recommendations section.

1.3. Broader Impacts

1.3.1. Health and Safety

Periodic insulin injection is necessary in diabetics. Such drug injection is unhealthy and unsafe for an individual due to a spike in concentration immediately after injection. The insulin concentration will then decrease until dangerously low and a person notices physical side effects, in which case another spike in concentration will occur upon injection. If a near constant necessary drug concentration could be held, the body would tolerate the presence of the drug much better [6]. Microfluidics can create a steady concentration of a needed drug within the body. Small consecutive reservoirs can be periodically evacuated, creating a more steady concentration within the blood stream. This would be ideal for diabetics that need daily injections to maintain healthy blood sugar levels.

1.3.2. Environmental Factors

Daily syringe disposal is tremendous due to the inability to reuse such an item that comes into contact with the blood stream. New Jersey alone estimated a total of 700,000 syringes used daily

[7]. With billions of syringes being used per year in the U.S. the waste created is immense. A microfluidic delivery method would create less material usage and less waste. The development of this microfluidic device would have a lesser environmental impact than the amount of syringes wasted each year.

1.4. Background

1.4.1. Microfluidics

The microfluidics field has been around since the 1950s. During this period it was of interest to try and dispense small amounts of liquid on the nanoliter scale, which has revolutionized today's inkjet technology [8]. It was revolutionized in 1979 when a miniature gas chromatograph was developed on a single silicon wafer [9]. It is often associated with the terminology "lab on a chip" which has to do with fluid dynamics on the micro/nano scales. Typical microfluidic devices have channel width of 10 to 1000 μm . Applications of such devices range from medical analysis, environmental monitoring, biochemical analysis, and microchemistry with fluid volumes on the order of 10^{-6} to 10^{-18} liters [10], [11]. A drip of water would be considered large scale to microfluidics, possibly thousands of times larger. At this scale, fluids do not behave the same way; energy dissipation, surface tension and fluid resistance becomes dominant. New behaviors can be observed and utilized to perform new functions that would be otherwise impossible on the macroscale. Quantities of molecules can be controlled in space and time for precise mixing and limited waste. Well known uses of microfluidics include ink jet printers, DNA sequencing, chromatography, and electrophoresis. Ink jet printers use "tubes" to deliver the ink that are about 70 μm in diameter and will create an ink blot close to 12,000 times per second [12]. Macroscopic mixing of liquids occurs easily due to turbulent flows, or eddy currents, that are present in the liquid. In microfluidic applications the flow is laminar and mixing predominantly by diffusion. The Reynolds number (Re) determines the type of mixing (Equation 1). Turbulent flow is when $Re \geq 2000$. Laminar flow is common amongst microfluidics due to the channel height and width being much smaller than its length.

Equation 1. *Equation to determine Reynolds number for laminar vs. turbulent flow* [13]

$$Re = \rho V D / \mu$$

In the Reynolds Number equation, ρ = fluid density (kg/m^3), V = velocity (m/s), D = hydraulic diameter (circular) or $D = 2ab/(a+b)$ where a & b are sides (rectangular), μ = dynamic viscosity of fluid (Ns/m^2). The development of soft lithography helped microfluidics emerge as a research focus. Polydimethylsiloxane (PDMS) is the primary material used in microfluidics. The use of PDMS has made it possible for prototypes to be created quickly, usually two days shorter than silicon technology [10]. PDMS is an elastomer which can be easily molded, cured, and bonded to quickly create a working device. The general shift from bench top lab equipment to microfluidic chips is that analysis is faster, cheaper, and requires less sample volume. Hydrodynamic driven flow is normally achieved in two ways: closed reservoir and peristaltic pumping. Closed reservoir commonly refers to a syringe pump, which requires the entire reservoir to be removed and replaced/refilled when empty. In contrast, peristaltic pumping has an external reservoir, which can be refilled without stopping the pumping process [14]. Both of these methods produce a parabolic velocity profile while pumping in a microfluidic channel. This is the result of attractive surface forces at the solid/liquid interface that inhibit the molecular mobility close to the channel walls. An alternative to hydrodynamic driven flow utilizes electrokinetic principles which can be used to induce electroosmotic flow (EOF). EOF utilizes the solid/liquid interfacial interaction to generate flow with an externally applied electric field. This will be discussed in more detail in the electrokinetics section. The field of microfluidics has grown to incorporate all instrumentation into a microfluidic chip to perform such sequences as: pretreatment and transport, chemical reactions, separation, and detection. This is where the term “lab on a chip” (LOC) came from. LOC systems are a subgroup of microanalysis systems, sometimes referred to as micro total analysis (μTAS).

1.4.2. Lab on a Chip

LOC systems include mixing systems (comparisons here [15]), micro-pumps [16], micro-valves, and integrated electrodes for electrochemical detection [17], all in one chip. An example of this is a fully integrated LOC device used for influenza detection on a 1.5 cm x 1.6 cm substrate [18]. LOC systems have been used for polymerase chain reaction (PCR) diagnosis of diseases, environment surveillances, food processing industry, agricultural researches, and forensic identifications [19]. A major consideration when designing a LOC device is the manipulation of flow by such methods as pressure driven, capillary effects, electric fields, magnetic fields/Lorentz forces, centrifugal forces, and acoustic streaming [20]. Such effects used to drive flow are shown in Figure 1. These components can be integrated locally within the microchannel or connected to input and outputs of the chip. Additional considerations would be the rheology of the fluid and can have a significant impact on performance, whether Newtonian or non-Newtonian.

Figure 1. Typical microfluidic mixer (top) in which is shown to have chemical surface modification (bottom left) and topological changes (right). These types of techniques can be combined to obtain three dimensional flows, and potential mixing [20].

1.4.3. Micropumps

Micropumps include various closed system ways of transporting material in a microfluidic device. For such devices to be successful the pump must be comparable in size to the fluid being transported. There is significant research put forth to progress micropumps when considering

their performance and cost. Fields such as biotechnology and aerospace have the most interest in the development of micropumps. Biotechnology looks to advance research for drug dispensing devices and for the ability to scale down analysis tools. Aerospace is interested in micropumps for the application of miniature mass spectrometer systems as well as micropropulsion systems for miniaturized space exploration (< 5 kg) [21]. As stated in this thesis project's overall goal to create an drug dispensing pump, a similar concept that uses a three valve peristaltic pump is described here [22]. Figure 2 shows the categorization of various micropumps.

Figure 2. Various classifications of micropumps by a Stanford University research group [21].

In this review by a Stanford University research group, numerous drivers are discussed with valve type, construction, materials, dimensions, working fluids, voltages necessary, frequency obtained, ΔP output, and volumetric flow rates [21]. An exotic pumping technique utilizes sound waves

have also been used to create acoustic streaming motions which distort channel walls and can cause a sort of peristaltic pumping [20].

1.4.4. Electrokinetics

Electrokinetics can be used in various ways for controlling microflows and can be divided into four sub-categories: electrophoresis, electroosmosis, streaming potential, and sedimentation potential. All electrokinetic phenomenon depend heavily on the zeta potential. The zeta potential (ζ) is the electric potential difference between a liquid medium and the stationary layer of fluid attached to a solid surface. This will be described in greater detail in Section 1.4.7. Electrokinetic phenomenon have been described by the help of two equations: Poisson equation and Navier-Stokes equation [23]. Electro-osmosis is the result of fluid motion relative to an applied electric field; dielectrophoresis is the motion of charged particles which are suspended in a liquid, relative to an electric field; and electrowetting is the modification of surface properties and wettability with the application of an electric field. AC and DC fields have both been used in electrokinetics applications, and microfluidic manipulation depends highly on the frequency and amplitude of the electric field. Capillary and electrokinetic flow both show advantages over pressure driven flow as chip features decrease. However, these effects can be inhibited due to surface contamination or heterogeneities. These alternative pumping techniques are useful in microscale pumping devices, because of the strength of surface forces becomes a large factor in pressure driven flow. When the surface area to volume ratio of the molecules in a liquid system is high enough, it will eventually become near impossible to pump fluids before a catastrophic failure occurs. A velocity profile of a large-channel mechanical pumping system can be seen in Figure 3. These profiles are nearly always parabolic.

Figure 3. Velocity profile inside a microfluidic channel [24].

Electrokinetics is a way to get around the difficulty of mechanical pumping on the micro/nanoliter scale. This phenomenon is only obtainable at microliter and submicroliter levels and is a great example of the scaling laws of physics [25]. Obtaining electro-osmotic flow would result in a nearly uniform velocity profile, as opposed to parabolic. The Hagen–Poiseuille equations can be used to predict the pressure drop required for a channel of certain dimensions to reach a desired flow rate.

Equation 2. *Hagen-Poiseuille equations are used to predict required pressure to obtain a particular flow rate in a microchannel*

$$R = \frac{\Delta P_{\mu}}{Q_{flow \ rate}}$$

Circular channel	Rectangular channel
$R = \frac{8\mu_d L}{4\pi r^4}$	$R = \frac{12\mu_d L}{wh^3}$

In this equation, R = fluid resistance, ΔP_{μ} = Pressure difference, Q = volumetric flow rate. μ_d = dynamic viscosity, L = channel length, r = channel radius, w = channel width, and h = channel height.

1.4.5. Microfluidics Mixing

In microfluidics, at low Reynolds numbers, turbulent flow is not present. With turbulent flow eddy currents exist in the fluid and aids in rapid mixing. “T-mixers” are used widely in microfluidics for mixing and rely solely on diffusion (Figure 4).

Figure 4. A representative microchannel displaying diffusion dominated mixing.

A T-mixer can be used to estimate the diffusivity of one fluid into another. By knowing the width of the channel and knowing the diffusivity of the fluid, an estimation can be made on the percentage of mixing (Equation 3). Additional modeling of mixers and equations necessary to calculate mixing efficiency of various channel geometries can be found here [26], [27].

Equation 3. *Empirical equation on the mixing in a microfluidic T-mixer* [28]

$$t_{50\%mix} \cong \frac{w^2}{D}$$

In this equation, W = width of channel, D = diffusivity (m²/s), t = amount (%) of mixing.

1.4.6. Electroosmotic Flow

Electroosmotic flow can occur due to an electric double layer formation at the solid liquid interface, when the mobile layer is set into motion with the application of an electric field. Most electric double layer models are based on or variations of the Gouy-Chapman-Stern model [29]. The electric double layer is an array of charged particles as well as oriented dipoles, which is theorized to exist at every interface. The theory proposes that at every interface there are two layers of charge, one positive and one negative, and that a surface is left inherently charged while counterions are released into the liquid. The surface charge is balanced by oppositely charged ions in the fluid (zeta potential). The layer that has been adsorbed onto the surface is called the Stern layer and the mobile layer is called the diffuse layer (Figure 5). This arrangement of abnormal ion concentration decreases rapidly as the distance from the interface increases [30]. An example of a charged surface at a solid/liquid interface is glass (SiO_2). Glass actually terminates from the bulk to a phase boundary with SiOH . At neutral pH (ex. water) this group deprotonates and ionizes to form surface groups SiO^- [20], [31]. Figure 6 shows the surface chemistries of SiO_2 with and without chemical treatment.

Figure 5. Diagram of electric double layer formed between a solid and liquid

interface with substantial electroosmotic effect taking place [31].

Figure 6. SiO_2 surface chemistries at a) untreated b) treatment with sodium hydroxide (aq.) c) treatment with HCl (aq.) and air (oxygen) plasma treatment d) after interaction with water at neutral pH [31].

If a potential is now applied across the channel, the diffuse layer will flow due to the electrostatic force (Figure 7). Due to the cohesive nature of hydrogen bonding on a water molecule, the bulk fluid is attracted to the diffuse layer and will flow. This results in a uniform velocity profile. In fused silica systems, the porous glass structure provides a high surface area to volume ratio which maximizes the electroosmotic effect [32].

Figure 7. Electroosmotic flow obtained due to the electric double layer formation of the representative material [33].

At equilibrium a charge density profile is formed in which the liquid is electrically neutral, but there exists a charged layer adjacent to the interface. This charged layer bears the opposite charge of the charge bound on the surface and will be equal in amplitude at the interface. The Debye layer, is known as the ionic concentration and potential distribution that is adjacent to a charged solid surface when in contact with an electrolyte medium. The Debye length (λ_d) decreases as the inverse square root of ion concentration in the liquid. The λ_d is commonly ~1-100 nm in water [20]. In the Debye layer exists a net electrical charge density which creates a local electric field that is tangent to the solid/liquid interface. This creates a force on the liquid that induces shear. When an electric field is applied across the length of the channel, fluid velocity increases from zero at the surface to a finite value of $-m_{EO} E$ (m_{EO} = electro-osmotic mobility of the surface). Equation 4 relates m_{EO} to the surface charge density (σ_{el}) [20].

Equation 4. *Used to calculate electro-osmotic mobility* [20]

$$m_{EO} = \frac{\sigma_{el} \lambda_D}{\mu} = \frac{\zeta \epsilon \epsilon_0}{\mu}$$

In this equation, σ_{el} = the surface charge density, μ = shear viscosity, ϵ = dielectric constant, ϵ_0 = permittivity of a vacuum, ζ = zeta potential of the surface, all defined at the location of no-slip boundary condition. The magnitude of fluid velocities in electroosmotic flow are set by the interfacial slip phenomenon and are independent of channel cross sectional dimensions, if λ_D is < channel width. Typical surface potentials are the 10-50 mV range for aqueous solutions. This means that to obtain fluid velocities of millimeters per second requires electric fields on the order of kilovolts per centimeter range [20].

1.4.7. Zeta Potential

The zeta potential (ζ) is generally the potential at the solid/liquid interface. It is a fundamental parameter in models of electric double layer. The zeta potential is dependent upon pH and concentration. The pH attributes primarily to the protonation and adsorption at the wall and the concentration is attributable primarily to electrical shielding by the overconcentration of

counterions in the diffuse layer [34]. Zeta potential differs from the surface charge in that it is defined at the hydrodynamic interface, while the surface charge is defined at the solid/liquid interface. It has been found that zeta potential measurements of systems, which are reportedly published in literature, have a large variation. Figure 8 shows the estimation of the zeta potential at a SiO_2 /electrolyte (aq.) interface.

Figure 8. (Not to scale) Depiction of a SiO_2 /aqueous electrolyte interface. An electro-osmotic velocity profile is also included (right). The shear plane is where hydrodynamic motion becomes possible and ζ is the potential at this plane [34].

In Figure 8, Ψ = the local potential and ζ = the zeta potential at the shear plane.

1.4.8. Streaming Potential

Streaming potential is thought of as the inverse of electroosmosis. The build-up of a streaming potential happens when liquid is pressed through a capillary, or along a charged wall, and drags the charges of the electric double layer with it. This results in counterion build-up at the end of the capillary and will generate a potential difference across the length of the microfluidic channel. If the width of the channel is much larger than the λ_D , it should be fairly possible to calculate the

streaming potential of the system [23]. An equation relating the measured potential difference along the length of a microchannel to the zeta potential is given in equation 5.

Equation 5. *Equation from streaming potential which relates zeta potential to a measured potential difference across a microchannel*

$$\Delta U = \frac{\epsilon \epsilon_0 \zeta \Delta P}{\eta \kappa_E}$$

In this equation, ΔP = applied pressure, κ_E = electrical conductivity of the electrolyte ζ = zeta potential, ϵ = dielectric constant, ϵ_0 = permittivity of a vacuum, η = viscosity, ΔU = measured potential difference.

1.4.9. Measuring Flow in a Microchannel

Measuring flow in a microchannel can be relatively difficult. Shear stress sensors and thermal anemometers have been used as integrated probes to measure flow rate in a closed loop. Most other measurements have been developed using optical microscopy. Particle image velocimetry (PIV) can provide information on the flow of micro and nano sized particles. This can be done either by tracking particles with an optical microscope or probing fluorescence at a specified time interval and tracking particle displacement. PIV uses a fluorescence of the particles and a known shutter speed of a CCD camera to track particle displacement per frame. Hg-arc lamps have been used to continuously illuminate particles as well as Nd:YAG lasers to illuminate sub-micron particles that will fluoresce ~1 mJ of energy [35]. Laser confocal microscopy has also been used to probe a more three dimensional flow profile within microchannels and can achieve fluorescence (see Recommendations).

