CHARACTERIZATION OF MIXING IN A T-STYLE MICROFLUIDIC CHIP

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TITLE: CHARACTERIZATION OF MIXING IN A T-STYLE
MICROFLUIDIC CHIP

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ABSTRACT

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The goal of this study is to characterize the mixing that occurs in a microfluidic chip. To characterize the mixing, the minimum length to complete mixing and evolution of mixing will be investigated. There are two types of mixing that occur within a microfluidic channel, diffusion and advection. In the beginning of the microfluidic chip, diffusion is the dominant form of mixing, and in the later portion of the microfluidic chip advection is the dominant form of mixing. The type of design used for this experiment was a zig zag geometry microfluidic chip with channel dimensions of 60 μm X 500 μm X 522 mm. The minimum length for complete mixing was $361 \pm 3.475 \mu\text{m}$ at a flow rate of 25 mL/hr. The mixing was measured using optical light microscopy. For all flow rates less than 20 mL/hr the flow rate was too low to mix the two fluids. The pressure produced by the 30 mL/hr flow rate caused the microfluidic chip to fail.

Keywords: Microfluidic chip, diffusion, advection, laminar, turbulent, photolithography, softlithography

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Chapter 1: Introduction and Background

1.1 Microfluidics

The goal of this study is to characterize the mixing that occurs in a T-style microfluidic chip. T-style microfluidic chips have two input channels that converge at 90 degrees into a single output channel, Figure 1. This study will first investigate the minimum distance needed to have complete mixing of two fluids at various flow rates and concentrations. Then this study will characterize how the fluids are mixing as they flow through the entire length of the output channel. After the completion of this study, future students looking to design microfluidic chips will be able to determine the necessary length a device will need to be in order to achieve complete mixing for a range of flow rates.

Figure 1: The basic design of a T-style microfluidic chip.

1.1.1 Definition of Microfluidics

The field of microfluidics deals with the behavior, control, and manipulation of fluids on the microscale, ¹. The behavior of the fluid is controlled by designing microfluidic chips that have specific dimensions to control the structure of the fluid flow. Pressure controls the fluid to achieve specific flow rates which move the fluid through a microscale channel. The fluid is manipulated by designing specific geometries into the microfluidic chip to influence the degree of fluid mixing.

Fluids are a sub-category of the phases of matter. They encompass liquids, gases, and plasmas. For this study the fluids used are liquids only. Newtonian fluids are defined as a substance that will deform continuously under an applied stress. The most important property of fluids when dealing with microfluidics is viscosity ¹. Viscosity is the fluid's resistance to flow in the form of internal shear stresses. The viscosity of a fluid within a microfluidic channel will control the structure of the flow, which will be critical in designing a functioning microfluidic chip that mixes both fluids completely.

There are three flow regimes that are experienced in a microfluidic channel, laminar, turbulent and creep. Laminar flow is the structured ordered flow of a fluid through a pipe. In laminar flow the viscous forces are greater than the inertia forces. This results in the fluid moving in lamina that are parallel to the wall of the pipe. By increasing the velocity of the fluid the flow structure will change to turbulent. This is where small packets of fluid called eddies move

laterally or forward. This is due to the inertial forces dominating the viscous forces of the fluid. Creep flow is an extreme version of laminar flow where the velocity of the flow of the lamina nearest the wall is equal to zero, due to the much higher force of viscosity to inertia forces.

The structure of the flow dictates the which mixing mechanism is most important inside of the microfluidic channel. Advection is the transport mechanism that is dependent on the large scale motion of fluid currents. Advection relies on inertial forces of the fluid and is dominant form of mixing in turbulent flow. Molecular diffusion is the dominant for of mixing in laminar and creep flow because of the lack of lateral flow present. Molecular diffusion relies on the random motion of molecules from a high concentration to a low concentration of molecules.

Fluids are not fixed to a lattice structure, like a solid material and the molecules are free to move relative to each other. These molecules are widely spaced out compared to their molecular diameter, Figure 2. The molecules move around by Brownian motion, or the random motion of the molecules due to their individual thermal energy, ¹. This means it is difficult to track the motion of an individual molecule, so the concept of a continuum is used to analyze fluids.

Figure 2: Body centered cubic structure of a solid, Left. Fluid molecules contained in a unit cell, Right,².

To determine if a fluid can be treated as a continuum, the Knudsen number is used, Equation 1.1.

$$Kn = \frac{\lambda}{D} \quad (1.1)$$

Where Kn is the Knudsen number, λ is the molecular diameter, and D is the characteristic dimension. If the Knudsen number is less than one then the fluid can be analyzed as a continuum substance. That is, the fluid can be divided an infinite number of times and no molecular interaction will affect the mechanics of

the fluid. For our application water will be flowing through a microfluidic channel. The molecular diameter for water is $1.9 \text{ \AA}$ and the characteristic dimension is $500 \text{ }\mu\text{m}$, the width of the microfluidic channel. This results in a Knudsen number of 3.8×10^{-7} , which is much less than one. This means that the fluids running through the microfluidic chip can be treated as a continuum and a classical fluid dynamics approach can be used.

1.1.2 Microfluidic Applications and Broader Impacts

Microfluidic devices have shown promising uses in the chemical and biological sciences. The field of microfluidics got its start as an analysis technique that had many useful advantages to traditional analysis techniques,¹. For example, a microfluidic device uses less sample volumes and has a shorter analysis time than traditional techniques.

One of the most important microfluidic devices currently being used is the lab on a chip device. The goal of this device is to completely automate chemistry and biology analysis lab procedures,¹. The hand sized microfluidic chip would replace lab benches, beakers, test tubes, large amount of reagents, and trained personnel with a single autonomous device. These microfluidic lab on a chip designs would utilize sample wells, channels, valves, mixers, and assay regions to characterize chemical reactions.

