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Tesi di Laurea Magistrale

Porous monoliths for microfluidic cell trapping and filtering devices

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Acknowledgments

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Abstract

Immunoassay is a challenging factor, that must be dealt with by means of novel and optimize methodologies, in a sense that being able to filter the cells targeting in a best way so as to study their effect and existence in the well-defined concentration of biological entities; recently researchers focused on a very novel method for cell culturing studies through introducing a defined path for better concentrate the cells in a restricted area, this geometry was introduced as Microfluidic tube or channel focused for channeling and filtering the bio entities an especially cells for assay studies.

In studying cell-cell communication at colonial level, providing a physical barrier with chemical permeability to allow and track transport of signaling molecules is necessary for prolonged experiments. Here, fabricating and integrating hydrogel filtering membrane module into the microfluidics chip for communication studies have been developed.

In this thesis, three level PDMS microfluidic chip with different geometries were designed and fabricated successfully by soft lithography technique. Three level PDMS microfluidic chip including parallel deep channels, shallow cell traps and filtering compartment between interconnected traps. Filtering module consist on an array of pillars boxed in a cubic space and a monolithic hydrogel matrix engulf the entire module including pillars allowing passage of signaling biomolecules. Thereafter, porous monolith microstructures were integrated into the microchips as a filtering barrier via photolithographic patterning. Selected membrane materials are biocompatible and photoactive hydrogels including Polyethylene Glycol –Diacrylate (PEG-DA) and 2-hydroxyethyl methacrylate-co-ethylene dimethacrylate (HEMA-EDMA). In order to increase the adhesion and stability of the membranes, a silanization step 3-(Trichlorosilyl) Propyl Methacrylate, right after plasma bonding of chips was added prior to the gel injection.

The primary filtering efficiency tests have been successfully performed with 2µm Polystyrene microbeads. The bacterial communication tests are currently under development. In comparison with other methods of Nano-filter fabrication such

as direct 3D printing in channels with two photon polymerization technology with submicron resolution, our method renders faster and more cost effective prototyping cycles.

Monoliths were stable during prolonged experiments with constant supply of flow. In primary tests, 2 μm diameter PS microbeads are used as a substitute for E-coli. Both PEG-DA and HEMA-EDMA monolith form stable membranes with PEG-DA exhibiting more volumetric shrinkage after polymerization.

Chapter 1. Introduction

“Microfluidics, a technology characterized by the engineered manipulation of fluids at the submillimeter scale, has shown considerable promise for improving diagnostics and biology research. Certain properties of microfluidic technologies, such as rapid sample processing and the precise control of fluids in an assay, have made them attractive candidates to replace traditional experimental approaches.” [1]

Microfluidic chips can be identified as a micro device that deals with the flow of liquid in one or more tiny channels with at least one dimension of Nano/micrometers size. Concerning the terms of microfluidic, it can be considered to both science and technology (including: research on theoretical of flows, transport phenomena, interaction in tiny space with the high surface area to volume ratio; microfabrication technology of microfluidic chip for application in chemical/biological analysis- such as Lab-On-a-Chip).

In synthetic biology, one of the main challenges in studying the cell-cell communication at colonial level has been to provide a physical barrier with chemical permeability to allow and track transport of distinct signaling molecules necessary for communication, while maintaining colonial physical separation for prolonged experimental times. In this work, a method for fabricating and integrating filtering membrane module into the microfluidics chip for communication studies have been developed. In comparison with other methods of Nano-filter fabrication such as direct 3D printing in channels with two photon polymerization technology with submicron resolution, our method renders faster and more cost effective prototyping cycles.

Chapter 2. Literature review

2.1. Microfluidic systems

2.1.1. Historical development of Microfluidics

The history of microfluidic date back to 1950s, mainly in manufacturing of inject printers. The mechanism of these printers is based on microfluidics which involves very small tubes transport the ink for printing. In the late 1970s, a miniaturized gas chromatograph was realized on a silicon wafer. By integration of fluidic and optical components in microdevices, micro system is a more accurate description. In 1980s, the early stage of microfluidics dominated with development of micro flow sensors, micropumps, and microvalves. Within the following years several silicon-based analysis systems have been presented. Afterwards researchers spent a lot of time in developing new microfluidic components for fluid transport, fluid metering, fluid mixing, valving, or concentration and separation of molecules within miniaturized quantities of fluids within the last two decades.