2. Materials Selection

Materials used in microfluidics include: glass, hydrogels, polymeric films, silicon, silicone elastomer (PDMS), and thermoplastics such as poly (methyl methacrylate), polycarbonate, etc. Pyrex glass, SU-8, and polydimethylsiloxane (PDMS) are all viable materials available at Cal Poly for this application. PDMS is probably one of the most widely used in microfluidic applications. It is cheap, transparent, and easy to manufacture prototypes. The problems with glass and PDMS fabrication at Cal Poly will now be individually discussed.

2.1. Glass

Glass (SiO_2) has been used extensively for creating microfluidic devices. The disadvantages to using glass at Cal Poly include:

- Wet etching to create channels is an isotropic process which creates “slanted” side walls and uses hydrofluoric acid which is particularly dangerous and can cause death by less than 2.5% total body area (dermal exposure).
- Laser cutting to create channels is not available.
- Dry etching by deep reactive ion etching (DRIE) to create channels is difficult and not well characterized at Cal Poly. Additional limitations are involved in available gases (only SF_6 and O_2) due to safety concerns.
- Anodic Bonding is required to enclose channels. This process also poses safety concerns of high voltage and heat.
- Sputtered metals on glass & silicon can pose adhesion issues.

2.2. Polydimethylsiloxane (PDMS)

PDMS has also been used extensively for creating microfluidic devices via soft lithography. PDMS is usually used to create channels by using photolithography of SU-8 to create a mold, casting PDMS over the mold to create three sides to the fluidic channels, and using plasma

exposure to functionalize the surface of the PDMS to bond to materials such as SiO₂ or an additional sheet of PDMS. The disadvantages to using PDMS at Cal Poly include:

- It has high hydrophobicity which requires large pressures to obtain hydrodynamic driven flow on the microscale. The surface can be modified to be more hydrophilic with surface oxidation techniques, but is not permanent.
- It has less desirable surface properties than that of glass for potential electrokinetic processes or else it is limited by surface oxidation treatment which is not permanent.

Table I shows reported electroosmotic mobilities from literature. The values reported for PDMS are all after surface oxidation. This would not be ideal for a drug dispensing device for consumer sales.

- PDMS will adsorb many hydrophobic compounds and biomolecules.
- Cal Poly's plasma treatment process for surface functionalization to create a covalent bond does not always allow for a completely bonded device. Recommendation: Tri-Star Technologies Plasma Treatment System PT-2000P owner's manual has detailed information about integrating an oxygen source into current atmospheric plasma gun

Figure 9 shows the Tri-Star technology recommended set up to incorporate O₂ gas.

Table I. *Reported electroosmotic mobilities in microchannels made from various materials*

Material	EO Mobility (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	Buffer Conditions	Source
SU-8	4.5	20 mM Phosphate (pH 7)	[2]
Glass (Schott Borofloat)	4	20 mM Phosphate (pH 7)	[2]
Silica (capillary)	6.7	20 mM Phosphate (pH 7)	[36]
Glass (Schott Borofloat)	7.7	Borate buffer (pH 9.2)	[37]
PDMS (oxidized)	1-3	20 mM Phosphate (pH 7)	[36]
PDMS (oxidized)	4-6	21 mM Phosphate (pH 9)	[38]
Polystyrene, imprinted	1.8	22 mM Phosphate (pH 7)	[39]
PMMA	2.5	23 mM Phosphate (pH 7)	[40]
Copolyester	4.3	24 mM Phosphate (pH 7)	[39]

Figure 9. Tri-Star recommended setup for O₂ gas addition to Ar [41].

For the electrokinetic pumping application attempted in this work, PDMS is considered unfit. Its high hydrophobicity makes it difficult to pump fluid through PDMS microfluidic channels and it also has less desirable electroosmotic surface properties than that of SU-8 or glass [14]. Glass is also a widely used material; however, processing is expensive. Wet etch processes result in a highly isotropic profile and deep reactive ion etch (DRIE) processes are slow and limited in depth. SU-8 is a readily available material that is relatively cheap, easy to process, and displays electroosmotic surface properties comparable to that of glass (see Table I). SU-8 was therefore chosen to fabricate this microfluidic pump. The substrates to deposit the microfilm of SU-8 will be a silicon wafer and a Pyrex substrate. Silicon wafers are cheap and readily available while Pyrex substrates are more expensive. Pyrex was necessary for drilling vias and so testing could be empirically observed through the Pyrex.

2.3. SU-8 by MicroChem

SU-8 is a negative photoresist MicroChem product. (The mechanism for SU-8 polymerization and molecular changes with processing steps is discussed in much greater detail in section 3.3). It is used for micromachining and other microelectronic applications due to its chemical stability and high aspect ratios. The polymer has high optical transmission for wavelengths > 360 nm and can create near vertical side walls, shown in Figure 10. SU-8 also displays advantageous properties of low molecular weight, high transparency, low glass transition temperature, high viscosity, uniform coatings, vertical side walls, aspect ratio > 50 , excellent chemical resistance, and good biocompatibility [42]. It can be used to create $1\text{ }\mu\text{m}$ to $100\text{ }\mu\text{m}$ layers in a single coat by using the various SU-8 formulations. Each formulation differs in solvent content to alter viscosity [43]. The various formulations of SU-8 2000 series include: 2000.5, 2002, 2005, 2007, 2010, 2015, 2020, 2025, 2035, 2050, 2075, 2100, and 2150. As the subsequent digits after the first digit “2” increase, the viscosity increases which will allow for spin coating of thicker films.

Figure 10. Showing the high aspect ratio of SU-8 2000 series. Features are $50\text{ }\mu\text{m}$ tall [43].

The photoresist consists of a polymeric epoxy resin dissolved in an organic solvent with the addition of a photoacid generator. The SU-8 consists of three components [42]:

- An epoxy, called Epon SU-8 (Shell Chemicals)
- A solvent, called gamma-Butyrolactone
- A photoacid generator from triarylium-sulfonium salts

2.3.1.SU-8 Processing

MicroChem SU-8 processing involves multiple baking steps, an exposure step, and chemical treatment. The recommended processing specified by MicroChem datasheets is shown in Figure 11. The various molecular changes that take place during each processing step were carefully studied for bond implementation. This will be discussed more in Section 3.3.

Figure 11. MicroChem suggested process flow chart [43].

An SU-8 molecule can be seen in Figure 12. When the resist is exposed to UV light, a chemical reaction occurs that can be seen in Figure 13. Then, a subsequent thermally crosslinking bake makes the exposed portions of the film insoluble in the SU-8 liquid developer (Figure 14). SU-8 2050, a relatively viscous version of the polymer was used to create the channels for this prototype.

Page 21

Figure 15. Major SU-8 processing steps. (1) spin coating, (2) exposure, (3), post exposure bake, and (4) development.

2.3.2.SU-8 Applications

SU-8 has been used extensively for microelectromechanical systems (MEMS). The average price of SU-8 per liter is \$600 [44]. It can be used for temporary or permanent applications; however, due to the nature of the coating it is not easy to remove. Permanent applications include: microfluidics, advanced packaging, and optoelectronics as an optical waveguide. Temporary applications include: etch masks for DRIE and plating/deposition for MEMS and soft lithography. Microfluidic applications of SU-8 can be found here ([45], [46], [2], [47], [48]) most of which include bonding procedures implemented for microfluidic channel encapsulation. These procedures were taken into consideration; however, many involved a precisely controllable bond apparatus or other methods that provide their own difficulties.

3. Microfabrication Procedures

There were four different bond methods that were attempted throughout the duration of this project. These four bond methods will first briefly be explained. Method 4 was the only method that resulted in complete encapsulation with minimal to no reflow of SU-8, blocking the channels. Refer to section 3.6 for a description of Method 4 processing procedures. Along with a description of bond Methods 1-4, test methods and research performed to investigate SU-8 crosslinking will also be discussed.

3.1. Microfabrication Bond Iterations

As discussed in the section 1.2 (Project Scope & Summary) the direction of the project shifted many times. The project started off with (Method 1) trying to bond a patterned (microfluidic) SU-8 layer to Pyrex with MicroChem suggested processing (Figure 16). Only basic aspects of device are shown for simplicity (Silicon, Pyrex, & SU-8).

Figure 16. Bond method 1 cross sectional view.

With method 1, implementing MicroChem suggested processing steps, this resulted in < 10 % bonded area. After conducting research and tests to better understand the molecular changes occurring in the film, the next attempt was to try altering the post exposure bake step to inhibit crosslinking of the film to enhance molecular mobility. This was done in hope of receiving more wetting of bond interface and a better bond. The post exposure step implemented was 55°C for 3 minutes. This process is shown in Figure 17.

Figure 17. Bond method 2 cross sectional view.

With the reduced PEB step, the bond area increased significantly. Bonded area approached 90% in some cases; however, with multiple attempts a complete bond was not achieved. This method was deemed better, yet not suitable for repeatability.

The next step was to spin coat a thin layer of SU-8 (2007, ~8 μm thick) on the Pyrex wafer/slide, soft bake the Pyrex, bring the Pyrex + SU-8 2007 in contact with the SU-8 2050 (channel layers) with its reduced PEB to allow for a complete bond. This was assuming that the SU-8 2007 would have much more mobility due to un-crosslinked SU-8 molecules in the film. This was assumed to allow for plenty of mobility, and again, better wetting of the bond interface. This process is shown in Figure 18.

Figure 18. Bond method 3 cross sectional view.

Method 3 resulted in a complete bond, but there was significant reflow of the SU-8 2007 into the channels. It was found that the Pyrex would initially wet the SU-8 2050 in some areas and not others. Significant pressure would be required to wet the remaining areas of the SU-8 2050, but

when this occurred the initially wetted areas would be forced to flow into the channels because there was no other place for it to go. It was concluded that the stiff Pyrex substrate was not allowing for complete initial contact between the two surfaces, and only by applying significant pressure to the Pyrex would it allow for complete wetting, but simultaneously caused the SU-8 2007 to reflow in the channels.

Figure 19 shows method 4, which utilized a thin, flexible, polymer substrate upon which to spin coat the SU-8 2007. The flexibility of the substrate would allow for complete wetting of the SU-8 2007 to the SU-8 2050 without requiring significant pressure. The substrate used was a cell phone screen cover, most likely poly (ethylene terephthalate), and is in the process of being confirmed.

Figure 19. Bond method 4 cross sectional view.

Method 4 resulted in 100% bonded area. This was the method that was implemented for final device fabrication for fluid leakage testing, electroosmotic flow tests, streaming potential, and electrophoresis. Each described method will now be discussed in more detail.

3.2. Bonding Method 1

Initial investigation of an SU-8 bonding procedures started out with a 100 mm silicon wafer and a 100 mm Pyrex wafer (Corning 7740). MicroChem suggested processing steps were implemented for silicon wafer processing, up until the wafer bonding step. The silicon and glass wafer processing steps are shown in Figure 20.

Figure 20. Method 1 silicon and glass wafer processing steps used.

A large wafer sized T-mixing style mask was used to test method 1. Bond temperatures started at 100°C and went as high as 180°C. Processing was performed in the Cal Poly Materials Engineering Department class 1000 clean room.

3.2.1. Mechanical Drilling of Fluid Input/Output Holes Through Pyrex Wafer

The T-mixing photolithography mask was used to draw dots on the Pyrex wafer where fluid holes were needed. A 1.5 mm diameter diamond coated drill bit was used to create the holes at marked locations at a spin speed of 3000 RPMs (max speed). Constant drips of water were applied to cool the bit and wash away debris with minimal pressure applied to the substrate. Constant backing out of the bit kept contact area clean and the bit from overheating. Wafers were securely held with clamps on a rigid plastic backing. Drill set up is shown in Figure 21. These holes were aligned to be in the same location as the masked region of future photolithography steps. This will

eventually create a complete passage through the wafer for fluid input/output attachments. PDMS plasma bonded (syringe pressure fit) attachments were made for fluid connection. Idex Nanoports were used in the final design and will be described in greater detail in Section 4.1. Glass wafer drilling is a tedious process and must be done with extreme care to assure survival of the wafer. Drill times of up to 10 minutes on a single hole were performed, depending on the status of the bit.

Figure 21. Glass wafer/slide drill set up.

3.2.2. Silicon & Pyrex Wafer Clean and Dehydration

The machined Pyrex wafer and silicon wafer are then subjected to a 12 minute basic clean in Piranha solution. Piranha solution is a 9:1 ratio of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). The basic clean was performed at 70°C . Chemical gloves, apron, and face mask were used. The wafers are then rinsed with the spin-rinse-drier and dehydrated on a hot plate at 200°C for 30 minutes to evaporate all solvents from the wafer to prevent improper adhesion of SU-8.

3.2.3. Spin Coating of SU-8

Spin coating was then performed to obtain a uniform SU-8 layer. The bottle of SU-8 was taken out of the fridge a day (~12-24 hours) ahead of spin coating to allow for the bottle to reach room temperature. Upon finishing the spin coating processes the bottle was put back in the fridge. This

is to prevent solvent evaporation because if left out for too long the viscosity can increase dramatically. This creates for thicker films (with the same film speed), films that are harder to receive complete coverage, and SU-8 can difficult to pour onto a wafer as well. Additionally, the interior of the spin coater was covered with aluminum foil prior to each use. SU-8 is very difficult to remove so foil is used to create an easily disposable mask for the spin coater. A spread cycle of 500 rpm at 100 rpm/s for 1 minute was used to distribute the SU-8 2050 across a silicon and Pyrex wafer, and a following thickness dependent cycle of 1500 rpm at 300 rpm/s for 1 minute was used to obtain a thickness of $\sim 100\text{ }\mu\text{m}$ [43]. A model WS-400-8N/L spin coater was used to perform the spin coating processes (Figure 22).

Figure 22. Model WS-400-8N/L spin coater was programmed to complete the spin cycles and obtain a 100-120 μm thick SU-8 layer.

SU-8 2050 (5 mL) was deposited onto the wafers to assure adequate coverage of the polymer due to its high viscosity. SU-8 2050 is close to the viscosity of room temperature honey. The SU-8 had to be poured straight from the bottle onto the wafer in a careful circular pattern to prevent bubble entrapment from the liquid “folding” over on itself and trapping air. It is extremely important to get the SU-8 in the very center of the wafer and allow it to settle before starting the spin cycle

or else it may not result in complete coverage. Spin coating was performed quickly after the dehydration step to prevent contamination. Dehydration and spin coating were performed under a fume hood to limit particle contamination. Acetone was used to clean the spin coater.

3.2.4.Edge Bead Removal

An edge bead removal step can be implemented directly after spin coating. This involves leveling and covering the wafer on a surface for ~ 24 hours in a dry environment. This allows the film to level out and form a more uniform surface [49]. Additionally, the wafer was covered with an upside down petri dish and aluminum foil to prevent light from penetrating the film. Additional methods have been found to remove edge bead which include an additional spin cycle in which the SU-8 developer is used to spray the outer edge of the wafer. This will dissolve the outer rim of the film [50]. Figure 23 shows various substrates before and after edge bead removal.

Figure 23. Edge bead removal of various substrates [49].

3.2.5. Soft Bake

The soft bake involved a transfer from a hot plate of 65°C for 5 minutes to a 95°C hot plate for 15 minutes. The stepping process is used to prevent rapid thermal expansion and thus micro-cracking within the newly formed SU-8 layer (Figure 24). The soft bake is meant to slowly drive solvents from the polymer film and to dry the film for subsequent handling. Hot plates were monitored closely with a thermocouple to ensure temperature stability during the bake (Figure 25).

Figure 24. Micro cracking in an SU-8 layer due to rapid thermal expansion during a wafer bake step.

Figure 25. Hot plates used for dehydration at 200°C, and step baking at 65°C and 95°C are monitored with a thermocouple. The hot plates are located in a fume hood to prevent contamination during baking procedures.