However lab on a chip devices are only one of the many applications that is utilizing microfluidic technologies. Pharmaceutical sciences are using microfluidic technologies to discover new drugs as well as improve drug delivery

systems,¹. Instead of using a pill to give a single dose of drugs, a microfluidic chip can be used to dispense multiple exact micro liter doses over a long period of time and improve the drug's performance, Figure 3. Cellular biology is also taking advantage of microfluidic technology. Currently cell culturing and testing can be done using microfluidic devices, and microfluidics are even being used to simulate organ-level functions,⁴. This type of technology could speed up the testing process for new drugs and eliminate the need for animal testing.

Figure 3: A microfluidic drug delivery system. The drug container uses capillary pressure values to deliver the drug in a controlled manner.

1.1.3 Advantages of Microfluidics

The reason for microfluidics' wide range of uses is because it offers so many advantages to the traditional techniques for doing the same processes. As the name implies the most useful advantage is the reduction in the amount of fluid volumes needed to complete a test. Microfluidics can be utilized to reduce the amount of samples and reagents by orders of magnitude, ¹. This means that if samples are difficult to acquire a microfluidic chip can be used to run a test when the traditional process may require more samples than can be collected ².

Because fewer fluids are input for the process, the output product and waste of running a test are minimized. This will reduce the amount of impact each test has on the environment, and if the test requires the use of hazardous materials, reduce the hazard and cost to the testing facility.

Aside from volume reductions, microfluidics also have multiple advantages as a mass processing tool. Microfluidic chips can be designed to use parallel processing so that multiple tests can be run, reducing the amount of time needed to run a series of tests, ^{3,4}. For example, a microfluidic chip could take in one sample of blood and test for white blood cell count, diagnose a disease and evaluate organ function. Also due to the small amount of volumes used in a microfluidic chip, less power is needed to run a test. This results in a large net reduction in costs required per test.

1.1.4 Flow Dynamics

1.1.4.1 Type of Flow

On the microscale fluids do not flow the same way as they do on the macroscale. On the macroscale fluids have turbulent flow. Turbulent flow is the random disordered mixing of two fluids. On the microscale fluids experience laminar flow. Laminar flow is the ordered smooth flow of fluids caused by the domination of viscous forces, Figure 4.

Figure 4: A diagram of turbulent flow and laminar flow.

At small enough velocities, the fluids can also experience creep flow also called Stokes flow which results in the velocity of fluid nearest the wall being zero, ⁵. To determine the flow type of a microfluidic system the Reynolds number can be calculated, Equation 1.2.

$$Re = \frac{\rho V D_h}{\mu} \quad (1.2)$$

The Reynolds number, Re , is a dimensionless number that is used to characterize the structure of the fluid flow. In the Reynolds equation ρ is the density of the fluid in g/cm^3 , V is the linear flow rate in m/sec , D_h is the hydraulic diameter of the system in m and μ is the dynamic viscosity of the fluid in $kg/(m \times sec)$. For hydrodynamic systems with a Reynolds number of less than 1000, the structure of the flow is characterized as laminar. Anything greater than 1000 is characterized as turbulent. A Reynolds number of less than one is defined as Stokes flow. For microfluidic chips, the flow rate and geometries are low enough to have a Reynolds number of less than 100. The hydraulic diameter for rectangle microfluidic channels is calculated using Equation 1.3.

$$D_h = \frac{2wh}{(w + h)} \quad (1.3)$$

In the hydraulic diameter equation for rectangular channels w is the wide of the channel and h is the height of the channel. Figure 5 shows a cross section of a typical microfluidic channel. The microfluidic channels for this investigation had a width of $500 \mu m$ and a height of $60 \mu m$.

Figure 5: Example of a typical rectangular microfluidic channel.

The flow structure is important to consider when designing a microfluidic chip due to the fact that flow structure will have a large effect on the amount of mixing that will occur inside of fluid channel, ^{6,7,8,9}. Larger channel with a Reynolds number of 1000 or higher will mix faster than small channels due to the increase in advective mixing. Often times it is critical to completely mix two fluids to ensure a uniform chemical reaction inside the chip. If different concentration gradients are present across the channel during chemical synthesis, for example, a varying quality of chemical products would form.

1.1.4.2 Pressure driven flow

In order to move fluid through the microfluidic chip the fluids need to be pumped using a pressure system. The advantages of using a pressure driven system are the ease of the system's operation and fabrication of the microfluidic chip. Alternative methods for moving fluids through microfluidic channels, such as

electrokinetics, require additional and difficult fabrication techniques that can decrease the effectiveness of the microfluidic chip if fabricated incorrectly ¹⁰.

There are very few design limitations when designing a microfluidic device to pump fluids using a pressure driven system. The one important design consideration is not to exceed the maximum pressure, called the burst pressure, the microfluidic chip can withstand, ¹¹. The pressures that the microfluidic chip experiences are defined by Equation 1.4, ⁷.

$$\Delta P_{Total} = \Delta P_{\mu} + \Delta P_{\lambda} \quad (1.4)$$

This means that the total pressure that the microfluidic chip experiences is equal to the pressure due to the viscous nature, ΔP_{μ} , of the fluid moving through it and the surface tension, ΔP_{λ} of the fluid moving through it. The surface tension pressure, ΔP_{λ} , is only important when there is a liquid gas interface and becomes negligible when liquid is moving continuously through the microfluidic chip, ¹².

The surface tension pressure, ΔP_{λ} , is calculated using Equation 1.5, ⁷.

$$\Delta P_{\lambda} = \frac{2\lambda}{r} \quad (1.5)$$

In the ΔP_{λ} equation λ is equal to the surface tension of the fluid in N/m and r is the hydraulic radius of the microfluidic channel.

The viscous pressure, ΔP_{μ} , is given by the Hagen-Poiseuille equation and is dependent on the fluid resistance in microfluidic channel, R , and the volumetric flow rate of the fluid, Q , Equation 1.6, ⁷.

$$\Delta P_{\mu} = RQ \quad (1.6)$$

The viscous pressure, ΔP_{μ} , is the dominant pressure experienced by the microfluidic chip during testing. The fluid resistance, R , for a square channel is defined by Equation 1.7, ⁷,

$$R = \frac{12\mu L}{wh^3} \quad (1.7)$$

where μ is the dynamic viscosity of the fluid, L is the length of the microfluidic channel, w is the width of the microfluidic channel, and h is the height of the microfluidic channel. This means that the viscous pressure is dependent on the fluid's resistance to flow, the geometry of the microfluidic channels and how fast the fluid is being pumped. After a microfluidic chip has been manufactured the only variable the tester has control of is the flow rate, Q . A typical T-style microfluidic chip has very small width and even smaller height. This means that normally the length is the largest dimension by orders of magnitude. This geometry of 522 mm X 500 μ m X 60 μ m results in very high fluid resistance, and high pressures of ~100-700 kPa.