A primary goal for much of the microfluidic community is to develop technologies that enhance the capabilities of investigations in biology and medical research. Many microfluidic studies describe methods that aimed to replace traditional macro scale assays, and usually performs proof-of concepts experiments. [2]

Biotechnology is closely linked to microfluidics. Biological targets are nearly always transported by a buffer fluid or carrier fluid, as well in vitro and in vivo. In the human body, any bio-MEMS has to deal with body fluids. In *in vitro* microsystems, the target molecules/particles are nearly always transported by a buffer fluid for many reasons: first, the target molecules/particles are most of the time extracted from a liquid (e.g., DNA and cells); second, the biochemical reactions on these targets are performed in an aqueous environment; and third, confinement of the targets is easier in a liquid than in a gas. Very few examples

of biotechnological microsystems exist that do not require the use of microfluidics. One counterexample might be the “electronic nose,” where detection of target molecules transported by ambient air is done directly on a dry contact surface by mass spectrometry.

2.1.2. Physical concepts of Microfluidics

Laminar Vs Turbulent flow

The Reynolds number is a dimensionless quantity that describe the ratio of inertial to viscous forces in a fluid. [3] It is proportional to the characteristic velocity of the fluid and the length scale of the system; it is inversely proportional to the fluid viscosity. (Re) is used to determine the transition from laminar to turbulent regimes, with Re < 2100 considered laminar for flow in cylindrical channels. The Reynolds number for this flow is defined as

$$Re = \frac{ud}{v}$$

- u is the flow velosity
- v is the kinetic velocity
- d is the channel diameter

In fluid mechanics terms, the Reynold number compares the magnitudes of inertial force to viscose force in a flow. Because Re $\propto$ d, the small dimensions of microfluidic channels are responsible for very low Reynolds numbers, resulting in laminar flows.

For microfluidic systems, (Re) is almost always in the laminar flow regime, allowing for highly predictable fluid dynamics. Molecular transport also changes dramatically at this scale because convective mixing does not occur, enabling predictable diffusion kinetics.

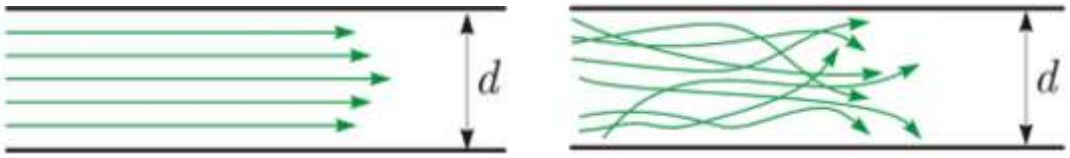


Figure 1: Microfluidics laminar (left) vs Turbulent flow (right)

Surface and interfacial tension

Surface tension describes the tendency of a fluidic in a surface to reduce its free energy by contracting at the surface -air interface. Interfacial tension is a similar phenomenon, but it generally applied to two immiscible fluidics (for example oil in water). These forces play dominant roles on the microscale (figure 2) compared to gravity, which is much more dominant on the microscale. Researchers have used these phenomena to conduct protein crystallization, and passively drive fluids through microchannels. [2]

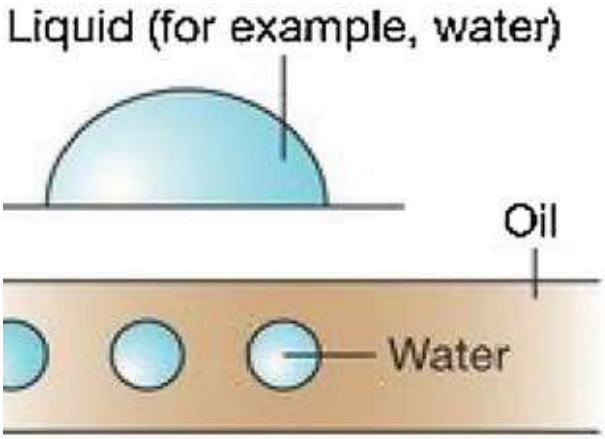


Figure 2. Surface and interfacial tension in microfluidics

Capillary forces

Capillary action describes the movement of a fluid through a narrow construction, such as a narrow tube or porous material. At the microscale, capillary action is a more dominant force, allowing fluids to advance in opposition to gravity. Capillary forces have been used to manipulate fluids in many applications, the most famous examples perhaps being the at-home pregnancy test and portable glucometers to monitor blood glucose levels. [2]