3.2.6.Exposure

After soft baking the wafers and sufficient hardening of the SU-8 layer was observed upon cooling, photolithography was performed to pattern the SU-8 layers. Photolithography is an exposure process that is used to preferentially remove material. SU-8 is a negative photoresist. This means where UV light comes into contact with the photoresist it will start a chemical reaction, which upon a subsequent PEB makes the polymer insoluble in a liquid developer. UV light exposure < 350 nm to photosensitive resist results in degraded features, therefore an i-line long pass filter was used to eliminate these wavelengths. The silicon wafer was exposed to form microchannels within the SU-8 layer. A light integral of 34 (287 seconds) was used on the Canon (PLA – 501FA) aligner which produces 6.5 mW/cm^2 . The light integral is related to the time the photoresist is exposed to the UV light. The higher the light integral is the longer the time. The mask used to pattern the silicon wafer can be seen in Figure 26. The mask displays where UV light will hit the SU-8 layer as the light regions, and where the light will be masked from the SU-8 as the dark regions. The microchannels can be seen as the “T-shape” down the center of the mask. The microchannels are $200 \text{ }\mu\text{m}$ in width.

Figure 26. Mask used during photolithography of silicon wafer
to produce microchannels within SU-8.

Wafers were manually aligned and stacked within the aligner. This is due to automated alignment problems that were experienced and multiple fractured wafers. Additionally, glass wafers and slides are unrecognizable by the aligner and manual alignment is necessary. Manual alignment was performed by isolating the wafer in between a glass mask blank and the mask (Figure 27). It is important to have contact between the SU-8 and transparency mask so that diffraction of light around the mask does not occur. This will greatly affect the outcome of features (Figure 28). This is another reason why edge bead removal is important and will allow for complete contact between the SU-8 and transparency mask. For exposure to occur, the aligner needs to run a dummy through the aligner to initiate the exposure process.

Figure 27. Manual alignment set up to expose SU-8 layer. Dummy wafer was sent through the aligner to initiate exposure.

Figure 28. SU-8 2050 that was exposed with “spacers” between the polymer film and transparency mask causing detrimental effects upon development.

3.2.7. Post Exposure Bake

A post exposure bake (PEB) was then performed to further continue the polymer crosslinking process of the exposed regions prior to development. The PEB was also stepped from 65°C for 3

minutes to 95°C for 8 minutes to prevent micro-crack formation. An outline of the mask appeared visible during the PEB and confirmed adequate exposure of the polymer. Exposure time is correct when features are not visible until ~10 seconds after placing on the hot plate.

3.2.8. Development

Wafers were fully submerged upside down in a 3000 mL beaker of developer, polypropylene glycol methyl ether acetate (PGMEA). They were developed for 14 minutes with occasional irritation of the developer. Wafers were then individually rinsed with isopropyl alcohol (IPA) and dried with a nitrogen gun. If the dried wafers looked hazy or formed a white residue, they were replaced in the developer for another half minute and then re-rinsed with IPA, dried with nitrogen, and checked again.

3.2.9. Pre-Bond

The silicon and Pyrex wafers were simultaneously baked at 85°C for three minutes to prevent micro-cracking during the bonding bake. The wafers were then hastily brought together. They were visually aligned by placing the input/output holes above the fluid reservoirs. While ensuring alignment, the wafers were then tightened between two plates by a large C-clamp (Figure 29). The plates were previously heated in the bond bake oven to the bond temperature so heat gloves were used.

Figure 29. Wafer clamping setup. Wafers are clamped in between the top and bottom plate. Plates previously heated to 85°C.

3.2.10. Bond Bake

Bonding was carried out at multiple different temperatures for 20 minutes. To gain an understanding of SU-8 bonding, bond temperatures of 100°C, 120°C, 140°C, 150°C, 160°C, 170°C, and 180°C were performed to conceive the best parameters. A small oven was used to perform bake (Figure 30).

Figure 30. Bond bake furnace that would contain entire bond apparatus. Temperature was monitored by a thermocouple.

3.2.11. PDMS Microfluidic Hookup

Polydimethylsiloxane (PDMS) was cured and cut into small squares for microfluidic hookup.

Syringe needles were pressed through the PDMS to create a squeeze fit to prevent leakage. The PDMS was then bonded to the glass wafer using argon plasma. The syringe tip was positioned directly above the drilled input/output holes to create a passage from the syringe, through the needle, and into the microfluidic channels. Figure 31 shows a picture of a complete silicon to Pyrex microfluidic chip with PDMS syringe hookups.

Figure 31. Silicon-SU-8-Pyrex microfluidic chip with PDMS fluid hookups.

3.2.12. Method 1 Silicon to Pyrex Wafer Bonding Results

It was found that the wafers did not hold liquid. It was thus concluded that the bond must be further characterized. It thus remained whether the SU-8 withheld the integrity of the features produced during photolithography, as well as the strongest bond strength. Bond strength was measured by prying the silicon wafers apart with wafer tweezers and investigating the SU-8 features. This bond strength test method is known as the “open-crack method” and only gives a rough estimate of the bond strength [51]. The results of these tests gave poor results at all bond temperatures. Bond temperatures started at 100°C and found poor bond quality, yet signs of wetting across the two substrates. Slightly noticeable increases in bond strength continued up through bond bake temperatures of 170°C. Wafers were broken apart with tweezers and observed. In addition, channel integrity was withheld up until a bond temperature of 180°C was

implemented. A JEOL scanning electron microscope (SEM) was used to assure that the SU-8 structure of the channel walls were not degrading. It was found that around bond temperatures of 180°C, SU-8 began to reflow. Figures 32 & 33 show scanning electron microscopy images of channels at bond temperature 170°C after detachment from Pyrex wafer.

Figure 32. Cross section SEM image (30x magnification) of SU-8 microchannels and reservoir. Image shows channel integrity withheld at bond temperature 170°C.

Figure 33. SEM high magnification cross section image of microchannels.

Image shows channel integrity withheld at bond temperature 170°C.

3.2.13. Method 1 Silicon to Pyrex Cross Sectional SEMs

Wafers were sectioned and different bond areas were investigated. Figure 34 shows an example of different bond areas that were investigated.

Figure 34. Silicon/SU-8/Pyrex wafer post method 1 bond. Red circles indicate fully bonded areas.

Blue circles indicate partially bonded areas. Bond temperature used was 160°C.

The dark areas marked by red circles of Figure 34 represent fully bonded regions of SU-8 to Pyrex. The lighter areas that are marked by blue circles of Figure 34 represent areas of partially bonded SU-8 to Pyrex. This conclusion was drawn by sectioning the wafers and polishing their cross sections. Figure 34 represents the dark region marked by the red circle in Figure 34.

Figure 35. Complete bond of SU-8 to SU-8 without void space. This is the dark region marked by red circles in Figure 33. Bond temperature 160°C.

Figure 36 represents the lighter region marked by blue circles in Figure 34. It can be seen here that the liquid was able to disperse through the wafer through this matrix of voided area. The partial bond near the channels left an easy path for the fluid to break the microfluidic channel boundary to disperse freely between the wafers.

Figure 36. Partially bonded SU-8 to SU-8 with void space allowing for fluid passageways.

This is the light region marked by blue circles in Figure 34. Bond temperature 160°C.

With these results it was found that parts of the wafer began to bond, where other parts had a matrix of partially bonded regions that create voids. These voids lead to easy fluid passageways that allow fluid movement throughout the wafer. Microfluidic implementation is impossible while this problem exists.

3.2.14. Method 1 Results & Conclusion

Results show that there is incomplete SU-8 to Pyrex bonding. Voids are present in the bond which is letting the fluid easily disperse between the wafers. An investigation of molecular changes in the SU-8 processing steps was needed to understand the crosslinking reaction and molecular mobility changes.

Hypothesis Created of SU-8 Mobility: If the post exposure bake time/temperature is reduced, the crosslink density will decrease, which result in a higher molecular mobility for the chains to wet a secondary substrate and obtain a better bond.

Investigation will include:

1. **Differential scanning calorimetry** to relate post exposure bake step to the degree of crosslinking in the film.
2. **Monitoring development** to see if films still exhibit good solvent resistance in exposed regions.
3. **Visual inspection** of bond (% wetted to the substrate)

3.2.15. Plan for Testing & Continuing to Method 2

Before continuing on to method 2, research was done to better understand the crosslinking reaction and molecular level changes occurring in the film upon each processing step. This will discuss in much greater detail the exposure and cationic chain growth reaction of the SU-8.

3.3. Investigation of SU-8 Crosslinking

3.3.1. Description of Crosslinking Investigation

Highly crosslinked films display good chemical resistance and mechanical properties. Control of crosslinking reaction is critical for processing thermoset plastics; both the reaction prior to the gel point and after need to be finely controlled for a useful desired product. The gel point is when one first observes the visible formation of a gel or insoluble polymer fraction. The gel is insoluble in all solvents, even at elevated temperatures, and polymer degradation does not occur. The gel point also corresponds to the formation of an infinite network of crosslinked polymer molecules which have created a macromolecule. Essentially, the entire film is connected and can be thought of as one giant molecule. During the processing of SU-8 too slow or fast of crosslinking can be detrimental to the film and bond strength can be significantly affected. Thermoset polymers are classified as A-, B-, and C-stage polymers depending on their extent of reaction p (0-100%) compared to the critical gelation point (P_c). The polymer is considered in A-stage if $p < p_c$, and has properties of still being soluble and fusible. The B-stage is when the system is near the gel

point ($p \approx pc$) and is still fusible but barely soluble. The C-stage is when it is well past pc , a highly crosslinked film that is both insoluble and infusible [52].

The crosslinking (curing) process of SU-8 occurs in two steps upon exposure: (1) formulation of a strong acid during the exposure step, and (2) an acid-catalyzed, thermally driven epoxy crosslinking during the post exposure (PEB) step. A photomask is used during photolithography to mask it from the UV light in order to develop these unexposed regions away. In this experiment the epoxy crosslinking process is observed with differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). If the crosslinking process can be better understood it may be used to optimize a bonding procedure for fabrication of MEMS devices.

3.3.2. Scope of Experiment

The aim of this experiment was to understand the crosslinking process of a photodefinable epoxy for the development of a bonding process. The bond process consists of bonding the photodefinable epoxy based polymer to another substrate. This however, requires mobility of the polymer chains to be able to move and flow into another surface. This will depend heavily on the degree of crosslinking. The crosslinking process occurs in two steps upon exposure: (1) formulation of a strong acid during the exposure step, and (2) an acid-catalyzed, thermally driven epoxy crosslinking during the post exposure (PEB) step. The theory behind the ability to bond the epoxy film to another substrate is that if the degree of crosslinking can be controlled and inhibited, it will allow mobility of the polymer chains to form a sufficient bond; however, they must be crosslinked at a high enough degree to be chemically stable against the developer. The development step must be performed to develop the unexposed regions which in this case are the microfluidic channels. For this case study, four different films will be created and heat treated during the PEB step at different temperatures and times and thermally tested with differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). The DSC test was used to track the degree of crosslinking and the TGA to track residual solvent (cyclobutanone) left in the film.

3.3.3. Crosslinking

3.3.3.1. Step 1: Initiation

The curing process starts when the thin film is exposed to an irradiant source. Unlike a free radical mechanism, arylsulfonium salts decompose to yield acid catalysts. These salts consist of complex aromatic-sulfur structures with non-nucleophilic anions so that the reaction goes uninterrupted. If the anion was nucleophilic it would terminate the reaction. Epoxy resins are the most common resins used in cationic curing. Saturated cycloaliphatic epoxies are used most widely due to their high rate of cure and environmental resistance [53]. The UV radiation causes the photoinitiator to ionize by photon absorption. The ionization process creates active radicals of R^+ and R^- . The chemicals are strategically picked so that only R^+ radicals react with the monomer, hence the name cationic polymerization. The irradiated protonic acids on photolysis of the photoinitiator causes direct protonation of the monomer to form sulfur-centered cationic species resulting in the generation of a polymer chain [54]. The photochemical decomposition of a sulfonium salt by cationic polymerization is shown in Figure 37. The cleavage of the molecule occurs dominantly homolitically. The photoinitiator is a tri-arylsulfonium salt that is cleaved when exposed to UV radiation ($h\nu$) to form the cationic initiator. Cationic polymerization has an advantage over free radical polymerization of the epoxides due to their insensitivity against air, mainly oxygen, compared to radical photopolymerization of acrylates [55].

Figure 37. UV cleavage of a sulfonium salt that becomes a sulfur centered cationic species that reacts with SU-8 monomers [55].

Figure 38 shows the initiation of representative epoxy groups attached to an –R group, which represents the rest of the SU-8 molecule.

Figure 38. Initiation of the photoinitiator and representative SU-8 monomer reaction with the cationic species [55]. H^+ represents the cationic species.

3.3.3.2. Step 2: Propagation

Further polymerization of the chain growth reaction takes place by the addition of monomers to the growing cationic end. Cationic cure of epoxides progresses through a ring opening process which creates relatively minimal shrinkage compared to other polymeric coatings [53]. Although the shrinkage is minimal, it does occur and causes a final wafer to bow inwards. Figure 39 shows the chain growth of epoxy groups to the cationic species. Oxygen does not inhibit polymerization but water can via chain transfer of the cationic species with a water molecule. This is the reason for dehydration of the substrate prior to spin coating to drive out water and solvents.

Figure 39. Chain growth and the chain transfer of the epoxide polymerization [55].

3.3.3.3. Step 3: Termination

The polymerization chain can be terminated by three different ways: uni-molecular rearrangement of both negative (R-) and positive (R+) charges on the chain, chain transfer, or termination due to impurities [54]. The post exposure bake (PEB) renders the film partially crosslinked, but will need further heat treatment to complete the cure and become chemically stable for a permanent device (hard bake/bond). Studies indicate that there is no trace of catalytic acidity once the cure is complete [53].

3.3.4. Experimental Methods & Materials

To test the effect of the post exposure bake step on the crosslinking reaction, SU-8 thin films were exposed with a T-mixer transparency mask subjected to four different post exposure bakes. Kapton tape was placed on the wafer prior to spin coating so samples could be removed from the substrate, adjacent to the tape. TA Instruments differential scanning calorimetry (DSC) tool was

used to observe the degree of crosslinking that had taken place in the epoxy film. A TA Instruments thermogravimetric analysis (TGA) was used track residual cyclopentanone (solvent) and propylene glycol methyl ether acetate (developer) in the two of the films. Table II shows the four films that were deposited, exposed, heat treated, developed, and then tested by DSC. Film 4 is MicroChem's suggested post exposure bake which will heavily crosslink the epoxy film. Films 1-3 were inhibited PEB's (reduced temperature and time from MicroChem's suggested PEB) conducted to try to inhibit high degree of crosslink density. The degree of crosslinking must only be high enough for exposed (initiated) regions of the film to form a partial crosslinked matrix so that it is insoluble in the developer. A partial heat treatment must take place for this to occur or else the unexposed regions of the film, as well as the exposed regions, will develop away.

Table II. *Four films were deposited, exposed, post exposure heat treated, and developed for DSC and TGA testing*

Film	Post Exposure Bake Temperature		Post Exposure Bake Time	
1	55°C		3 min	
2	65°C		3 min	
3	75°C		3 min	
4/Control (MircoChem Recommended)	65°C (first step)	95°C (second step)	3 min (first step)	8 min (second step)

Percent weight loss was also tracked to determine if there was significant development of exposed regions when exposed to PGMEA due to an insufficient degree of crosslinking.

3.3.4.1. DSC Analysis

The DSC scans taken were equilibrated around room temperature and then heated at a rate of 10°C/min to a temperature of 200°C and 220°C. The sample was then cooled at a rate of 10°C/min to ambient temperature and ramped once more at 10°C /min to a temperature of 200°C and 220°C (it was noticed after the first couple samples that a higher temperature was needed to

ensure full reaction of the films and due to time constraints they were not rerun). This cycle should display crosslinking reactions occurring on the first heat cycle, and then no other reactions should take place on the subsequent heating or cooling cycles.

3.3.4.2. TGA Analysis

TGA was used to determine the amount of residual solvent in the epoxy film after development.