1.1.4.3 Velocity of Flow

When a fluid is moving through a microfluidic channel we can see that there is a velocity gradient across the width and height, figure 6, ². Because a fluid is defined as a substance that will deform continuously under the application of a shear stress and know that the flow structure of fluid in a microfluidic channel is

laminar Newton's law of viscosity can be used to show the relationship between shear stress and deformation rate, Equation 1.8, ².

$$\tau = \mu \frac{d\mu}{dy} \quad (1.8)$$

τ is the shear stress, μ is the viscosity of the fluid, μ is the velocity of the fluid in the x direction and $d\mu/dy$ is the velocity gradient in the y direction. This results in the highest velocity being the portion of the fluid that is furthest from the wall. Also there is a no slip condition for the portion of the fluid that is adjacent to the wall of the channel where the shear force is the greatest, ⁵. The no slip condition is circled in red in Figure 6.

Figure 6: Diagram of velocity gradient inside a microfluidic channel.

This velocity gradient gives a distribution of Reynolds numbers across the microfluidic channel. The center of the channel will have a higher Reynolds number meaning stronger inertial forces, more likely to create eddy current and promote advective mixing.

1.1.4.4 Mixing

The mixing of fluid on the microscale is comprised of two parts, advection of the fluid and diffusion. A dimensionless number called the Peclet number describes the ratio of advection versus diffusion mixing, and is calculated using Equation 1.9, ¹³.

$$Pe = \frac{VL}{D} \quad (1.9)$$

Where V is the velocity, L is the characteristic hydraulic dimension, and D is the diffusion coefficient. Because microfluidic chips have a low flow regime and laminar flow structure the effects of advection on mixing are small in comparison to the effects of diffusion on mixing. Unlike the Reynolds number where there are specific cutoff points to characterize the flow structure, the Peclet number is just a ratio for comparison. However designs for microfluidic chips are chosen based on both the Reynolds and Peclet number. A large Peclet number would indicate higher advection mixing, where a low Peclet number would indicate higher diffusion mixing. A Peclet number of zero would indicate a mixer that relies entirely on diffusion to mix. Typical Peclet numbers for microfluidic devices are generally around 10 – 200. Due to the large characteristic hydraulic dimension of this design the Peclet number is about 4500, which indicates that there is a larger ratio of advection to diffusion than normally exists in a microfluidic device.

1.1.4.5 Diffusion

To calculate the diffusivity of the two combining fluids, statistical modules of random walk diffusion, or Brownian motion, have been created. Equation 1.10 describes a simple relation of random walk distance to diffusivity and residence time,¹⁴.

$$x^2 \propto Dt \tag{1.10}$$

This equation can be used to calculate the diffusivity by dividing the square of the characteristic dimension by the time to complete diffusion. Once the diffusivity is known it can be used to estimate the minimum length to complete diffusion of a simple microfluidic reactor by Equation 1.11,¹⁵.

$$L \cong \frac{wQ}{hD} \tag{1.11}$$

This equation gives an estimate of length to complete mixing based on diffusion. Figure 7 shows a diagram of a perfect diffusion mixer microfluidic chip, that is zero advective mixing present.

Figure 7: A perfect diffusion T-style microfluidic chip.

1.2 Design and Fabrication of a Microfluidic Test Chip

1.2.1 Material Choice/PDMS

To make the microfluidic chips a material called polydimethyl siloxane, also known as PDMS, was chosen. PDMS is very commonly used as the base material for making microfluidic chips for its many advantages,^{16,17}. These advantages include, optical transparency, low cost, flexible, and biocompatible.

PDMS is silicone elastomeric polymer. It is comprised of a silicon oxygen backbone with two methyl groups on each silicon atom. The chemical formula for PDMS is $\text{CH}_3[\text{Si}(\text{CH}_3)_2\text{O}]_n\text{Si}(\text{CH}_3)_3$. Figure 8 shows the chemical monomer of PDMS.

Figure 8: PDMS chemical structure.

The PDMS used to make microfluidic chips is produced by Dow Corning and called Sylgard 184. It is a two part silicone elastomer. The Sylgard 184 silicone elastomer is mixed with the Sylgard 184 silicone elastomer curing agent in a 10:1 ratio and thermally cured. Because PDMS is mixed as a liquid and cured into a solid, it can be cast into any shape, such as microfluidic channel features. PDMS has a size resolution in the nanometer range, which enables it to take the shape of any micro size and shape channel, ²⁰.

PDMS has many advantageous mechanical, optical and thermal properties for being used as a microfluidic device. PDMS is a very flexible polymer which makes microfluidic chips robust, ¹⁸. Its flexibility is also utilized to create compression seals for the inlet and outlet connections of the microfluidic chip. This enables the chips to have easy slip in and out connections to the fluids that need to be pumped through the channels. PDMS is optically transparent which is useful so that the fluids can be observed while flowing through the microfluidic channels, ¹⁹. This property enables the use of light microscopy imaging

techniques through various point of the microfluidic chip while a test is being run. Figure 9 shows an example of the physical properties of PDMS as well as a full function microfluidic chip. Thermally PDMS is an insulator with a thermal conductivity of 0.16 W/mK . For comparison copper has thermal conductivity of 401 W/mK and soda lime glass has a thermal conductivity of 1.3 W/mK . This insulator property creates a thermal isolation between the fluids and the environment.

Figure 9: A PDMS microfluidic chip, Left and an example of the physical properties of PDMS, Right.