2.2. Microfluidic applications in biology

Recently, great methodologies introduced for the biological entities to be identified and being assayed; among all microfluidic technique as a noble method pioneered in term of fast analysis and modern issuance imported for bioassay to be exploited in biology and cell studies. Conceptually, the idea of microfluidics has been extracted from semiconductor industry and later expanded by the micro-electromechanical systems (MEMS) field. Microfluidic technology is characterized for precise manipulation of fluidics at the submillimeter length size.[3] These devices, commonly referred to as miniaturized total analysis systems (μTASs) or lab-on-a-chip (LOC) technologies, could be applied to biology research due to certain properties, such as rapid sample processing (shorter reaction time), small requirement for solvents, reagents, and cells critical for valuable samples and for high-throughput screening), precise control of fluidics in an assay, low cost, portability, versatility in design which made them attractive candidate to replace traditional experimental approaches.

"Microfluidics can provide more realistic in vitro environments for small-scale biological species of interest. Figure 3 provides comparative length scales for several biological structures, as well as common micro-fabrication structures used in microfluidic and MEMS technology." [3]

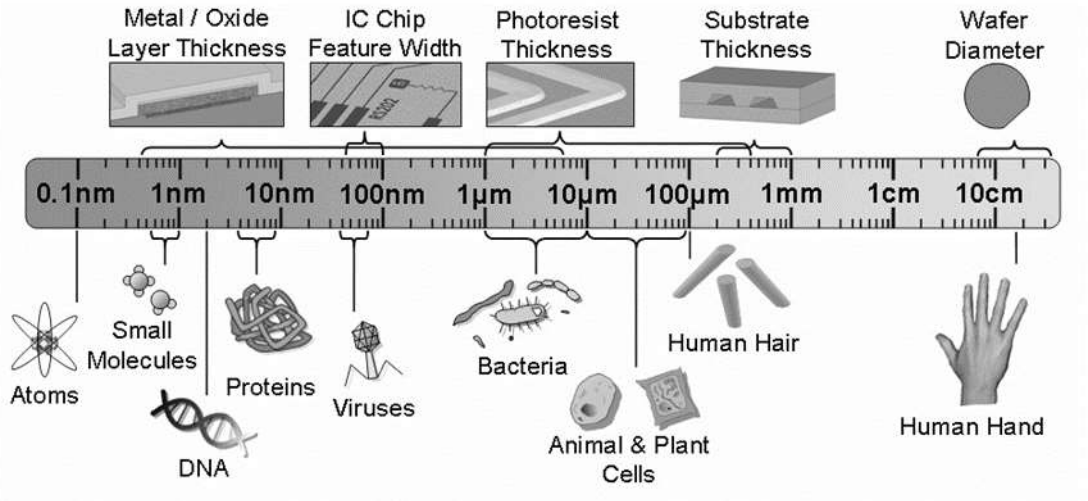


Figure 3. Approximate length scales for several biological and micro-fabrication structures.

2.2.1. Microfluidic cell trapping

Several techniques have been established for cell analysis, including flow cytometry [4], array-based methods [5], and microfluidics. Microfluidics has emerged as a promising tool for cell analysis by providing capabilities of cell handling, environmental control, high resolution imaging and integration of multiple functional components. There are several well-established isolation techniques based on trapping forces, including hydrodynamic, dielectrophoretic, magnetic and acoustic trapping. Most of the cell trapping techniques have adopted a hydrodynamic trapping method because of its passive separation [6]. Hydrodynamic mechanisms are based on dynamic changes in the flow field before and after trapping, which are determined by particle size. [7]

2.2.2. Filtering techniques

The need for efficient cell separation, an essential preparatory step in biological and medical assays, has led to recent development of numerous microscale separation techniques. Microfluidic-based sorting offers numerous advantages, including reducing sample volume, faster sample processing, high sensitivity and spatial resolution, low device cost, and increased portability [8].

Other advantages of using microfluidic in biological experiments are low cost material for fabrication of chips. PDMS is one the most useful materials with easy fabrication. The techniques presented are broadly classified as being active or passive depending on the operation principle. Developing efficient microscale separation methods that offering greater control over cell population distribution will be important in realizing true point-of-care (POC) lab-on-a-chip (LOC) system.

The early development of microfluidics as a field was strongly motivated by the need to analyze biomolecules more efficiently and accurately, which was culminated by the human genome project. However, the focus of the field has now been shifting more toward addressing the need for cell biology studies.

While the number of molecular species to be tracked keeps increasing with the progress of different “omics”, individual cells are more and more regarded as the basic “unit” of our biological understanding. With the appropriate length scale that matches the scales of cells, microfluidics is well positioned to contribute significantly to cell biology.