The residual solvents that are expected to be present are cyclopentanone, the SU-8 solution solvent that the material comes in, and PGMEA, the unexposed material developer.

Cyclopentanone has a boiling point of 131°C and PGMEA has a boiling point of 146°C. TGA was performed on films 2 and 3 (due to machine time limitations). The samples were tested with a ramp of 20°C/min from ambient temperature to 600°C. The expected result is that there will be less residual solvent in the film that was baked at 75°C as compared to the 65°C.

3.3.5.DSC & TGA Results & Discussion

3.3.5.1. DSC Results

The DSC data showed the chain growth reaction of epoxy chains occurring in two exothermic steps. Each sample was concluded to be undergoing a reaction, which after completion of the first heat cycle was not observed on either the cooling cycle or second heating cycle. On all DSC graphs exothermic reactions in the negative y-direction. Figure 40 shows the film 1 (PEB: 55°C, 3 min) DSC scan.

Figure 40. (PEB: 55°C, 3 min) Film 1 DSC scan. The scan shows the initial heating of the sample with a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.

The film 1 DSC scan did not provide clear data after the first step of the reaction. A problem occurred around the second step of the reaction which resulted in poor data. There was also some discrepancy with this sample which will be seen on the combined graph displaying all DSC scans in that it looks like an outlier where the reaction starts at a higher temperature than the others do. This sample showed the reaction starting around 65.55°C. This could have been due to a high ramp rate which caused inconsistencies. Figure 41 shows the DSC scan of film 2.

Figure 41. DSC scan of film 2 (PEB: 65°C, 3 min). The scan shows the initial heating of the sample with again a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.

The DSC scan of film 2 shows a much better curve than that of film 1. The reaction of this film is shown to start at 55.05°C which is at a much lower temperature than film 1. This data clearly shows the two step reaction of the epoxy film. Figure 42 shows the DSC scan of film 3 (PEB: 75°C, 3 min).

Figure 42. DSC scan of film 3 (PEB: 75°C, 3 min). The scan shows the initial heating of the sample with again a two-step crosslink reaction, the cooling cycle with no reaction, and the secondary heating cycle with no reaction.

Film 3 also has a lower initial reaction temperature (57.81°C) than that of film 1. This scan also clearly shows a two-step reaction on the initial heat cycle. Figure 43 shows the DSC scan of film 4, the MicroChem recommended baking procedure (PEB: 65°C 3 min, 95°C 8 min). The initial heating cycle and cooling cycle are included while the second heating cycle left out for clarity. The cooling cycle and second heating cycle displayed no sign of further reaction.

Figure 43. the DSC scan of film 4, the MicroChem recommended baking procedure (PEB: 65°C 3 min, 95°C 8 min).

The DSC scan of film 4 shows the degree of reaction is much further along than in films 1-3. The arrow describes how film 4 (green) has been driven to a higher degree of crosslinking, as described by the absence of the first exothermic reaction. Figure 44 shows an overlay of films 1-4 for comparison. Table III compares the amount of heat released (J/g) for each film which correlates to each DSC scan.

Figure 44. Overlay of DSC scans for films 1-4. The DSC scan of film 4 only shows the second step of the reaction.

Table III. Amount of heat released from each film for the first and second exotherm. Data correlates to the DSC scans

Film	Temperature & Time of Bake	1st Exotherm (J/g)	2nd Exotherm (J/g)
1	55°C, 3 min.	30.61	30.91
2	65°C, 3 min.	40.74	43.27
3	75°C, 3 min.	27.8	36.26
4	Normal (65°C, 3 min; 95°C, 3 min)	0	67.38

Film 4 doesn't show any heat release for a first exotherm because the reaction has already completed. Films 1-3 do not show a comprehensible trend with the first exotherm, assuming that there would be less heat released in the higher baked films. Additionally, in films 1-3 it would be assumed that the second exotherms would have similar heat release. Further investigation with a larger sample size would be necessary to understand whether a trend can be found in this data. If significant differences can be found by this method of data collection it could be roughly correlated to the degree of crosslinking in the film, and ultimately to molecular mobility. If samples were to be rerun, a slower ramp would be suggested as well as a standard way to calculate (integrate) for heat flow. The conclusions that can be drawn from these experiments are that the MicroChem suggested bake dramatically increases the degree of cure compared to the inhibited bakes.

3.3.5.2. TGA Results

TGA was performed on the samples with heat treatments of 65°C 3 min and 75°C 3 min to determine the amount of residual solvent left in each film. The data obtained for the PEB of 65°C for 3 minutes is shown in Figure 45.

Figure 45. The TGA graph for PEB of 65°C and 3 minutes. The amount of weight loss from ambient to >300°C is ~6.4%.

The TGA graph obtained for PEB 75°C and 3 minutes is shown in Figure 46.

Figure 46. The TGA graph for PEB of 75°C and 3 minutes. The amount of weight loss from ambient to >300°C is ~7.9%.

The graphs were then combined overlaid (Figure 47) and analyzed with increasing temperature from ambient to around 300°C to observe the weight loss trend (Figure 48).

Figure 47. The TGA data overlay for PEB 55°C 3 min and 65°C 3 min.

Figure 48. The TGA data overlay for PEB 55°C 3 min and 65°C 3 min from ambient temperature to ~300°C.

The TGA data overlay from ambient to ~300°C shows that there is more weight loss occurring for the lower baking temperature. This is due to residual solvent evaporation of cyclopentanone and possible PGMEA. However, this data is only produced from one sample each and could be entirely due to normal variation in sample. The weight percent loss is actually less than 1% and a larger sample size will be necessary to conclude this result. This method is purposely noted as a potential method of designing experiments to further explore SU-8. Solvent loss will become crucial in bond Method 4.

3.3.5.3. Weight Loss Experiment in Developer (PGMEA)

During processing of each sample, weight loss was tracked before and after development to determine whether there was sufficient weight loss with low PEB temperatures. This was done to ensure exposed areas were had reacted enough to become insoluble in the developer. Each wafer was exposed with the T-mixer mask. The weight loss was tracked and compared across the recommended MicroChem specified PEB and the low baking temperature and times used in this experiment. Table IV shows the weights tracked before and after development and the percent weight loss of material due to development.

Table IV. *Weight loss of each film was tracked to determine percent weight loss due to development*

Wafer #	Pre-PEB Processing	Pre-Weight Avg. (g)	PEB	Post-Weight Avg (g)	Difference in Weight Pre/Post	% Weight Loss	Develop Time (min.)
1	Normal (SP)	10.098	55°C 3 min	9.857	0.241	2.387	12
2	Normal (SP)	8.269	65°C 3 min	7.983	0.286	3.459	12
3	Normal (SP)	10.168	75°C 3 min	10.015	0.153	1.505	12
4	Normal (SP)	10.314	65°C 3 min, 95°C 8 min	10.017	0.297	2.880	12

This data shows that there is no significant trend between material losses in each film. The variation is most likely due to wafer handling with wafer tweezers. The tweezers get coated with polymer and can actually redeposit small amounts of polymer back onto the wafer at various untraceable amounts. This must be controlled to determine minute differences between weight loss due to development and differing PEB temperatures. Visual inspection showed no significant material loss due to inhibiting the PEB. However, optical microscopy revealed slight surface pinholes (Figure 49).

Figure 49. SU-8 2050 showing surface pin hole features with reduced post exposure bake (55°C, 3 min).

3.3.5.4. Conclusions of SU-8 Crosslinking Experiment

There are many beneficial conclusions that can be made with these experiments:

1. A lower PEB can be reduced to 55°C for 3 minutes and still develop well enough. This means, exposed regions are insoluble in developer and unexposed regions are insoluble in developer with slight pin hole development of the surface.
2. The cationic chain growth crosslinking reaction occurs in two steps, as seen with the DSC data.
3. Lower PEB temperatures decrease the degree of reaction significantly.
4. It may be that lower PEB temperatures have more residual solvent left in the film; however, more samples must be run for confirmation. This technique may be useful for soft bake residual solvent tracking (Bond Method 4 recommendation).
5. Weight loss due to development is inconclusive.

3.4. Method 2 Bond Processing

As discussed in the overview of bonding procedures, method 2 involved altering the post exposure bake step. Additionally, a new mask was received for the fabrication of a new design.

3.4.1. Base Layer Processing Steps & New Mask Design

The processing steps remained generally the same except new masks were integrated which incorporated for multiple SU-8 layers. The bond design explained in the bond overview section neglected most of these steps. Figure 50 displays a cross section view of the full bond Method 2 design. This design consists of a base layer of SU-8 2050 fully “flood” exposed, gold electrodes that were patterned with positive resist/photolithography, the SU-8 2050 microchannel layer, and a “bonded” Pyrex slide with predrilled fluid input/output holes. Process flow charts for SU-8 2050 base layer processing and Au electrode patterning are shown in Figure 51.

Figure 50. Bond method 2 cross section.

Figure 51. Processing flow chart for SU-8 2050 base layer and gold electrode patterning.

These processing steps will now be discussed before continuing to processing of the microchannel layers.

3.4.1.1. Silicon Wafer Piranha clean/Dehydration

Previously discussed in 3.2.2.

3.4.1.2. Spin Coat

Spin coating was then performed to obtain a uniform SU-8 layer as the base layer of the system. Main spin coating procedures are discussed in 3.2.3. The spin coating recipe used here was a 500 rpm spread cycle for 1 minute with acceleration 100 rpm/s and a 2200 rpm final cycle for 1 minute with a 300 rpm/s acceleration.

3.4.1.3. Edge Bead Removal

Edge bead removal was performed overnight in a covered, dark, dry environment. Edge bead removal was previously discussed in section 3.2.4.

3.4.1.4. Soft Bake

A stepped soft bake was then performed of: 65°C 5 minutes, 95°C 15 minutes to ensure a rigid film. The wafer was then left to cool for ~5 minutes. Soft Baking was previously discussed in Section 3.2.5.

3.4.1.5. Exposure

A “flood” exposure was performed at LI=34, meaning no transparency mask was used and the entire wafer was exposed. The wafer was stacked in the aligner via dummy wafer method. Exposure was previously discussed in 3.2.6.

3.4.1.6. Post Exposure Bake

A post exposure bake was performed of 65°C 3 minutes, 95°C ramped to 200°C over ~ 20 minutes and slowly cooled. If the ramp up and down (especially down) are not performed at a slow rate, the film can delaminate from the wafer. This essentially hard bakes the base layer. It is not well understood how adhesion of the microchannel layer is affected by the extent of cure of this step. Adhesion did pose a problem in later steps when developing the microchannel layer. It may be beneficial to inhibit this layer from hard baking for potential crosslinking with the microchannel layer which could enhance adhesion. Another factor that could be affecting adhesion is SU-8 base layer cleaning, which will be discussed in microchannel processing section. Post exposure baking was previously discussed in Section 3.2.7.

3.4.1.7. Physical Vapor Deposition of Gold

A Denton desk model V sputter coater was used to deposit a nanometer scale thick gold layer. Physical vapor deposition (PVD) was performed at 9 mTorr, power level of 50 watts, for 5

minutes. This resulted in a 200-300 nm thick gold film. Titanium was also sputtered and patterned for electrodes and resulted in sufficient adhesion. No other metal PVD processes were attempted.

3.4.1.8. Positive Resist Spin Coating

A positive resist (Shipley S1813) was used for the gold wet etch mask [56]. A Brewer 200X spin coater was used with spin recipe (Positive Resist 2): 100 rpm 15 seconds (deposit), 200 rpm for 10 seconds, 500 rpm for 10 seconds, 4000 rpm for 20 seconds (thickness dependent step), and 300 for 5 seconds.

3.4.1.9. Positive Resist Soft Bake

A stepped soft bake of 65°C 30 seconds, 95°C 1 minute was used to drive out remaining solvent. The wafer was stepped up and down to reduce thermal shock effects.

3.4.1.10. Positive Resist Exposure

The positive resist was exposed with the dummy wafer and manual stack method described previously in 3.2.6. A light integral of 4.5 was used for exposure. The transparency mask was visually aligned and could be manipulated to avoid any defects such as bubbles, edge bead, and even insufficiently coated wafers. Positive resist works in the opposite manner as that of a negative resist. Where positive resist is exposed to light, these regions become soluble in liquid developer. Additionally, this positive resist does not require a post exposure bake before development. Figure 52 shows the transparency mask used for electrode exposure.

Figure 52. Transparency mask CAD drawing of electrode placement which will align to reservoirs of fluid channels.

3.4.1.11. Positive Resist Develop

The wafer was developed in a Microposit developer (MF-CD-26). Warning: contains Tetramethylammonium hydroxide (TMAH), molecular formula of $N(CH_3)_4^+ OH^-$. Development time was usually around 4 minutes with slight agitation. The wafer was then rinsed in DI water (x4) and subjected to a spin rinse dry (SRD) cycle. Electrodes were then tested for resistance. Various electrodes were categorized whether they were on the interior or exterior location on a wafer (Figure 52) and the resistance measurements are shown in Table V.

Table V. Resistance measurements for various electrode locations

Electrode	Resistance (Ω)	Location (Figure 52)
1	135	Outer
2	132	Inner
3	136	Outer
4	131	Inner
5	135	Outer
6	131	Inner
7	137	Outer
8	131	Inner
Average	134	
Std. Dev.	4.24	

It is shown here that the resistance of electrodes located the outer edge of the wafer had slightly higher resistance. This is most likely attributed to the fact that the gold layer deposited on the wafer is thicker towards the middle and thinner towards the edges which causes the resistance to be slightly higher.

3.4.1.12. Hard Bake

A hard bake step of the resist may or may not be used. The hard bake is performed at 150°C for 1 minute (stepped from 65°C). It was found that this step is not necessary for adequate development of gold electrodes. Additionally, a rapid bake like this can cause stresses between the various layers of materials. Skipping this process would be advised.

3.4.1.13. Gold Wet Etching

Gold etching was performed in a small wafer-sized petri dish for no more than 1.5 minutes. Gold etchant used is a Transene product (GE-8148) containing a KI-I₂ complex. Etching was performed at room temperature. Prolonged etch times may cause swelling of base layer SU-8 and can be seen by a darkening of the base layer. This may also cause problems with

delamination and subsequent microchannel layer adhesion. The shortest gold etch is recommended with an immediate DI rinse and SRD cycle.

3.4.1.14. Positive Resist Strip

The positive resist stripping process is performed with a Microposit Remover 1165 at 50-60°C for ~5 minutes (or until resist has been dissolved). This is to ensure removal of positive resist from remaining gold electrodes. A picture of the wafer thus far is shown in Figure 53.

Figure 53. Wafer with SU-8 base layer and Au patterned electrodes.

3.4.2. “Functional” Microchannel Layer Processing Steps & Bond

Now that the base layer and electrodes have been patterned, it is time to create the fluid layer and discuss bond Method 2. The process flow chart for the second half of processing is shown in Figure 54. This is usually broken up into a two day process from what has been discussed previously and finalizing the design. The only issues that arise are cleanliness and/or degree of cure of the base layer which will be discussed in the subsequent sections.

Figure 54. Process flow for the functional microchannel layer and bond procedure.

3.4.2.1. Wafer Clean

This is a controversial step in the process flow that has been previously mentioned. With an “old” Piranha solution the wafer with a base layer of SU-8 2050 and gold electrodes were subjected to ~4 minute Piranha clean at 70°C. This sometimes caused marks to form on the wafer (SU-8) surface (Figure 55) and caused discoloration of the Piranha (Figure 56). This only happened after the Piranha had been replaced with new solution. It was hypothesized that the increased amount of hydrogen peroxide in the solution caused an increased “attack” on the wafer. An old Piranha solution would not have as high of hydrogen peroxide levels due to the evaporation that occurs over time. Additional considerations which could not be tested were that the degree of cure of the base layer SU-8 film. It would be recommended to investigate the effect of not hard baking the film until the final wafer bond procedure to possibly enhance chemical bonding between the fluid channel layers and base layers of SU-8. Additionally, it was also hypothesized that the absorption of Au etchant could be causing the discoloration of the Piranha. It was shown with long exposure to Au etchant that SU-8 films discolor (Figure 57).