1.2.2 Geometries

When designing a microfluidic chip once the size of the channels are picked, the Reynolds number and Peclet number dictate how fluids will flow and mix during testing, ^{20, 21, 22}. The only way to impact fluid mixing will be to have special geometries that will enhance advection in the mixing of fluids. If a single straight

channel is used the fluid will tend to stay very laminar and have no advective mixing. However if twists bends and turns are integrated into the channel design, mixing improves. There are many basic designs that can be chosen to help increase advective mixing in a microfluidic channel, ²³. The process for choosing which type is best is based on the Reynolds and Peclet numbers for the system. For the Reynolds numbers and Peclet numbers that exist in microfluidic chips, there are designs that will aid in advective mixing. The lower the Reynolds and Peclet number, the more complex the microfluidic channel's designs are to improve advection. The only consideration when choosing an exotic quick mixing geometry is the ease of manufacturing the chip. Figure 10 shows a few varieties of simple geometries that can be implemented into microfluidic chips to aid in mixing. Option C was the geometry chosen for the microfluidic device used in this study on mixing. The main purpose for the selection of design C was the ease of fabrication, and the ability to increase the length of the channel on the chip. However the zig zag design should aid in advective mixing due to the abrupt changes in flow direction.

Figure 10: A variety of geometries used to increase the mixing of fluids.

1.2.3 Fabrication

After the basic design of the microfluidic chip has been selected it is important to take into consideration the problems that can be encountered during the fabrication of the chip, ²⁴. Geometries with a width to height ratio of 20:1 or greater can cause sagging in the PDMS channel and potentially bond the two walls together during the bonding phase of production, which will be discussed in greater detail in section 1.3.4. If the channels are too close to the edge of the chip complete bonding can be difficult to achieve and could result in a low pressure failure of the chip. The feature size of the channel must be large enough to make using photolithography. Generally the features need to be greater than $\sim 10\ \mu\text{m}$ for the photolithography process to be able to print molds that will yield usable channels. This will be discussed in greater detail in section 1.3.2.

1.2.4 Pressure

Using a pump to push fluids through a microfluidic chip has a disadvantage due to the massive pressure that can build up inside the channels. From equation 1.6 the fluid flow rate and resistance dictate the amount of pressure needed to move a fluid. Fluid flow is controlled by the tester and the necessary residence time the fluid needs to exist inside the chip. This means that fluid resistance needs to be tuned by designing a microfluidic chip with correct size and geometry that can withstand the pressure,²⁵. Figure 11 shows the resulting pressure that occurs inside the microfluidic chip at the flow rates that are tested.

Figure 11: Graph of Flow Rate and the resulting internal Pressure.

The maximum pressure that can be contained by a PDMS microfluidic chip is ~ 500 kPa. The pressure is limited by the weakest section of the PDMS microfluidic chip. For the majority of time the most likely point of failure due to burst pressure was the fourth wall of the microfluidic chip. This is due to the

fourth wall being thinner than the rest of the chip. However, other common points of failure were at the connection of the inlets, and the bonded region. The connection inlets failed due to ripping of the PDMS around the hole during connection. The small rips in the PDMS during connection hook ups were exacerbated when the fluids were running, lengthening the rips and leaking fluid , permanently destroying the microfluidic chip. The bonding was the point of failure when a contaminant particle was found between the microfluidic chip and the fourth wall.

1.3 Fabrications of a Microfluidic Reactor

Microfluidic chips are designed with a goal in mind, be it mixing characterization, cell culturing, nanoparticle synthesis, or any other application of microfluidics. This means every chip needs to be designed to complete its function using the proper flow rates and geometries, without exceeding the limits of pressure or manufacturability.

1.3.1 Design Mask for Lithography

A 2D CAD drawing of the microfluidic chip can be utilized to create a transparency of the design called a mask, Figure 12. The CAD drawing of the chip is printed on a mylar sheet at a resolution of 20,000 dpi.

Figure 12: Typical mask used in the production of a microfluidic chip.

1.3.2 Mask to Mold(Photolithography)

Once the mask is fabricated a master mold is made from a silicon wafer and SU-8 2050, a negative photoresist,²⁰. The silicon wafer is used as a flat non porous surface to act as a substrate for the SU-8. The SU-8 is spun on and exposed in the areas desired to create a positive pattern of the microfluidic channels, Figure 13.

Figure 13: The mask is used to expose the SU-8 film to light and create the master mold.

SU-8 2050 produced by Microchem is a negative photoresist,²⁰. This means that the area of SU-8 that is exposed to light is hardened and crosslinked to create the features and the rest is dissolved away chemically. Photoresists have 4 chemical components. SU-8 uses a resin called poly(cis-isoprene)synthetic rubber, a photoactive compound bis-arylazide, a solvent xylene, and surfactants to improve the wetting and adhesion to the silicon,²⁶. The SU-8 is developed using an organic developer produced by Microchem specifically design for SU-8 2050. SU-8 has a resolution of surface features of 2 μm or greater.

1.3.3 Mold to Chip(Softlithography)

Once the microchannels are laid down on the silicon wafer using SU-8 softlithography is used to create the microfluidic chip. Softlithography is the technique of using a soft elastomeric material to replicate small features,²⁰. To make a microfluidic chip, PDMS is cast onto the master mold, where it takes on the negative image of the master mold. This gives the first three walls of the microfluidic chip, Figure 14.

Figure 14: PDMS cast on top of master mold. The three walls of the PDMS microfluidic chip once removed from the master mold.

1.3.4 Bonding

The three wall PDMS chip is then bonded to a fourth wall of PDMS using argon plasma bonding. Both the three wall and fourth wall PDMS pieces are exposed to a high purity atmospheric pressure argon plasma. The argon plasma dissociates the methyl groups and oxidizes the surface forming silanol groups [Si-OH]. When the two oxidized groups come into contact an irreversible Si-O-Si bond is formed and the PDMS is sealed, Figure 15,^{27, 28, 29}.

Figure 15: The PDMS microfluidic chip being treated with high purity atmospheric pressure Ar plasma.

The tip of the plasma dispenser is about $\frac{1}{2}$ " from the surface of the PDMS surface. The plasma is then passed over the PDMS surface in a serpentine pattern making sure to expose the entire surface area and paying close attention to go all the way over the edge of the surface as well. Pass over the surface of the PDMS two times taking about 1 minute for both passes.