Figure 55. Discoloration of SU-8 2050 base layer due to Piranha exposure.

Figure 56. Discoloration of Piranha solution after removal of wafer (wafer shown in Figure 55).

Figure 57. Discoloration of SU-8 due to prolonged Au etchant exposure.

Picture of embedded mask design (not discussed in this report).

3.4.2.2. Wafer Dehydration

The wafers were then subjected to a SRD cycle and dehydrated at $\sim 120^{\circ}\text{C}$ which was obtained with a temperature ramp for 20 minutes. Wafers were then cooled down slow to 95°C , stepped to the 65°C hot plate. They remained on the 65°C hot plate for 1 minute and then were cooled to room temperature.

3.4.2.3. Functional Microchannel Layer Spin Coating

After cooling to room temperature, wafers were centered on the negative resist spin chuck with a 100 mm wafer aligner. The spin coater was previously covered in aluminum foil for easy clean up.

A sufficient amount of SU-8 2050 was deposited straight from the bottle into the center of the wafer and let to settle with the lid closed. This set up is shown in Figure 58. This was previously described in 3.2.3.

Figure 58. Silicon wafer with SU-8 base layer and gold electrodes previously patterned and the SU-8 2050 material for the microchannel layer ready to be spin coated.

The spin coating procedure used to create the microchannel layer was: 500 rpm at 100 rpm/s for 1 minute and 1500 rpm at 300 rpm/s for 1 minute. Table V shows profilometer scans with an Ambios XP-1 profilometer, which were done to determine the film thickness and thickness variation.

Table VI. *Profilometer scans measuring SU-8 2050 microchannel layer thickness of two wafers, at various points on the wafers*

Scan	Wafer 1	Wafer 2
1	99.5	99.4
2	99.2	98.8
3	99.7	99.8
4	99.9	99.3
Avg.	99.575	99.325
Std. Dev.	0.299	0.411

The profilometer scans were made at four varying locations on two separate wafers. The thickness was near 100 microns and a 2-sample T-Test comparing the means concluded no significant difference between the thicknesses with a 95% confidence interval. Edge bead removal is skipped during the processing of the functional layer due to the fact that most of the transparency mask accounts for dissolution of the outer region of the wafer by inhibition of exposure.

3.4.2.4. Soft Bake

Soft baking temperatures ranged from 65°C for 3 minutes stepped to 95°C for 8 minutes to 65°C for 5 minutes stepped to 95°C for 15 minutes. This depends upon how rigid the film needs to be with handling. The MicroChem suggested bake is the latter. Residual solvent can act as a plasticizer of the film but could also potentially react during the cure process and could cause deleterious effects. Wafers were stepped down to 65°C for 1 minute and cooled for 5 minutes at room temperature.

3.4.2.5. Exposure of Functional Layer

SU-8 2050 microchannel layer was exposed using the manual wafer stack method and dummy wafer. The wafer was stacked on a glass mask blank with the transparency mask in direct contact on top of the wafer and i-line filter on top of that. Visual alignment of microchannels to electrodes was necessary. A light integral of 34 was used for exposure.

3.4.2.6. Inhibited Post Exposure Bake of Functional Layer

A post exposure bake of 55°C for 3 minutes was used. An exposure pattern was visible in the SU-8 after this bake. The wafer was left to cool for 5 minutes.

3.4.2.7. Development of Functional Layer

Development was performed in PGMEA at room temperature with the wafer upside down in a wafer cassette. Slight agitation was used. In unused PGMEA development times were ~ 4 minutes. It would take much longer in a petri dish with the wafer right side up. Wafers were quickly removed and rinsed with IPA. Do not use water or acetone! Visual inspection allows for analysis of complete development. Upon compressed N₂ drying, if white streaks are formed the wafer can be re-rinsed with IPA and even re-submerged in PGMEA, rinsed with IPA, and dried with N₂.

Adhesion Problem: Upon rinsing the wafer with IPA is when delamination occurs (Figure 59). To reiterate, possible factors include: base layer extent of cure, absorbance of Au etchant, cleanliness, and even perhaps residual solvent from reduced soft bake.

Figure 59. Severe adhesion failure occurring upon microchannel layer development. Root cause unknown (worst case observed).

3.4.2.8. Drilling Pyrex Wafer Input/Output Fluid Holes

Previously discussed in 3.2.1. Small, individual slides were used to separately bond to each microfluidic chip instead of an entire glass wafer. These slides were Pyrex (Esco Glass, Corning 7740) glass of dimensions 31 mm X 8 mm +/- .13MM X 0.50 mm.

3.4.2.9. Cleaning Pyrex Slide/Dehydration

The cleaning process was performed by submerging the Pyrex in Piranha at 70°C. Additional methods used an IPA ultrasonic clean. A difference in cleaning methods did not show any substantial difference in bond results.

3.4.2.10. Bond Method 2

Bond Method 2 included alignment of Pyrex slides with microfluidic channels and then placing the aligned wafer between a stiff glass slide (top plate) and aluminum wafer plate (bottom plate) as

shown in Figure 60. This was done at room temperature. Immediate wetting of (some of) the surface could be seen in certain areas. Applied pressure was not well monitored. The bond apparatus was assembled at room temperature and placed in an oven at 70°C. The temperature was then slowly ramped to 170°C over ~20 minutes and baked for an additional 20 minutes. The oven was then shut off and left to cool to room temperature (slowly).

Figure 60. Method 2 bond set up.

3.4.3. Method 2 Bond Results

Bond Method 2 resulted in much higher percent bonded area, but none reached 100%. Bonds were characterized by percent bonded area (dark portions) to unbounded areas (lighter portions), discussed in section 3.2.13. Pictures of various bond trials are shown in Figures 61-66.

Figure 61. Insufficient bond result of bond method 2 (1).

Figure 62. Insufficient bond result of bond method 2 (2). Clamp method: c-clamp.

Figure 63. Pyrex cracking as a result of bond method 2 (1). Clamp method: c-clamp.

Figure 64. Pyrex cracking as a result of bond method 2 (2). Clamp method: c-clamp.

Figure 65. Pyrex cracking as a result of bond method 2 (3). Clamp method: c-clamp.

Figure 66. Pyrex cracking as a result of bond method 2 (4).

All iterations of bond Method 2 produced an insufficient bond or resulted in Pyrex cracking. It was confirmed that the Pyrex would crack during the bond bake temperature ramp with c-clamp style pressure application, normally around 120-130°C. It is hypothesized that the thermal expansion of materials in the bond apparatus and wafer caused a catastrophic pressure to be obtained, which resulted in fracture. With the application of spring clamps this affect was avoided; however, these clamps did not apply even or high enough pressure to obtain a complete bond.

3.4.4. Method 2 Conclusions & Hypothesis Revision 1

After multiple trials it was concluded that Method 2, even if a complete bond was obtained, was not a suitable and most importantly, not a repeatable procedure for microfluidics fabrication at Cal Poly. With a reconstructed bond set up that applies even pressure and can produce repeatable pressures, it may be possible to use Method 2 as a repeatable fabrication technique. Future investigation of Method 2 should involve careful study of surface roughness of (both) substrates, wafer bow, and spring clamp/elastic bond head material for the bond apparatus to account for thermal expansion. For this project, instead of investigating a sophisticated bond apparatus, attention was refocused to molecular level changes in SU-8 film during processing. A potential apparatus is discussed here [57].

Hypothesis Revision of SU-8 Mobility: With current methods, the reduced PEB greatly increased mobility of the film but is not enough to sufficiently wet a secondary substrate. An uncured SU-8 2007 film (~8 microns) that will be spin coated on the glass slide will provide for enough mobility of the chains to wet the microchannel layer of SU-8 2050 and provide for a complete bond.

Plan: The SU-8 2007 film will be spin coated, partially soft baked, cooled to room temperature, brought into contact and aligned with the silicon wafer, and slowly reheated to soften the SU-8 2007 and control wetting/bonding of the substrates.

3.5. Method 3 Processing Steps

Method 3 will include all processing steps mentioned in Method 2 (section 3.4) including the inhibited PEB. The glass slide, subsequent to the cleaning steps was then spin coated with an SU-8 2007 film, soft baked, and then brought into contact with the silicon wafer with developed functional microchannel layer (Figure 67). Processing description will continue from Method 2, completion of developed SU-8 2050 microchannel layer and Pyrex slide cleaning.

Figure 67. Bond method 3.

3.5.1. Pyrex Slide Spin Coating

After cleaning, Pyrex slides were mounted on silicon “dummy” wafers via double sided tape. Minimal tape was used, but ensured that all corners of sides of Pyrex were contacted so that SU-8 2007 could not flow underneath Pyrex and coat the other side. Additionally, adhesion of double sided tape should be inhibited by repeatedly adhering/un-adhering it to a Kimwipe to allow for degradation of the adhesive. If this is not done it will be extremely difficult to remove the Pyrex slide from the silicon and will often result in fracture of one or both. Pouring SU-8 2007 is difficult in that it requires the full coverage of the slide prior to the spin cycle; otherwise it will not coat the entire slide. The spin coating recipe used is: 500 rpm for 20 s (100 rpm/s acceleration), 3500 rpm for 45 s (300 rpm/s acceleration). Slides were then carefully peeled off of the silicon without touching the coated surface. The double sided tape was then peeled from the back of the slide

and the fluid input/output holes were inspected for SU-8 coverage. Holes were not usually covered but tweezers were used to clear any SU-8 out of the hole, if necessary.

3.5.2. Soft Bake of Pyrex

Pyrex slides were soft baked at 65°C for 3 minutes. With a much thinner film than the SU-8 2050, the SU-8 2007 had significant solvent loss during this bake which resulted in a rigid film upon cooling to room temperature. TGA experiments or hardness tracking during soft bake would be recommended for better understanding of effects on the SU-8 film as solvent is evaporated.

3.5.2.1. Method 3 Bond

After the Pyrex slide had been sufficiently cooled to room temperature (~ 5 minutes) it was aligned and put into contact with the silicon wafer with developed microchannel layers. At this time there should be no wetting of the SU-8 layers. The wafer was then placed on a hot plate at 45°C with weight stacked on top. Temperature was slowly ramped to 85°C maximum (< 5°C/minute) while the bond was frequently monitored.

3.5.3. Method 3 Bond Results

This method of bond resulted in full bonding, but significant SU-8 2007 had begun to flow in the channels which would cause fluid blockage. Sequential pictures of Method 3 bond are shown in Figures 68-74. In the sequence, the left slide is the bond Method 3 described here and the right slide is a slide that was coated and not subjected to a soft bake. The right slide resulted in immediate wetting of the substrate and began to drift which caused misalignment. Additionally, the right slide completely filled the microchannels with the SU-8 2007.

Figure 68. Method 3 bond sequence (1).

Figure 69. Method 3 bond sequence (2).

Figure 70. Method 3 bond sequence (3).

Figure 71. Method 3 bond sequence (4).

Figure 72. Method 3 bond sequence (5).

Figure 73. Method 3 bond sequence (6).

Figure 74. Method 3 bond sequence (7).

As seen in Figure 74, (7) the SU-8 2007 had significant reflow in the microfluidic channels in certain areas and near incomplete bonding in others. Reflow of SU-8 2007 in a 200 micron wide channel can be seen in Figure 75.

Figure 75. Optical Microscopy (top down) of a 200 micron wide channel with significant SU-8 2007 reflow.

3.5.4. Method 3 Conclusions

The SU-8 2007 adhesion layer provided sufficient mobility to wet the microchannel layer and start a bond; however, the SU-8 2007 experienced significant reflow in late stages of the bond process. The soft baked Pyrex, as compared to the non-soft baked Pyrex produced much better control of the bond yet had problems with uneven pressure distribution across the bond interface.

Hypothesis of bond interface: The silicon wafer may be experiencing significant bowing (visually concluded) due to multiple layers of SU-8 with residual stresses from the curing process. This results in a slightly bowed microchannel layer which does not allow for even contact of the Pyrex slide. It can be seen from the sequential bond pictures that the contact is first made on the outer portions of the wafer and increase inward as the bond progressed, or as SU-8 is forced outward and into the microchannel which brings the two closer together in the middle.

It is now hypothesized that if a flexible substrate is used in place of the Pyrex, the SU-8 2007 adhesion layer will be able to conform to the bowed microchannels and sufficiently wet the surface and obtain selective bonding with minimal reflow. This will be Method 4.

3.6. Method 4 Final Bond

Method 4 is the method that completed the SU-8 bonding investigation and received a near 100% bond to create microfluidic chips.

3.6.1. Method 4 Processing

Due to the redundancy of the processing steps, most will be left for reference from bond Method 2 (Section 3.4). The process flow charts of all bond Method 4 processing can be seen in Figures 76 & 77). Processing will pick up after development of the microchannel layers on the silicon wafer, and start on the preparation of the flexible polymer substrate.

Method 4 (1/2)

Figure 76. Method 4 processing steps (1/2).

Method 4 (2/2)

Figure 77. Method 4 processing steps (2/2).

3.6.2. Flexible Polymer Substrate (PET) Selection

Due to the results and notion that was received from bond Method 3 the search began for a flexible substrate to perform the spin coating. The ideal material was not sought out for, but simply aiming to prove the concept of using a flexible substrate rather than a relatively stiff one. Proof of concept for this idea quickly led to the conclusion that a cell phone screen cover was a sufficient material for testing this concept. The reasons for using a cell phone cover is that it comes with both sides of the film covered to maintain cleanliness, it will “stick” to glass well enough for spin coating but can be peeled off with minimal effort, it can be easily cut to conform to multiple fluid chip shapes, and fluid holes can be simply punched through the substrate with minimal deformation. The material was minimally researched and was found to most likely be polyethylene terephthalate (PET), but will be shown later to have multiple layers.

3.6.3.Fluid Input/Output Hole Punching & cutting

Fluid holes were simply punched out with a crafters hole punch that was 1 mm in diameter. The holes were visually aligned with a transparency mask to make markings where the punches need to be made and to cut out films to conform to various chip sizes. The films were then visually aligned to punch the fluid holes. Both protective sheets were kept on the cell phone cover during this process. Numerous holes can easily be made with this process. The punching process did cause minimal deformation around the reservoir region which will cause problems with completed bonding in later steps. This problem could be avoided by reheating the polymer substrate and trying to reform the material to obtain a completely planar sheet. It will be mentioned in the Recommendations Section to separate the input/output reservoirs from the functional chip regardless for an increase in space on the chip as well as issues that occur with spin coating and bonding with a through hole in the substrate being coated.

3.6.4.Spin Coating Polymer Substrate

The spin coating process took a lot of careful handling to not disrupt the coated substrate. The protective films were removed from the polymer substrate (cell phone cover) and placed on a glass microscope slide that had been adhered to a dummy silicon wafer with double sided tape. The same forces that hold a cell phone cover onto the screen of your phone held the films on the glass slide. Figure 78 shows a top-down image of this wafer set up that can be used for spin coating.

Figure 78. Dummy silicon wafer set up for spin coating polymer substrates with SU-8 2007.

Once the silicon wafer is positioned on the vacuum chuck of the spin coater, the SU-8 2007 is poured onto the wafer. This process does require a significant amount of SU-8 2007 to ensure coverage of all individual films. The spin coating recipe employed was: 500 rpm for 20 s (100 rpm/s acceleration), 3500 rpm for 45 s (300 rpm/s acceleration).

3.6.5. Soft Bake

The glass slide was then removed from the silicon wafer and placed on a hot plate at 65°C for 3 minutes. This simultaneously soft baked the coated polymer substrates to drive out solvent and cause a “hardening” of the films. The glass slide was then cooled at room temperature for 5 minutes. Tweezers were used to ensure that the films had gained sufficient rigidity. Prior to soft baking tweezers would cause viscous flow of the film if brought into contact, but after baking, the films held their shape and could be scratched and deformed with enough pressure.

Recommended experiments to track solvent loss would include TGA and micro hardness or viscosity measurements with respect to temperature and time of soft bake. Theoretically, even if all solvent was driven from the film the chains are still not crosslinked and will be able to flow at a certain temperature. Due to the complexity and aromatic structure of the SU-8 monomer it is

hypothesized that as solvent, which acts as a plasticizer, leaves the film it will become more rigid and “solid-like.” This was found to be true in the SU-8 2007 with a 65°C 3 minute soft bake. After this bake and cooling the film to room temperature it was found that reheating to around 45°C - 50°C the SU-8 began to re-soften, and had enough thermal energy for the monomer to begin to reflow. This mobility will continue to increase and the film will become more “liquid-like” as the temperature is continually increased. This will be used to bond the polymer substrate to the silicon wafer with developed microchannels.

3.6.6.Method 4 Bond

The coated polymer substrates on the glass slide were carefully peeled off and each film was aligned with the respective microchannel on the silicon wafer. As the coated polymer substrates are peeled off, any SU-8 2007 that had filled the fluid holes is left attached to the glass slide which leaves a clear passage through the polymer substrate for fluid connections. No wetting should occur at this stage of contact between the microchannels and coated polymer substrate. Figure 79 shows a cross sectional depiction of this process.

Figure 79. Method 4 cross section of bond interfaces being brought together.

Figure 80 shows an image of initial contact of coated polymer substrates with the silicon wafer.

Figure 80. Method 4 bond, initial contact of coated polymer substrates with silicon wafer microchannels. (1)

Wafers were then placed on a hot plate at 45°C and slowly ramped to 75°C (or less) while visually monitoring bond progress. Ramp speed was < 5°C per minute. To help contact of the bond interface, wafer tweezers were used to lightly induce wetting by applying pressure to the top of the polymer substrate. Too much pressure can cause unwanted reflow of SU-8. Wetting increased as temperature increased. Sequential picture of this bond, relating to Figure 79, are shown in Figures 80-83.

Figure 81. Method 4 bond with wetting started. Temperature at time of photo 55°C. (2)

Figure 82. Method 4 bond with wetting nearly complete. Temperature at time of photo 65°C. (3)

Figure 83. Completed bond with IDEX Nanoport connections made (discussed in Section 4.1). Left chip, particle shown trapped between a polymer substrate and silicon chip which resulted in an incomplete bond. (4)

3.6.7. Method 4 Bond Results

By visual inspection bond results were positive with near 100% wetting. Optical microscopy top-down imaging of SU-8 2007 and SU-8 2050 interface was used to evaluate SU-8 bond area and reflow into channels. The fluid reservoirs were shown to have poor bonding due to insufficient spin coating and deformation of the polymer substrate from fluid hold punching. Optical microscopy pictures of the channels are shown in Figures 84-87.

Figure 84. Top-down optical microscopy of 200 μm channel.

Figure 85. Top-down optical microscopy of 200 μm wide channel.

Figure 86. Top-down optical microscopy of 500 μm wide channel.

Figure 87. Top-down optical microscopy of 500 μm wide channel.

Additional images of intricate mixing structures and channels are shown in Figures 88 & 89.

Figure 88. Top-down optical microscopy image of a fluid mixing reservoir containing mixing posts (SU-8 2050 features) with 200 micron channels leading in and out of the reservoir.

The mixing posts shown in Figure 88 are used to create turbulent flow as fluid passes around them. SU-8 2007 bonded to the posts with few voids and minimal reflow.

Figure 89. Top-down optical microscopy image of a Z-shape channels (far right) entering S-shape channels to increase length fluid flow in chip. Channels are 200 microns in width.

The reservoirs are the location where main bonding problems occur. This has been previously discussed with the hypothesis that polymer substrate deformation from fluid hole punching and spin coating. Figures 90-91 show results of bond quality surrounding the fluid reservoirs.

Figure 90. Top-down optical microscopy image of a fluid reservoir etched in the SU-8 2050 microchannel layer with patterned gold electrode and coated polymer substrate bonded on top. (Polymer substrate misaligned with fluid reservoir).

Figure 91. Top-down optical microscopy image of a fluid reservoir etched in the SU-8 2050 microchannel layer with patterned gold electrode and coated polymer substrate bonded on top.

It can be seen that many scratches on the polymer surface result from using the wafer tweezers to create contact of the two layers between the bond interface. This method could be improved to prevent damage of the polymer film that would allow for better visibility of the fluid channels. The bond was also investigated by obtaining a cross section of the channel layers to check for SU-8 2007 reflow into the channels. A scanning electron microscopy picture of a 500 μm wide channel can be seen in Figure 92. This sample was not polished but was taken from a wafer that was accidentally fractured.

Figure 92. SEM cross section of a 500 μm channel that had been detached from the silicon wafer (should be on bottom). The SU-8 layers show signs of complete bonding; the polymer substrate/SU-8 2007 layer however shows signs of delamination.

The sample used for the SEM cross section was taken from a fractured wafer and caused detachment of SU-8 base layer and rest of chip from the wafer. The sample can be seen in Figure 93. The amount of bowing in the SU-8 chip should be noted from this picture.

Figure 93. Sample used for SEM cross section was taken from fractured wafer. Top-down view and side view can be compared with noted amount of bowing in SU-8.

3.6.8. Method 4 Conclusions

Method 4 bond processes resulted in (nearly) 100% bonded area. Few voids can be seen between the bond interface, concluded by optical microscopy. SEM concluded that the SU-8 layers showed signs of a full bond between the interfaces. Additionally, it was shown that various intricate designs could be used in future microfluidic devices.

4. Testing

Microfluidic chips were tested for:

1. Working fluid pressures.
2. Ability to obtain electroosmotic flow.
3. Streaming potential.
4. Electrophoresis of glass particles.

Plan for testing will also include an explanation of IDEX Nanoport attachment, syringe pump integration, and method of testing volumetric flow rates by weight.

4.1. IDEX Nanoport Attachment

IDEX Nanoports used for fluid connection were commercially purchased products. They are meant to provide an easy attachment and detachment method of fluid connections. A cross section of a Nanoport setup is shown in Figure 94.

Figure 94. IDEX Nanoport cross section. [58]

Nanoports came with minimal instructions so fabrication was improvised. Nanoports were clamped over the fluid via with an inserted O-ring (for proper fluid seal) and epoxy adhesive ring (to bond to the substrate). The instructions specify the Nanoports must then be heated to $\sim 177^{\circ}\text{C}$ for 1 hour to cure the epoxy ring [59]. The Nanoports used in this study were part numbers: N-121S (Nanoport assembly 1 mm x 1 mm), N-100-01 (Adhesive rings), P-622 (Luer adapter assembly), and 1572 (320 μm inner diameter PEEK tubing). The Nanoport assembly is shown in Figure 95.

Figure 95. Nanoport assembly 1 mm x 1 mm, part numbers N-121S [59].

The Luer fitting assembly that connects a syringe to the 320 μm inner diameter tubing to the Nanoport assembly is shown in Figure 96.

Figure 96. Luer adapter assembly, part number P-622 [59].

Bonding did not form a sufficient bond to the polymer substrate. Adhesion of the epoxy rings was insufficient and delamination occurred. Recommendations for a better bond would include additional pressure, scoring the polymer surface to enhance adhesion, and slow heating and cooling of the system, otherwise the SU-8 layers can delaminate or crack. Due to time constraints, PDMS was used to seal the Nanoports to the fluid chips. Idex clamping mechanism shown in Figure 97 (before and after baking).

Figure 97. Four Nanoports bonded to two microfluidic chips (one input, one output on each chip).

PDMS was casted around each Nanoport to increase durability. PDMS was chosen so that the Nanoport could eventually be removed and reused, otherwise a stronger epoxy would be recommended. Capillary forces resulted in the PDMS to flow underneath the port but with the O-ring in place and clamping pressure applied, fluid vias were not compromised. Nanoport parts (except for the epoxy rings) are reusable many times. The adhesion rings must be stored in a refrigerator to prolong lifetime. Complete microfluidic chip to syringe pump set up is shown in Figure 98 with a close up of the Nanoport attachment via PDMS in Figure 99.

Figure 98. Full Nanopore assembly.

Figure 99. Microfluidic Chip with Nanopore connection via PDMS reinforcement.

It was found that fluid leaks occurred at Nanoport (threaded) connections into microfluidic chip. The implementation of Teflon tape on the threaded section aided in a tighter seal and fixed the leakage problem. Additionally, 320 μm ID tubing was eventually replaced with larger ID tubing (1 mm) which caused less resistance to flow. Tubes were connected to the Nanoport threaded connections with Loctite glue. This created far less resistance in fluid delivery and output setup to minimize pressure increase of the system from components that were not the microfluidic chip.

4.2. Fluid Leak Syringe Pump Tests

4.2.1. Fluid Leak Test Setup

Fluid leakage tests began with various flow rate trails (5 $\mu\text{L}/\text{min}$, 15 $\mu\text{L}/\text{min}$, 25 $\mu\text{L}/\text{min}$, 50 $\mu\text{L}/\text{min}$) to determine whether the fluid chips could maintain a workable fluid pressure. All tests were run for at least 30 minutes (>2000 seconds). An NE-300 New Era Syringe Pump and 30 mL syringe was used to pump the fluid. The pump was connected to a t-splitter directed (1) to a pressure sensor and (2) the microfluidic chip. The pressure sensor was used to track pressure within the system while the fluid pumped through the chip was excreted from an output tube onto a scale to measure weight over time. A 30 mL syringe inner diameter setting of 23.30 mm was used. Both pressure and weight were monitored every second. The pressure sensor is meant for gas pressure measurements only, so it was connected to fluid tubes and used an air bubble in which the fluid would compress to take pressure measurements. A Vernier gas pressure sensor (GPS-BTA) was used for testing [60]. This unit's resolution is around 0.05 KPa and has a maximum pressure reading of 210 KPa ($\sim 30 \text{ lb}/\text{in}^2$). Unit resolution was approached in many measurements. Weight measurements were taken with a constant drip of fluid into a cup on the scale. This weight measurement was then converted to a volume by the density. Volume change was plotted vs. time and a linear fit was used to obtain a volumetric flow rate. An Adam PGW precision balance was used with resolution of 1 mg [61]. The system used is shown in Figure 100. Microfluidic channel used for all tests was 200 μm in width, 100 μm in height, 20 mm long. Deionized water was used for the test at a constant (average) temperature of 22°C. These tests

were run with 320 μm ID tubing for the input and 1 mm ID tubing for the output. A system picture of the chip-to-scale connection is shown in Figure 101.

Figure 100. System setup used to track flow rate versus pressure.

Figure 101. Output tube shown running from microfluidic chip to scale.

4.2.2. Results of Fluid Leak Test

A dual y-axis plot of volume of liquid displaced through the microfluidic channel and pressure difference across the channel is shown in Figure 102 at a syringe pump flow rate setting of 5 $\mu\text{L}/\text{min}$. Once again, volume was obtained from weight measurement conversion by the density.

Figure 102. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 5 $\mu\text{L}/\text{min}$.

As can be seen from the plot, the constant drips onto the scale caused a step pattern when the volume was plotted over time. This also caused a stepping pattern in the pressure readout due to the drip formation at the end of the (output) tube which caused a force pulling through the entire system. As soon as the drip released from the tube (and fell to the scale) the pressure would rise. Although this may be a rough method to measure flow rate, the fit of the line to volume v. time is a relatively good fit (R^2 value > 0.99). The maximum pressure difference obtained across the channel was ~ 3.45 kPa, a relatively low pressure. Additionally, it took >1000 seconds to see signs of pressure equalization (amidst the continuous jumps due to drips of fluid). Figure 103 shows a dual y-axis plot of volume and pressure monitoring for a 15 $\mu\text{L}/\text{min}$. flow rate.

Figure 103. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 15 µL/min.

The plot shows the same result of dripping water over time, with an increased occurrence of each drip. This is expected due to an increase in flow rate. Again, an excellent fit of the volume data is shown (> 0.99). A maximum pressure of ~4.9 KPa was reached and it took > 2000 seconds for pressure equalization of the system. Figure 104 shows a dual y-axis plot of volume and pressure monitoring for a 25 µL/min. flow rate.

Figure 104. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 25 µL/min.

The plot of a 25 µL/min syringe pump flow rate resulted in increasing frequency of fluid drips. A maximum pressure difference of 6.65 KPa was obtained and it took > 2000 seconds for pressure equalization of the system. Figure 105 shows a dual y-axis plot of volume and pressure monitoring for a 50 µL/min. flow rate.

Figure 105. Dual y-axis plot of volume and pressure monitoring over time at a syringe pump flow rate of 50 µL/min.

The plot shows increasing line fit to the volume data (>0.999). The maximum pressure difference obtained was 10.75 KPa and pressure equalization took > 2000 seconds, once again. The line fit data was used to create a volumetric flow rate from each graph. The slope essentially gives a volumetric flow rate and each was plotted against the pressure difference upon equalization (assuming it had equalized). The empirical volumetric flow rate as a result of pressure difference is shown in Figure 106 compared to theoretical flow rate values obtained for a certain pressure by the Hagen-Poiseuille equation.

Figure 106. Empirical vs. theoretical flow rate values for a given pressure difference across the microchannel.

This Figure (106) shows a vast difference in empirical vs. theoretical flow rate values. This is most likely attributed to non-ideal behavior of fluid chip solid/liquid interactions, 320 μm inner diameter input tubes used, and the additional tubing and complexity of the system from the syringe pump to the scale. Additionally, the Nanoports cause fluid to be driven vertically through many different connections. The empirical equation fit for the data shows excellent fit and can be used to calculate volumetric flow rates obtained at various applied pressure differences in the same system. Table VII shows the calculated flow rates at each syringe pump setting (one trial each).

Table VII. *Measured flow rate calculated compared to each syringe pump setting*

Syringe Pump Setting ($\mu\text{L}/\text{min}$)	Flow Rate Measured ($\mu\text{L}/\text{min}$)
5	4.8
15	14.1
25	24.1
50	51.5

Additional experimental work for working pressures of the microfluidic chip increased until the gas pressure sensor reached its maximum readout value. This concluded (with a sample size of one chip) that working pressures can reach values of 210 KPa corresponding to a ΔP of 107.41 KPa ($15.57 \text{ lb}/\text{in}^2$) and possibly higher.

4.2.3. Conclusions of Fluid Leak Test

There were no devices that suffered microchannel fluid leaks or failures. Main mode of leakage was observed in the Nanoport (threaded) fluid connections and was resolved with the application of Teflon tape. The fluid leak tests did result in an empirical equation that differed from the theoretical Hagen-Poiseuille equation of volumetric flow vs. pressure difference across the length of a microfluidic channel. Additionally, the gas pressure sensor reached its maximum readout value, concluding that the microfluidic chips have the capability of withstanding pressure of 210 KPa ($\sim 30 \text{ lb}/\text{in}^2$), and possibly higher.

4.3. Syringe Pump Repeatability & Variability Test

Due to the noticed pressure equalization time (> 2000 seconds), flow rate repeatability measurements were taken at a flow rate of $10 \mu\text{L}/\text{min}$ after pressure had equalized. Four trials were used to understand the repeatability and variability of these tests from the syringe pump setting. The same experimental set up was used in this reliability test as was used in the previous leak check test, except for an output tube tip (only the last 1 cm of output tube) made of Teflon with a decreased diameter of 0.41 mm. Teflon was used to obtain a material with a lower surface

energy for a more hydrophobic material. This decreased the weight of each drip from ~15-30 mg to ~4-12 mg.

4.3.1. Syringe Pump Repeatability & Variability Test Results

Flow rates obtained can be seen in Table VIII. Although the maximum resolution of the scale is 1 mg (which equates to 1 μL resolution) the various fits of data used were expanded to include two decimal places. This may cause some reliability issues in the method of comparison; however, the data will be analyzed anyways. Reliability could be increased by increasing resolution of the scale.

Table VIII. *Flow rate measurements (fit from data) for four trials at 10 $\mu\text{L}/\text{min}$ after pressure equalization*

Trial	Flow Rate ($\mu\text{L}/\text{min}$)
1	9.01
2	9.08
3	8.90
4	8.95
Avg.	8.99
Std. Dev.	0.07

Additionally, one trial can be seen in Figure 107 to show the pressure equalization vs. volume.