To bring the two surfaces into contact simply lay one on top of the other. This should be done within 10 seconds of completing treating the second PDMS surface. Once the two treated PDMS surfaces come into contact take care not to touch any of the section where microfluidic channels are present. You should be able to see the two surfaces bonding together. It would look much like putting a static cling sticker onto a clean piece of glass. If air bubbles appear, very lightly

touch the two surfaces together. As soon as the two surfaces begin to bond they will be stuck together permanently and cannot be moved or adjusted. After bonding, place the PDMS chip in the oven at 70°C for 10- 30 minutes to promote complete bonding.

Chapter 2: Experimental Procedures

2.1 Mask Design

The microfluidic chip was designed to be a T-style mixing device. These devices are most commonly used for two part chemical synthesis applications. The two input channels were 250 μ m wide and 8 mm long. All input and output ports used a 4 cm² square feature for ease of hole punching with a circular punch. The two input channels then converged at a 90 degree angle into a single mixing channel. The mixing channel utilized a zig-zag design to add in the turbulent mixing occurring as well as increase the length of the device. The zig-zag design would utilize short portions of the channel where the width increased creating a section that would have a more turbulent flow behavior. These zig-zag sections would increase the Reynolds number by 84%. The design had a total length of 522 mm long. The width of the mixing channel was 500 μ m. The negative of this design was then impregnated into a thin sheet of mylar with a resolution of 20,000 dpi from a company in Brandon, Oregon, CAD Art Services. This completed the mask that would be used to create master molds, Figure 16.

Figure 16: The design of the T-style microfluidic chip.

2.2 Photolithography

Photolithography techniques are used to create the master mold that is used to cast the PDMS. A four inch diameter silicon wafer is used as the substrate for the master mold. The silicon wafer must be cleaned to ensure that all surface contaminants are removed before any processing can be done. The wafer is cleaned in a Piranha solution of 98% sulfuric acid and 30% hydrogen peroxide, mixed in a 9:1 ratio. The Piranha solution is used to remove all organic material that may be present on the surface of the silicon wafer. The wafer is immersed in the Piranha solution for ten minutes at 70°C. Then the wafer is rinsed in DI water to remove the acid from the wafer. Next the silicon wafer is cleaned in a Buffered Oxide Etch, BOE. The BOE is a hydrofluoric acid mixed with water

produced by Transene Company Inc. The wafer is immersed in the BOE for five minutes at room temperature. This removes all oxides that have formed on the surface of the wafer while it has been sitting at atmospheric conditions. The silicon wafer is then rinsed in DI water to remove the acid from the surface. The wafer is then baked on a hot plate at 150°C for 5 minutes to dehydrate the wafer, removing all liquids that were introduced to the wafer during the cleaning phase.

After the wafer has been cleaned, SU-8 is spun onto the surface of the wafer to create a uniform thickness film of 60 μm . Four milliliters of SU-8 2050 negative photoresist is dispensed onto the center of the silicon wafer. The wafer is spun using a Laurell Technologies Corporation spin coater with integrated speed controller. The wafer is initially spun at 400 rpm at an acceleration of 100 rpm/sec for 20 seconds to spread the resist evenly over the entire wafer. Then the wafer is spun up to 2500 rpm at an acceleration of 600 rpm/sec for 35 seconds to remove excess SU-8 leaving a 60 μm thick film. Once the wafer is spun, a bead of SU-8 will have developed on the edge of the wafer and is removed using an acetone wipe. The wafer is then spun at ~10 rpm for 10 minutes to relieve any stress that has developed in the SU-8 film during the spinning process.

Once the SU-8 has been deposited onto the surface of the silicon wafer some of the solvents are driven off in a soft bake process. The wafer is placed on a hot plate at 55°C for 12 minutes and then transferred onto a hot plate at 85°C for 25 minutes. The wafer is then placed on a cooling plate to bring the wafer and SU-8 film to room temperature. If a wrinkle appears in the SU-8 film, place the wafer

back onto the hot plate for an additional 5 minutes and then cool back down to room temperature. Repeat the heating and cooling cycle until the wrinkle is removed.

The SU-8 film is then exposed to UV light to crosslink the polymer and render the resist insoluble in developer. The light source is a mercury vapor lamp which has a power output of 6.0 mW/cm^2 . A long pass glass filter is used to block all photons with a wavelength less than 350 nm which lowers the power output to 3.2 mW/cm^2 . The wafer is then exposed for 78.2 seconds resulting in an energy dose of 250.24 mJ/cm^2 . The silicon wafer is placed in contact with the mask to ensure high quality resolution when transferring the image from the mask. A diagram of the filter, mask and wafer stack is shown in Figure 17.

Figure 17: Stack of components used during the exposure phase of photolithography.

Once the wafer has been exposed another soft bake process is done to remove more of the solvent. The wafer is again baked at 55°C for 12 minutes and 85°C for 25 minutes. Once the wafer is transferred to the 85°C hot plate the channel features should become visible in the SU-8 film. Once the wafer is baked, it is placed on a cool plate to bring the temperature back down to room temperature.

Once the soft bake is complete, the wafer needs to be developed using Microchem SU-8 developer. Two solutions of developer should be set at room temperature in two Petri dishes. Immerse the wafer in the first developer solution and agitate the solution with wafer tweezers for five minutes. Instead of agitating the solution by hand, a sonicator can be used if the unexposed SU-8 is not

dissolving. After the first five minutes move the wafer into the second clean solution of developer. The second solution should be agitated for an additional two and a half minutes. A small amount of developer was then added to the second solution and the wafer was left to sit for two and a half more minutes. The wafer was then removed from the developer and rinsed with isopropyl alcohol. If a white film appears on the surface of the wafer, the wafer needs to be developed 2 minutes longer. The wafer is then rinsed in DI water to remove the isopropyl alcohol. The wafer is dried using low purity nitrogen, and placed on a hot plate at 150°C for 15 minutes for a hard bake. At this point the SU-8 is completely hardened and permanently bonded to the surface of the silicon wafer with the microfluidic channels patterned on the surface. The mold height is then checked using a profilometer.

This master mold can be used for making several PDMS microfluidic chips. As long as the silicon wafer does not break the master mold can be reused to make additional PDMS chips.

2.3 Softlithography

Once the master mold has been created the PDMS can be cast to form the two parts of the microfluidic chip. Thirty mL of the elastomer is mixed with 3 mL of the curing agent in a single cup which will be poured onto the master mold. Ten mL of the elastomer is mixed with 1 mL of the curing agent in another cup which will be cast onto the bottom of a Pyrex Petri dish which will be used as the fourth wall. The two cups of PDMS are then degassed for 30 minutes in a vacuum

chamber to remove all the air that has been absorbed during the mixing process. After degassing the PDMS is poured onto the master mold and Petri dish. If bubbles form during pouring they can be popped using tweezers. The PDMS is then placed in an oven at 70°C and left for 3 hours to cure the PDMS into a solid layer.

After curing the PDMS needs to be prepared for bonding. The PDMS is peeled from the master mold to reveal the channel features. The PDMS is then cut around the edges of the chips to create straight clean walls. This aids in the plasma bonding process, creating a stronger bond. Holes are then punched through the PDMS using a sharpened 16 gauge hypodermic needle. The needle is sharpened using a 1/16" drill bit, Figure 18. The needle is sharpened by placing the sharp end of the drill bit on the metal part of the needle. Then the drill bit is twisted and pressed against the needle by hand. It takes about 5-10 minutes to sharpen a dull needle. After each use the needle will need to be resharpened for about 1 minute. The needle is then held 90 degrees to the surface of the PDMS and punched completely through the PDMS layers without any twisting motion and a plug is removed from the needle with a pair of tweezers. Once all of the holes are punched and the walls are cut, the surfaces to be bonded are cleaned with scotch tape to remove any contamination that has been collected on the surface.

Figure 18: PDMS punch before sharpening, left, and after sharpening, right.

2.4 Plasma Bonding

The two bonding surfaces are treated with a high purity argon atmospheric pressure plasma to bond the two surfaces in a permanent bond. The surfaces are both exposed to the argon plasma for roughly one minute. Then the surfaces are brought into contact to form the bond. It is important not to touch the surfaces when the PDMS is bonding because the pressure supplied by touching could cause the top of the channel to contact the bottom of the channel and bond shut. Once bonding is complete the PDMS chip is placed back into the oven at

70°C for a half hour to aid in the bonding process. Let the chip cool back down to room temperature before introducing liquid.

2.5 Mixing Testing

At this point the PDMS microfluidic chip is made and the last thing to do is run fluids through the microfluidic chip. The fluids are prepared by mixing ratios of organic dye to water by 1:1, 1:10, 1:25, 1:50. The fluids are then put into 35 mL syringes and placed into the syringe pumps model, NE-300 New Era Pump Systems Inc. The syringes are then hooked up to tygon tubing with an OD of 1/8" and an ID of 1/16" and a 16 gauge needle to interface into the connectors to PDMS layer. The syringe pumps are then set to the desired flow rate and the fluid is pumped through the chip.

To find the minimum channel length for complete mixing, the microfluidic chip is placed under a reflection light microscope. The fluid is then inspected by eye to find the point of complete mixing, and the length is measured using the known values of the microfluidic chip and a caliper. These measurements were accurate within ± 2 cm.

To show evidence of the evolution of mixing in the microfluidic chip a reflection light microscope is used again. The mixing is captured at distinct location across the microfluidic chip. Then a luminescence profile is taken of the cross section of the microfluidic chip to show how the fluids have mixed at that length through the microfluidic chip. The luminescence profile is a graph of the intensity of light that is passing through microfluidic channel. Because the two fluids mixing were

optically transparent and the other optically opaque and soluble in each other, the luminosity profile aided in determining the amount of diffusion that had occurred.

Chapter 3: Results

3.1 Chip Conditions During Testing

For testing the microfluidic chip, flow rates ranging from 1 mL/hr to 30 mL/hr were tested in increments of 5 mL/hr. These flow rates were chosen to simulate a chemical synthesis application for the microfluidic chip. The 30 mL/hr flow rate produced an internal pressure of 835.8kPa which caused a failure in the fourth wall of the PDMS. The results of the chip condition during testing are listed in table 1.

Table 1: List of Testing Conditions

Flow Rate [mL/hr]	1	5	10	15	20	25	30
Re	0.99	4.96	9.92	15.15	20.2	25.25	30.3
ΔP [kPa]	16.8	81.2	161.8	418.3	557.5	696.6	835.8
ΔP [psi]	2.4	11.8	23.5	60.7	80.9	101	121.2
V[m/s]	0.0092	0.046	0.092	0.17	0.22	0.28	0.33
Residence Time [sec]	56.4	11.3	5.6	3.1	2.4	1.9	1.5

The Reynolds number for this microfluidic chip are in the laminar region over all. However when the flow rate is equal to 1 mL/hr the flow structure is Stokes flow. When the structure of the flow is Stokes flow, the inertia forces are assumed to be zero. This means that mixing will be entirely dependent on diffusion. The residence times produced by these flow rates are perfect for use as a microfluidic chemical synthesis device. The desirable residence times are between one minute and one second. The pressures for the 30 mL/hr flow rate were too high for the microfluidic chip and caused a failure in the form of the fourth wall bursting. The most common point of failure was the bulk PDMS layer.

3.2 Photolithography

During the photolithography processing, stresses develop in the SU-8 film due to the evaporation of solvent. This loss of solvent, called the evolution of solvent, is critical to the processing and end properties of the SU-8 film.

However the stress cracks that present themselves after development can destroy desired features. The hard bake step is used to alleviate these cracks by flowing the SU-8 film to fill in the cracks, Figure 19. A hard bake at 150°C for 15 minutes relieves all stress cracks without altering the feature geometry.

Figure 19: SU-8 Crack present pre hard bake step, top. Hard bake step flows the SU-8 cracks back together, bottom.