Figure 107. Representative trial (1) for syringe pump reliability and variability test.

The plot shows much more even pressure after equalization. The data then received from the four trials were compared to the syringe pump setting of 10 $\mu\text{L}/\text{min}$ with a 1-sample T-test to determine whether or not there was a significant difference of the measured flow rate vs. the set point flow rate of the syringe pump (Figure 108).

Figure 108. 1-sample T-test concluding a significant difference of measured flow rate vs. set point flow rate of syringe pump.

The 1-sample T-test showed a clear significant difference between the syringe pump setting and actual flow rate obtained. The statistical results from the 1-sample T-test are shown in Table IX, as determined by Minitab.

Table IX. 1-sample T-test statistical results from Minitab. (SE Mean = standard error of the mean)

N	Mean	St. Dev.	SE Mean	95% CI	T	P
4	8.9853	0.0806	0.0403	(8.8570, 9.1135)	-25.18	0.000

The variability of syringe pump settings to actual volumetric flow rates obtained is most likely due to an input setting of the syringe inner diameter. A syringe pump can be used for various syringe sizes and to account for this variation, an inner diameter measurement of the syringe tube must be programmed into the pump. This is a relatively rough way of determining a volumetric flow and with relatively large syringe inner diameters this can cause increasing variability. Additionally, the syringe pump is a screw driven pump which, at such low flow rates, is activated on and off. It is recommended to look into peristaltic pumping devices with greater sensitivity for future

microfluidic investigation and potential LOC implementation (ex: quantum dot synthesis flow through cell).

4.3.2. Syringe Pump Repeatability & Variability Test Conclusions

The syringe pumps showed somewhat significant variability in reproducing flow rates. A 10 $\mu\text{L}/\text{min}$ setting on the syringe pump produced a volumetric flow rate with 95% confidence of 8.8570 $\mu\text{L}/\text{min}$ to 9.1135 $\mu\text{L}/\text{min}$. Future recommendations for this kind of measurement would include better monitorization of syringe inner diameter and investigation of the variability of syringe sizes (plunger diameter).

4.4. Electroosmotic Flow Test

To test for electroosmotic flow, the 320 μm ID input tubing was replaced with 1 mm ID tubing to reduce any fluid resistance in the system outside the microfluidic chip. A Micromanipulator Prober was used with adjustable probe tips to contact the electrodes with an HP 6186C DC current source. If PDMS had covered the electrodes it was simply peeled off until electrodes could be accessed. Figure 109 shows the probe tips contacting the electrodes.

Figure 109. Probe tips making contact with electrodes.

Conductive epoxy was also used for a more permanent connection to wires; however, if the epoxy is not well mixed it can cause a large increase in resistance. Numerous solutions were used to investigate potential of electroosmotic flow, such as: DI water, DI water + salts (sodium chloride, sodium silico aluminate, sodium thiosulfate, potassium iodide) and a phosphate buffer solution of pH 7. Electric field strengths of 100 V/cm and 150 V/cm were used by the application of a 300 volt and 200 volt potential difference across the electrodes (channels of 2 cm in length). Fluid flow was attempted within the entire system (syringe to input tube, to chip, to output tube) by tracking volume flow from the output tube (weight method). Upon failure to produce flow (with the entire system hooked up) the system input and output tubes were disconnected after filling the microchannels with fluid, to reduce resistance to flow. Potential differences were then applied to attempt empirical observations of flow.

4.4.1. Electroosmotic Flow Results

Signs of electroosmotic flow were seen, yet no continuous flow was observed. At the first application of the electric field (with the complete system set up), a drip would immediately form and fall, but no further flow was observed. Additionally, none of these observations were quantifiable. It must be noted that this is a relatively weak electric field strength for electroosmotic applications, and stronger fields in the kilovolt range could be necessary to drive flow. Stronger fields were not attempted due to safety reasons and time constraints. Another thing to note is the microchannel cross section of $100\text{ }\mu\text{m} \times 200\text{ }\mu\text{m}$ is relatively large and could provide difficulty in obtaining a noticeable flow. Additionally, testing was limited due to the fact that the gold electrodes began to dissolve in solution, as discussed in section 1.2 and will be discussed in the recommendations section. This greatly limited the observations for testing the system after fluid connections had been removed, and main observations were the rapid dissolution of gold. Severe bubbling occurred and fluid was noticeably flowing, but it was hard to tell if it was due to the rapid dissolution of gold or electrokinetic effects. Recommendations will also include redesign of the chip to “design for testing” and will be discussed in the recommendations section.

4.4.2. Electroosmotic flow Conclusions

At a max electric field strength of 150 volts/cm an electroosmotic flow could not be observed and quantified. Recommendations include redesign for testing, reducing the channel cross sectional area, and using a different material for electrodes. Flow rate testing is difficult and will take careful redesign to provide for conclusive results (discussed in recommendations). This conclusion lead to testing streaming potential and electrophoresis of particles, to prove the chip could be used for electrokinetic applications.

4.5. Streaming Potential Test

Streaming potential measurements were attempted to obtain the zeta potential of SU-8 and various solutions. By obtaining the zeta potential, predictions can be made on various electrokinetic properties of SU-8 for electroosmosis applications. Streaming potential was

measured by pumping fluid at a constant rate through the channel while using an Agilent 34405A 5.5 Digital Multimeter to measure a potential difference. The multimeter was connected to the probe tips to ensure sufficient contact of electrodes. Flow rates of 10 $\mu\text{L}/\text{min}$ were used to measure streaming potential periodically for 6000 seconds. Potential readings were taken at 500 s, 2500 s, 4000 s, 5500 s, and 6000 s. Additional flow rates were attempted, but no significant difference in potential could be seen.

4.5.1. Streaming potential results

Streaming potential results were inconclusive due to the fact that a constant potential difference could not be measured. Streaming potential readings are shown in Table X, which were an estimation due to constantly varying potential readings, sometimes as large as ± 5 mV.

Table X. *Streaming potential readings taken at 10 $\mu\text{L}/\text{min}$*

Time (s)	Streaming Potential (mV)
500	20
2500	14
4000	12
5500	11
6000	9

Degradation of potential was shown over time which made it difficult to gather a reading for a flow rate. Streaming potential test repeatability had large variation, and thus, no conclusions could be drawn. The resulting data that was previously shown is representative of the best results from numerous streaming potential tests. Other various flow rates were tested but streaming potential did not show any significant change in potential. It was shown to stay the same, sometimes decrease, and sometimes increase, and could not be quantified.

4.5.2. Streaming Potential Conclusions

No conclusions were drawn from streaming potential tests. Consistent measurements were not able to be obtained. This could be due to varying concentrations in the solution used. It would

also be recommended with future chip design to deposit more electrodes at various locations within the chip to gain simultaneous readings of potential change across the length of the microchannel. Additional electrodes would not add processing steps, only redesign of the positive photoresist mask. Streaming potential testing is affected by cross sectional area within the channel, and with increasing surface area to volume ratio of the fluid, the surface interactions will have a larger impact on measurements. It would then be recommended to increase the length of the chip and consistency within the ionic strength of the fluid be verified, as this can cause major variation within potential readings. Design for testing, which will be discussed heavily in the recommendations section, will be key for gaining more accurate streaming potential measurements.

4.6. Electrophoresis of Glass Particle Test

The last test attempted was to try to create for electrophoresis of glass particles within the channel. This was attempted due to the fact that although electroosmotic flow could not be obtained, it may be easier for small particles to migrate through the channel than the fluid. Glass particles (obtained from the glass beads used for the Microabrasive blaster) were inserted into the system. Additionally, due to the degradation of gold electrodes from previous testing, wire was inserted through the tubing into the fluid reservoirs for application of an electric field. The larger 1 mm ID was used for this application. DI water with glass particles was pumped throughout the entire system until fluid began to drip out of the output tube. The syringe was then disconnected from the system and the pressure was allowed to equalize. A large working distance optical microscope was used to track fluid flow within the channels. Due to the insertion of glass particles they were able to be seen in motion and at rest. Normal testing did not allow for visual measurements of flow rate via optical microscopy because there was no way to “see” fluid movement within the channels. Potential differences of 200 volts and 300 volts were applied to the wires to observe particle movement due to the applied field. The optical microscope image was fed to a large TV monitor. The width of the microfluidic channel used for measurements was 200 μm , which was used to create a length scale across the screen. With applying forward and

reverse potential differences, particles were recorded flowing through the channels. Data was then later manually analyzed at half speed (to increase accuracy) and particles were individually timed from one location in the channel to another. Each measurement was taken three times and times were averaged to obtain a more accurate particle velocity. Also, due to the fact that videos were analyzed at half speed, the velocity was doubled. Particles observed were observed for velocity, location, size, and direction of flow with an applied electric field. Particle size was rated on an arbitrary scale from 1-5, 1 being the smallest and 5 being the largest. Figure 110 shows the interactions of a glass particle with an aqueous medium (pH 7) with an applied electric field.

Figure 110. Interaction of a glass particle submerged in an aqueous medium (pH 7) with an applied electric field.

With an applied electric field, the electrostatic force is greater than the friction force and electrostatic retardation force, which causes particle motion toward the positive electrode. A sample size of 16 was used for 200 volts and 20 for 300 volts.

4.6.1. Electrophoresis of Glass Particles Results

Electrophoresis of glass particles was successful. Particle velocity measurements ranged from 100 $\mu\text{m/s}$ to 1600 $\mu\text{m/s}$. Results comparing particle velocities at 200 V and 300 V can be seen in Figure 111, generated by a 2-sample T-test of particle velocities (95% confidence).

Figure 111. Velocity measurements taken from potential differences of 200 volts and 300 volts.

The 2-sample T-test statistical analysis that correlates to the above Figure (111) is shown in Table XI.

Table XI. 2-Sample T-test comparing the mean particle velocities at 200 V and 300 V

Potential	Sample Size	Mean	St. Dev.	SE Mean
300 V	20	610	408	91
200 V	16	232	184	46
Estimate for Difference:		378 $\mu\text{m/s}$		
95% CI for Difference:		(168, 588) $\mu\text{m/s}$		
T-Value:		3.7		
P-Value:		0.001		
Degrees of Freedom:		27		

This statistical test showed a significant difference of particle velocity between applying 200 V and 300 V of 378 $\mu\text{m/s}$. There is a large variation in the data, which is thought to be attributed to particle size as well as location in the channel (whether or not particles were flowing next to a channel wall). However, particle location could only be tracked in two dimensions because it could not be concluded whether a particle was near the top and bottom (solid/liquid) interfaces. An analysis of variance (ANOVA) test was run to compare variation between the input variables and particle speed. Input variables were: voltage applied, particle location, particle size, and forward/reverse potential difference. Figure 112 shows the ANOVA results.

Figure 112. Results for ANOVA test to analyze input variable effect on particle speed.

The residuals plot for this test is shown in Figure 113 and the statistical p-values of each test, to monitor confidence are shown in Table XII.

Table XII. *P-values for the ANOVA significance of input variables*

Variable	P-Value
Voltage	0.013
Size	0.705
Location	0.734
Direction	0.507

Figure 113. Residuals plot that was obtained with ANOVA analysis.

It is shown by the ANOVA residual plot that the residual fit is not very good, which can additionally be seen by the non-normal histogram plot. Additionally the p-values for each variable conclude that the only significant input variable shown is applied potential. It must be noted that due to the long working distance of the optical microscope and viewing of the particles through multiple layers of the chip, it was difficult to see the particles. A SEM picture of the glass particles was taken to determine uniformity of size (Figures 114-115).

Figure 114. SEM of glass particles used for electrophoresis tests.

Figure 115. SEM of glass particles used for electrophoresis tests.

It was found by the SEM pictures that particles were fairly regular and almost perfectly round with little defects. However, by empirical observations of particle size compared to channel width it was determined that particles sizes ranged from 10-20 μm . It was also found upon disassembly of the chip that most of the glass particles had been stuck in the reservoir. It must be noted that filters were not used and particles were not cleaned before testing. So electrophoresis was thought to have been of the glass particles; however, some data collection could have been from dirt or polymer particles from the syringe and/or tubing.

4.6.2. Electrophoresis Conclusions

Electrophoresis of particles (thought to be glass) was successfully observed. The particle size range was estimated to be (normally) from 10-20 μm in diameter. It was found that the only statistically significant factor attributing to particle velocity was found to be electric field strength. Particle velocities ranged from $\sim 100 \mu\text{m/s}$ to $1600 \mu\text{m/s}$. It is hypothesized that particle size and location would play a significant role in the velocity, but could not be confirmed here.

5. Conclusions and Continuation of Project

Further continuation of the project will be carried out by future microfluidics enthusiasts.

Implementation of SU-8 bonding procedures developed in this work can and are already being implemented into design for future projects. This creates for a novel way of bonding materials that spans far greater than microfluidics applications. However, selective bonding for microfluidic applications was proven here with the development of bond method 4 with working pressures proven up to 107.41 KPa (15.57 lb/in²) without failure. These procedures can be used for attempted fabrication of various lab on a chip projects for micro-chemical reactions, analysis, as well as electroosmotic flow chips.

5.1. Recommendations

5.1.1. Dissolution of Gold

The dissolution of gold was (accidentally) proven here to have the ability of dissolution in an aqueous solution in the presence of chloride ions. This can actually be used to one's advantage by designing reservoirs which are capped with gold, and can preferentially be dissolved for a drug dispensing device, etc. This could be a universal device which has various drug types and can be dispensed preferentially. A simple depiction of this design is shown in Figure 116 and would need electrical connection to each gold capped reservoir. (Examples here [5], [62]).

Figure 116. Gold capped reservoir for a drug dispensing device.

Figure 117 shows the dissolution of the gold electrodes used for the electroosmotic flow test. This was seen after removal of the Nanoports.

Figure 117. Dissolved gold electrodes (Nanoports removed). Left electrode completely dissolved, right is partially dissolved.

5.1.2. Future Testing for Streaming Potential & Electroosmotic Flow Chips

A new design that is designed for testing will be required for streaming potential and electroosmotic flow measurements. Designing for a test includes: smaller cross sectional area and moving Nanoports so they are physically out of the way. Right now, the chip is only 8 mm x 31 mm and the Nanoports are > 8 mm in diameter and 7 mm tall. Additionally, each chip requires two Nanoports for an input and an output. With most observations being visual by the naked eye and optical microscopy, the Nanoports significantly crowd the chip. In fact, only a large working distance (~ 10 cm) optical microscope could be used for these measurements. It would be recommended to move the input and output vias away from the chip and have them feed into the reservoirs from the side. This would allow for more visibility within the chip and would get rid of any problems that arise from SU-8 inconsistencies due to spin coating area around the open fluid vias. An overemphasized design is shown in Figure 118. Decreasing chip cross sectional area will allow for increased influence of surface interactions. This will allow for greater impact of the

SU-8/liquid interface to increase electroosmotic effect. This will also allow for a potentially easier streaming potential measurement. This could also be increased with increasing fluid channel length and better control of solution concentrations. Increasing length would allow for more electric field effect on the microfluidic channel, and a greater percent fluid being affected by the field.

Figure 118. Design for testing of a new electrokinetic chip that allows for more room due to moving the Nanoport connections.

Along with new design should be consideration of a new electrode material. It was shown here that gold dissolution happens relatively quickly. Most resources state that platinum is widely used for these applications. It was shown at Rochester Institute of Technology that platinum is easily sputtered with a DC Argon plasma on a Denton Desk V sputter coater, with relatively similar conditions as sputtering gold for SEM imaging. The only drawback is cost. A 50 mm x 50 mm x 100 μm Platinum foil was \$1,110 [63].

Other considerations include fabrication of microfluidic chips on a glass wafer. This would provide for the allowance of imaging the chip from the other side of the wafer. This could be explored with optical microscopy as well as laser confocal microscopy. The BMED department has one in which electrophoresis can be used to probe fluorescent particles and track their movement. Must check the fluorescent wavelength of the particles to see if the fluorescence (usually UV) will be

transparent to glass and SU-8. This would allow for space to be saved by not needing as much room for Nanoport relocation, although it would still be recommended due to spin coating issues.