The energy dose for the microfluidic device was 250.24 mJ/cm^2 , as recommended by Microchem. This energy dose created a resolution of features that was on average $4 \pm 1.8 \text{ }\mu\text{m}$ different from the printed mask, Figure 20. The minimum feature size needed for the microfluidic chip was

100 μm . However when the master mold was created the difference from the mask to the mold was only 0.5 μm smaller than expected.

Figure 20: Resolution chip, showing the effectiveness of the light integral to achieve the features.

The profilometer and reflection light microscope were used to determine the dimensions and geometry of the features produced. The profilometer found that the height of the channel walls was 60 μm tall, figure 21. This is 10 μm greater than was desired during the design of the microfluidic chip. This means that the spin speed was slower than needed to achieve the proper height. The light microscope was used to evaluate the surface of the SU-8 features in both width and height, figure 22. The width of the features was 0.5 μm smaller than designed. This is due to the SU-8 film being over developed.

Figure 21: Profilometer scan showing the cross section of the SU-8 layer of the master mold.

Figure 22: Light microscope image of the intersection of the input channels and the output channel in SU-8.

3.3 Softlithography

When the PDMS was cast to the shape of the master mold it was compared to the width dimension of the mold. The master mold had a width of $706.5 \pm 3.475 \mu\text{m}$ when the mask had a width of $707 \mu\text{m}$. The PDMS cast had a width dimension of $715.1 \pm 3.475 \mu\text{m}$, figure 23. The cast created a width that was 1.5% larger than expected from the mask. This is due to the tapering of the SU-8 walls on the master mold. Instead of the wall being a rectangle in cross section shape it was a trapezoid.

Figure 23: Light microscope image of the intersection of the input channels and the output channel in PDMS.

After the PDMS is bonded together the microfluidic chip needed to be able to withstand the pressure that moving fluid would be providing. From earlier investigations the microfluidic chip was designed to not exceed a pressure greater than 200 kPa. However during experimentation the failure pressure of the design was found to be near 800 kPa. The point of failure for the device at this pressure was the center of the fourth wall of the microfluidic chip not the bonded area. This means that the process used to bond the PDMS together far

exceeded the expectations that were set from prior experimentation. Also the point of failure was in the bulk PDMS not the bond location.

3.4 Minimum Length to Complete Mixing

The minimum length to complete mixing depends on the flow rate and the concentration of the fluid. According to equation 1.10, in a perfect diffusion mixer, it is expected that the fluids will complete mixing around 55 m. The minimum length of complete mixing occurred at the fastest flow rate and the lowest concentration, at a length of 361 ± 20 mm. That is over 100 times sooner than expected. At higher flow rates, the concentration gradient had a larger impact on the minimum length to complete mixing. As the flow rate increased the minimum length to complete mixing decreased. A higher concentration gradient resulted in a longer length to complete mixing. At a flow rate of 15 mL/hr the 50:1 concentration gradient was the only one to mix complete within 522 mm. At a flow rate of 20 mL/hr and higher all flow rates were able to mix completely. At the maximum flow rate that the device could withstand, the higher concentration gradient completed mixing in 9.5% less length than the lowest concentration gradient. Figure 22 shows the results of the average length complete mixing.

Figure 24: Minimum average length to complete mixing.

Because the two fluids are mixing by both advection and diffusion, it is also advantageous to look at the residence time to completion for each flow rate and concentration, figure 23.

Figure 25: Minimum average residence time to complete mixing.

From the graph, as the flow rate increases the residence time to complete mixing decreases. This means that there must be some advection occurring inside of the microfluidic chip and that it is not a perfect diffusion mixing device. Also for all concentrations the residence time is very similar for each flow rate indicating that the amount of diffusion mixing for the three concentrations must be similar as well. However the measurements for the three concentration gradients were so close together that the measurement tool could not predict a difference. This means that concentration gradient had a very small effect on complete mixing, and flow rate was the important variable to control mixing.

3.5 Mixing Evolution

As the fluid was flowing, another point of interest during mixing was the point where the dye first reached the other side of the microfluidic channel but the dye was not uniformly dispersed. This point was referred to as the point of initial mixing.

The lower concentration gradient takes the longest distance to initially diffuse and the highest concentration gradient diffused in the shortest distance. As the flow rate increased the length to initial diffusion decreased. As the flow rate increased the effect of the concentration gradient on the initial length of diffusion decreased. At a flow rate of 25 mL/hr the length to initial diffusion for all the concentrations was nearly equal, Figure 26.

Figure 26: Distance until initial mixing average.

Another interest is how the fluid mixes throughout the entire length of the microfluidic chip. A look at a single concentration of 25:1 for multiple flow rates, shows that the faster the flow rate the faster the fluids mix, Figure 27.

Figure 27: The amount the two fluids have mixed for given length throughout the microfluidic chip for a concentration of 25:1.

By the end of the chip at 522 mm only the 20 mL/hr flow rate has completed mixing. Comparing two different concentrations at a single flow rate shows the effect of diffusion and advection mixing, Figure 28.

Figure 28: Percent mixed as a function of distance for multiple concentrations at a flow rate of 1 mL/hr, top, and 10 mL/hr, bottom.

In both cases the high concentration gradient is mixing more and faster than the lower concentration gradient. In all four of these cases no combination mixes completely.

Chapter 4: Discussion

4.1 Chip Conditions During Testing

With flow rates ranging from 1 to 30 mL/hr the flow structure is mostly laminar.

However at a flow rate of 1 mL/hr the Reynolds number was less than one and the chip had a Stokes flow structure.

The chip design utilizes a width of 500 μm , but also includes a portion that has a wider width increasing the effect of turbulent mixing. Each zig zag portion of the chip creates a brief part where the Reynolds number of the flow is increased and then goes back into the normal 500 μm wide, Figure 29. With over 350 zig zags over the entire length of the chip this small portion of larger Reynolds number greatly influences the fluids mixing by advection.

Figure 29: Portion of the microfluidic reactor with an increased width for the fluid to mix more turbulently.

The pressure generated inside of the microfluidic channels was enough to alter the geometry while running. Due to PDMS's low yield strength the pressure generated inside of the channels caused bowing of the walls increasing the volume of fluid the microfluidic chip could hold. This bowing would also have the ability to increase the Reynolds number influencing the flow structure, Figure 30. At flow rates less than 15 mL/hr this bowing of the channel was not present due to insufficient pressure. This high internal pressure caused increase in channel

volume and should be accounted for in all designs where precise control of the mixing is important.

Figure 30: Bowing of the PDMS microfluidic channels during fluid pumping at high flow rates.

If the bowing of the microfluidic channels becomes a problem due to failure of the device or incomplete mixing, making thicker casts of PDMS can alleviate the problem. The thicker the PDMS cast the less the pressure will be able to bow the channel out creating a larger geometry.

Over time the tygon tubing used to move the fluid from the syringe to the microfluidic chip wear out due to the pressure. The tubes began to leak around the compression fitting causing a pressure drop that made the chip inoperable. The tygon tubing needed to be replaced after every 10 hours of use.

4.2 Photolithography

The height of the microfluidic channels was 20% taller than expected. This is likely due to the temperature of the SU-8 prior to spinning. The SU-8 is stored in a refrigerator to increase the shelf life. The SU-8 compound was left out for 3 hours to let it accommodate to room temperature, but Microchem, the manufacturer of the SU-8, recommends that it be left out over night. However for this application the height being 10 μm taller did not impact the final. To achieve the desired thickness of 50 μm the spin speed could be increased to 2750 rpm for the same amount of time. This would help to thin out the SU-8 during the spinning process.

4.3 Softlithography

The process of plasma bonding can be tricky to perfect. It is imperative that the surface of the PDMS be clean. Dust particles that occur at a critical bonding site such as over the PDMS wall can create a high probability failure point. The defect can create a gap in the bonding that under pressure split the two PDMS pieces apart. The PDMS thickness can also affect the ability for the PDMS to bond. The thinner the PDMS the less ineffective bond area is present. This means that although the thick PDMS is more advantageous for a final product, it is more difficult to bond and create a functioning microfluidic chip.

4.4 Minimum Length to Complete Mixing

On the macro scale mixing occurs nearly instantly. With this design it takes a much longer time for two fluids to mix completely. With the zig zag pattern at 522 mm long the two fluids will only mix at high Reynolds numbers and high flow rates. This means for the geometry designed into the chip the pressure is too high to be effective for a chemical synthesis application. To improve the design the channels need to be larger and run at higher pressure, or an alternative design would need to be used for mixing.

The zig zag design is a passive mixing design that attempts to make the fluid behavior more turbulent. This design would be more effective if the Reynolds number was larger. To keep the same geometry inside the chip an active mixing design would need to be utilized to work for a chemical synthesis application. An example of an active mixing would be to introduce acoustic waves to introduce oscillatory motion promote mixing.

4.5 Mixing Evolution

The results of the mixing evolution experiment are intuitive. The faster the fluids are moving or the higher the concentration gradient of the two fluids, the more mixing occurs. However the shape of the evolution of mixing graphs shows something interesting. As the two fluids initially mix, the shape of the beginning portion is exponential. This indicates that the dominant form of mixing is the diffusion of the two fluids. As the fluid continues down the microfluidic chip the rate of mixing becomes linear. This means that the effect of diffusion mixing is decreasing due to the decrease of a concentration gradient between the two

fluids. The mixing toward the end of the chip is dependent on the advection that is occurring because of the geometry of the zig-zag pattern.

The length to complete mixing was predicted to be 55 m if the microfluidic chip behaved as a perfect diffusion mixer. The minimum length to complete mixing was found to be 361 mm. This means that diffusion was not the only mode of mixing and that some type of advective mixing must be present to improve mixing so drastically. Figure 31 shows the appearance of multiple concentration zones created inside of the microfluidic channels. This is likely due to presence of a secondary flow.

Figure 31: Picture of the presence of multiple concentration gradients across the microfluidic channel.

This secondary flow would cause a rocking motion of the lamina in the height dimension, resulting in a folding of the lamina. This folding would aid in mixing by creating more surface area between multiple concentration gradients resulting in faster mixing. The presence of multiple concentration gradients was only observed at flow rates greater than 10 mL/hr. It is likely that the flow rates less than 10 mL/hr did not complete mixing due to the lack of presence of the multiple concentration gradients that would increase the effectiveness of the diffusion mixing to completely mix the two fluids.

Chapter 5: Conclusions and Recommendations

The zig zag microfluidic design failed for the application of a chemical synthesis device. For flow rates 1 through 20 mL/hr the two input fluids failed to mix completely within 522 mm regardless of concentration gradient. At the flow rates of 25 and 30 mL/hr the two input fluids did mix within 522 mm, however the pressure inside the channel was large enough to burst the microfluidic chip at 30 mL/hr.

The photolithography and softlithography processes both created the desired features of the microfluidic chip. The photolithography processing produced a width that was 0.5 μm smaller, and the height of the features was 10 μm taller. However this did not affect the final results of the mixing of the device. The soft lithography process produced a width of $8.1 \pm 3.475 \mu\text{m}$ larger than designed.

The fluids in this design relied on both diffusion and advection for mixing. In the first section of the microfluidic chip, diffusion was the dominant form of mixing due to a high concentration gradient of the two fluids. As the two fluids continued down the microfluidic chip the concentration gradient decreased and advection became the dominate form of mixing. Even though the structure of the fluid flow was laminar advection was present, especially at the wider portion of the microfluidic device. With over 350 wide zones within the microfluidic chip, there was enough advection to effectively aid in the mixing of the two input fluids.

There are several ways to improve the mixing, both diffusion and advection, in a passive T-style microfluidic mixer. For diffusion it is best to have the largest possible interface between two concentration gradients. This will increase the amount of diffusion that takes place at any time during the mixing. It is also important to reduce the characteristic diffusion dimension as much as possible. This will decrease the length that the diffusing species will need to travel to complete mixing. To improve the mixing through advection, running at the highest possible flow rate will promote the drive for a secondary flow structure to be present. Introducing the solute solution in the center of the channel would also aid in mixing by decreasing the characteristic diffusion dimension and the surface area of the diffusion solute in the solvent.

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