The idea also came up to score and separate the microfluidic chips or attempt delaminating them from the silicon and flexible polymer altogether. This could be done by chemical attack (most likely for the polymer substrate) and sacrificial layer deposition and removal such as OmniCoat (most likely for the silicon). The polymer substrate was chosen due to immediate availability, but could be tailored to easily be dissolved and not harm the rest of the chip in the process. The only drawback is the inconsistencies in the thin SU-8 layer which might not have enough mass to be robust enough for such a process. This could be solved by spin coating an SU-8 2050 layer on the flexible substrates, fully curing it, and then using a thin SU-8 2007 layer to bond the channels to the flexible polymer substrate (Figure 119).

Figure 119. Microchip design for polymer substrate dissolution for full SU-8 chip.

Also, thinner SU-8 adhesion layers could be investigated such as SU-8 2000.5 or 2002 which would provide for $\sim 0.5 \mu\text{m}$ thick film and $2.5 \mu\text{m}$ thick films, respectively. This could provide for less SU-8 reflow problems when shrinking channel width and height.

Other future work for streaming potential and electrochemical detection would include electrodes not only in the reservoirs of the microfluidic channels, throughout the span of the channel (Figure 120).

Figure 120. Additional electrodes for electrochemical detection for electrophoresis and streaming potential measurements.

Additional investigation that would be recommended for further characterization of SU-8 is described here: [64] where a UV-ozone/air treatment is used to increase the hydrophilicity of SU-8 channels this surface treatment of the SU-8. Dramatic increases in hydrophilicity and electrokinetic properties were shown.

References

- [1] Merriam-Webster, 2012. [Online]. Available: Merriam-Webster.com. [Accessed 29 May 2012].
- [2] T. Sikanen, S. Tuomikoski, R. A. Ketola, R. Kostainen, S. Franssila and T. Kotiaho, "Characterization of SU-8 for electrokinetic microfluidic application," *Lab on a Chip*, vol. 5, no. 8, pp. 888-896, 2005.
- [3] Sikanen, "Characterization of SU-8 for electrokinetic microfluidic applications," *Lab on a Chip*, vol. 5.8, p. 888, 2005.
- [4] R. Frankenthal and D. Siconolfi, "The anodic corrosion of gold in concentrated chloride solutions," *Journal of the Electrochemistry Society*, vol. 129, no. 6, pp. 1192-1196, 1982.
- [5] S. Smith, T. Tang, J. Stevenson, B. Flynn, H. Reekie, A. Murray, A. Gundlach, D. Renshaw, B. Dhillon, A. Ohtori, Y. Inoue and A. Walton, "Miniaturized drug delivery system with wireless power transfer and communication," *Nanobiotechnology, IET*, vol. 1, no. 5, pp. 80-86, 2007.
- [6] "Clinical: Novolog (insulin aspart)," Medpedia, 9 Apr. 2012. [Online]. Available: <[http://wiki.medpedia.com/Clinical:Novolog_\(insulin_aspart\)>](http://wiki.medpedia.com/Clinical:Novolog_(insulin_aspart)>)..
- [7] "Safe syringe disposal guide for home generated medical waste," New Jersey Department of Health and Senior Services, 4 Apr. 2012. [Online]. Available: <http://www.state.nj.us/health/eoh/phss/syringe.pdf>.
- [8] H. P. Le, "Progress and trends in ink-jet printing technology," *Journal of Imaging Science and Technology*, vol. 42, p. 49—62, 1998.
- [9] S. Terry, J. Jerman and J. Angell, "A gas chromatographic air analyzer fabricated on a silicon

- wafer,” *IEEE Transactions on Electron Devices*, vol. 26, no. 12, pp. 1880 - 1886, 1979.
- [10] G. M. Whitesides, “The origins and the future of microfluidics,” *Nature*, vol. 422, 2006.
- [11] J. C. McDonald and G. M. Whitesides, “Poly(dimethylsiloxane) as a material for fabricating microfluidic devices,” *Accounts of Chemical Research*, vol. 35, no. 7, pp. 491-499, 2002.
- [12] “Niherst: make way for fluidics!,” The National Institute of Higher Education, Research, Science and Technology, 19 Apr. 2012. [Online]. Available: <http://www.niherst.gov.tt/scipop/sci-bits/microfluidics.htm>.
- [13] “Low reynolds number flows,” Hitech-Projects, 30 May 2012. [Online]. Available: <http://www.hitech-projects.com/euprojects/artic/index/Low%20Reynolds%20number%20flows.pdf>.
- [14] A. Webster, J. Greenman and S. J. Haswell, “Development of microfluidic devices for biomedical and clinical application,” *Journal of Chemical Technoogy and Biotechnology*, vol. 86, p. 10–17, 2011.
- [15] C.-Y. Lee, C.-L. Chang, Y.-N. Wang and L.-M. Fu, “Microfluidic mixing: a review,” *International Journal of Molecular Science*, vol. 12, pp. 3263-3287, 2011.
- [16] H. T. G. Van Lintel, F. C. M. Van De Pol and S. Bouwstra, “A piezoelectric micropump based on micromachining of silicon,” *Sensors and Actuators*, vol. 15, pp. 153 - 167, 1988.
- [17] T. Huey Fang, N. Ramalingam, D. Xian-Duib, T. Swee Nginc, Z. Xianting, A. T. Lai Kuan, E. Y. Peng Huate and G. Hai-Qing, “Real-time PCR microfluidic devices with concurrent electrochemical detection,” *Biosensors and Bioelectronics*, vol. 24, p. 2131–2136, 2009.
- [18] R. Pal, M. Yang, R. Lin, B. N. Johnson, N. Srivastava, S. Z. Razzacki, K. J. Chomistek, D. C. Heldsinger, R. M. Haque, V. M. Ugaz, P. K. Thwar, Z. Chen, K. Alfano, M. B. Yim, M.

- Krishnan, A. O. Fuller, R. G. Larson, D. T. Burke and M. A. Burns, "An integrated microfluidic device for influenza and other genetic analyses," *Lab Chip*, vol. 5, p. 1024–1032, 2005.
- [19] V. M. Ugaz, R. D. Elms, R. C. Lo, S. F. A. and M. A. Burns, "Microfabricated electrophoresis systems for DNA sequencing and genotyping applications: current technology and future directions," *Philosophical Transactions of the Royal Society of London*, vol. 362, p. 1105–1129, 2004.
- [20] H. Stone, A. Stroock and A. Ajdari, "Engineering flows in small devices: microfluidics toward a lab-on-a-chip," *Annual Review of Fluid Mechanics*, vol. 36, p. 381–411, 2004.
- [21] D. J. Laser and J. G. Santiago, "A review of micropumps," *Journal of micromechanics and microengineering*, vol. 14, p. R35–R64, 2004.
- [22] "Piezoelectric micropump with three valves working peristaltically," *Sensors and Actuators*, vol. 21, no. 1, pp. 203-206, 1990.
- [23] H.-J. Butt, K. Graf and M. Kappl, *Physics and chemistry of interfaces*, second edition, Weinheim: Wiley-VCH, 2006.
- [24] B. Bhushan, "Biomimetics inspired surfaces for drag reduction and oleophobicity/philicity," *Beilstein J. Nanotechnol*, pp. 66-84, 2011.
- [25] P. K. Wong, "Electrokinetics in micro devices for biotechnology applications," *IEEE/ASME Transactions on Mechatronics*, vol. 9, no. 2, 2004.
- [26] M. S. Virk, A. E. Holdo and S. Kaennakham, "Numerical analysis of fluid mixing in t-shape micro mixer," in *Excerpt from the Proceedings of the COMSOL Users Conference*, Grenoble, 2007.
- [27] R. H. Liu, M. A. Stremler, K. V. Sharp, M. G. Olsen, J. G. Santiago, R. J. Adrian, H. Aref and

- D. J. Beebe, "Passive mixing in a three-dimensional serpentine microchannel," *Journal of microelectromechanical systems*, vol. 9, no. 2, JUNE 2000.
- [28] T.-R. Hsu, *MEMS and microsystems: design and manufacture*, Boston: McGraw-Hill, 2002.
- [29] R. Hunter, *Foundations of colloid science*, volume 2, Oxford: Clarendon Press, 1989.
- [30] D. C. Grahame, "The electrical double layer and the theory of electrocapilarity," *Chemical Reviews*, vol. 41, no. 3, p. 441–501, 1947.
- [31] W. L. Hau, D. W. Trau, N. J. Sucher, M. Wong and Y. Zohar, "Surface-chemistry technology for microfluidics," *Journal of micromechanics and microengineering*, vol. 13, pp. 272-278, 2003.
- [32] J. Santiago, "Electrokinetic bioanalytical systems," Stanford University, [Online]. Available: <http://microfluidics.stanford.edu/Projects/Archive/bioanal.htm>. [Accessed 30 Apr. 2012].
- [33] "Electrokinetics," ALChemE, [Online]. Available: http://alcheme.tamu.edu/?page_id=6823. [Accessed 29 May 2012].
- [34] B. J. Kirby and E. F. Hasselbrink Jr., "Zeta potential of microfluidic substrates: 1. theory, experimental techniques, and effects on separations," *Electrophoresis*, vol. 25, pp. 187-202, 2004.
- [35] C. D. Meinhart, S. T. Wereley and J. G. Santiago, "PIV measurements of a microchannel flow," *Experiments in Fluids*, vol. 27, pp. 414-419, 1999.
- [36] A. M. Spehar, S. Koster, V. Linder, S. Kulmala, N. F. deRooij, E. Verpoorte, H. Sigrist and W. Thromann, "Electrokinetic characterization of poly(dimethylsiloxane) microchannels," *Electrophoresis*, vol. 24, p. 3674, 2003.

- [37] S. A. Soper, A. C. Henry, B. Vaidya, M. Galloway, M. Wabuyele and R. L. McCarley, "Surface modification of polymer-based microfluidic devices," *Analytica Chimica Acta*, vol. 470, no. 1, pp. 87-99, 2002.
- [38] X. Ren, M. Bachman, C. Sims, G. P. Li and N. Allbritton, "Electroosmotic properties of microfluidic channels composed of poly(dimethylsiloxane)," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 762, no. 2, pp. 117-125, 2001.
- [39] K. Lee, S. LaBianca, S. Rishton, S. Zohlgarnain, J. Gelorme, J. Shaw and H. P. Chang, "Micromachining applications of a high resolution ultrathick photoresist," *Journal of Vacuum Science and Technology*, vol. 13, no. 6, pp. 2556-2560, 1995.
- [40] J. L. Dektar and N. P. Hacker, "Photochemistry of triarylsulfonium salts," *Journal of the American Chemical Society*, vol. 112, no. 16, pp. 6004-6015, 1990.
- [41] Owner's manual: Tri-Star Technologies plasma treatment system PT-2000, El Segundo, CA: Tri-Star Technologies, 2013.
- [42] D. M. Altpeter, "Description of SU-8," 13 4 2005. [Online].
- [43] "SU-8 2000: permanent epoxy negative photoresist," MicroChem, 26 Apr. 2012. [Online]. Available: www.microchem.com.
- [44] "SU-8: A versatile material for MEMS manufacturing, MEMS feature," in *SEMI*, www.semi.org/mems, Summer 2006.
- [45] S. Tuomikoski and S. Franssila, "Free-standing SU-8 microfluidic chips by adhesive bonding and release etching," *Sensors and Actuators A*, vol. 120, pp. 408-415, 2005.
- [46] S. G. Serra, A. Schneider, K. Malecki, S. E. Huq and W. Brenner, "A simple bonding process of SU-8 to glass to seal a microfluidic device," in *In Proc. 3rd Int. Conf. on Multi-Material*

Micro Manufacture (B, orovets, 3–5 Oct. 2007), pp. 43-6., 2007.

- [47] “An all SU-8 microfluidic chip with built-in 3D fine microstructures,” *Journal of Micromechanics and Microengineering*, vol. 16, pp. 2318-2322, 2006.
- [48] F. J. Blanco, M. Agirregabiria, J. Garcia, J. Berganzo, M. Tijero, M. T. Arroyo, J. M. Ruano, I. Aramburu and K. Mayora, “Novel three-dimensional embedded SU-8 microchannels fabricated using low temperature full wafer adhesive bonding,” *Journal of Micromechanics and Microengineering*, vol. 14, no. 7, p. 1047, 2004.
- [49] H. Lee, K. Lee, K. W. Oh and B. Ahn, “A new fabrication process for SU-8 thick photoresist structures by simultaneously removing edge-bead and air bubbles,” *Journal of Micromechanics and Microengineering*, vol. 21, no. 12, 2011.
- [50] J. Deng, “SU-8 photolithography process,” Harvard, 6 August 2008. [Online]. Available: http://www.cns.fas.harvard.edu/facilities/docs/SOP031_r2_6_SU-8%20photolithography%20process.pdf. [Accessed 21 May 2013].
- [51] L. Chen, T. Chen and L. Sun, “The bond strength measurement of silicon-silicon bonding wafers based on crack opening opening method,” *International Journal of Information Acquisition*, vol. 3, no. 4, p. 339, 2006.
- [52] G. G. Odian, Principles of polymerization. 4th ed, New Jersey: Wiley & Sons, 2004.
- [53] F. Vara, W. Brann, T. Berejka, B. Hanrahan, D. Cowell, L. Carlblom and K. Schaper, UV/EB curing primer: inks, coatings, and adhesives, Northbrook: Radtech International North America, 1994.
- [54] A. Darwish Al-Obaidani, “Development of a photocuring system for cationic epoxy formulations using side emitting optical fibres,” [Online]. Available: <http://etheses.nottingham.ac.uk/864/>. [Accessed 26 Nov 2012].

- [55] A. Hartwig, B. Schneider and A. Lühring, "Influence of moisture on the photochemically induced polymerization of epoxy groups in different chemical environments," *Polymer*, vol. 43, pp. 4243-4250, 2002.
- [56] MicroChem, "Microposit S1800 series photo resists," [Online]. Available: http://microchem.com/PDFs_Dow/S1800.pdf. [Accessed 23 May 2013].
- [57] Z. Chen, Y. Gao, J. Lin, R. Su and Y. Xie, "Vacuum-assisted thermal bonding of plastic capillary electrophoresis microchip imprinted with stainless steel template," *Journal of Chromatography A*, vol. 1038, p. 239–245, 2004.
- [58] C. Koch, J. Ingle and V. Remcho, "Bonding Upchurch® nanoports to PDMS," Department of Chemistry, Oregon State University, 8 Feb 2008. [Online]. Available: <http://blogs.rsc.org/chipsandtips/2008/02/12/bonding-upchurch%C2%AE-nanoports-to-pdms/>. [Accessed 27 May 2013].
- [59] F. P. & I. f. L. Applications, "Idex health & science," 2011-2012. [Online]. Available: https://www.idex-hs.com/about/data_sheets/NEW_IDX1106_IDEX_2011_Catalog_Complete_LoRes.pdf. [Accessed 25 May 2013].
- [60] "Gas pressure sensor," Vernier, 2013. [Online]. Available: <http://www.vernier.com/products/sensors/gps-bta/>. [Accessed 25 May 2013].
- [61] "Balances and Scales," ADAM, 2013. [Online]. Available: <http://www.adamequipment.com/am/balances/146>. [Accessed 25 May 2013].
- [62] R. M. Capito and L. A. Lucas, "Microchip for drug delivery," Northwestern University, 4 June 2000. [Online]. Available: <http://clifton.mech.northwestern.edu/~me381/project/drug.pdf>. [Accessed 26 May 2013].

- [63] D. L. Fuller, "Physical vapor deposition – evaporation and sputtering," Rochester Institute of Technology Microelectronic Engineering, Rochester, 2013.
- [64] C. J. Chang, C. S. Yang, L. H. Lan, P. C. Wang and F. G. Tseng, "Fabrication of a SU-8-based polymer-enclosed channel with a penetrating UV/ozone-modified interior surface for electrokinetic separation of proteins," *Journal of Micromechanics and Microengineering*, vol. 20, p. 11, 2010.