Separation and isolation of rare cell population from a heterogeneous suspension is essential for many applications, ranging from disease diagnostics and drug treatment analysis to conducting fundamental studies. Recently, various separation techniques have been successfully applied to separate stem cells based on their intrinsic properties to conduct fundamental studies.

Conventional cell separation systems employ membrane-based filtering schemes, which are limited by the membrane pore size and are easily susceptible to clogging. Microfluidics presents a functional tool-set for cell separation offering numerous advantages including, reduce samples and expensive reagent volumes, fast sample processing, increasing detection accuracy, integrated reference systems with little human intervention, reducing odds of sample contamination, increased portability -potential for point-of-care (POC) diagnostic in resource poor setting lacking clinical labs and skilled personnel, and low cost. Thus, developing efficient microscale separation methods that can offer greater control over cell size distribution is becoming increasingly important for realizing many lab-on-a-chip systems.

Traditionally, microscale cell separation techniques take advantage of the disparities in the intrinsic properties of the different cell populations to achieve separations. Mechanical and physical properties, including size, shape, density, adhesion, and deformability, are common markers for differentiation. Due to their high sensitivity and efficiency, cell separation based on polarizability and magnetic characteristics have become extremely popular in the recent years. Highly specific separation based on difference in cell affinity (surface biomarkers) have also been effectively exploited to demonstrate separation between cells with similar physical and electromagnetic properties. Cell separation principles can be categorized to active and passive separation techniques. Active techniques rely on an external force field for functionality, while passive techniques rely entirely on the channel geometry and inherent hydrodynamic forces for functionality.

Similar to other microfluidic components (e.g., micromixers, micropumps, and microvalves), microfluidic cell separation techniques can also be broadly classified as ***active*** and ***passive*** separation techniques. Active techniques rely on

an external force field for functionality, while passive techniques rely entirely on the channel geometry and inherent hydrodynamic forces for functionality.

Active separation techniques

2.Magnetic sorting

Recently developed method enables periodic spatial arrangement of cells in a modulated magnetic field based on diamagnetic cell response [9]. The advantage of this technique is that it is applicable to any diamagnetic particle, as long as its magnetic susceptibility is different from that of the medium, eliminating the need for cell labeling with ferromagnetic beads.

In this technique, sample cells are first incubated with magnetic beads with recognition molecules (antibodies), for "magnetic labeling" instead of fluorescence. Then, a magnetic field gradient is used to isolate the magnetic beads, which in turn picks out the cells.

magnetic cell sorting can be operated in either serial or parallel manner, resulting in higher throughput. Up to 10^{11} cells can be processed in 30 min. Magnetic field is largely permeable to biological tissues and cells and less likely to interfere with cell function or immunochemistry necessary for magnetic labeling [10].

3. Dielectrophoresis

Dielectrophoresis cell separation and sorting technique depend on the principle that when polarizable molecules such as large biomolecules and cells are placed in non-uniform electric field, the field can impart a net force on the particle due to an induced or permanent dipole. This force does not require the particle to be charged. All particles exhibit dielectrophoretic activity in the presence of electric fields. However, the strength of the force strongly depends on the medium and particles electrical properties, on the particles shape and size, as well as on the frequency of the electric field. Consequently, fields of a particular frequency can manipulate particles with great selectivity. DEP has been applied in a number of

systems based on selective particle trapping and elution or deflection in continuous flow [11].

Dielectrophoretic forces are caused when a non-uniform electric field interacts with the induced electrical polarization, or dipole, of a cell. Depending on the frequency and conductivity of cell cytosol and surrounding media, these forces tend to hold the cell in place near the high field region or push the cell away from high field region.

4.optical sorting

In optical sorting technique, Light has been used to manipulate and separate particles depending on their optical polarizability. For sorting, demonstration of this technique using interferometric patterns of light for separations based on size (protein microcapsules) and refractive index (separating polymer from silica spheres) has been accomplished, however, cell separation has not yet been provided [8].

5.Acoustic sorting

This technique is based on the fact that cells and particles suspended in fluid experience an acoustic radiation force when they are exposed to ultrasound. Separation of particles utilizing this force can be achieved by generating a standing wave over the cross section of a microfluidic channel. In this example configuration, while the fluid flow carries the particles through the channel, a radiation force pushes them towards either the pressure nodes or the pressure antinodes of the standing wave [12] [13]. Ultrasonic acoustic resonance within a microchannel, induced by piezoelectric material, can produce radiation to manipulate particles and molecules.

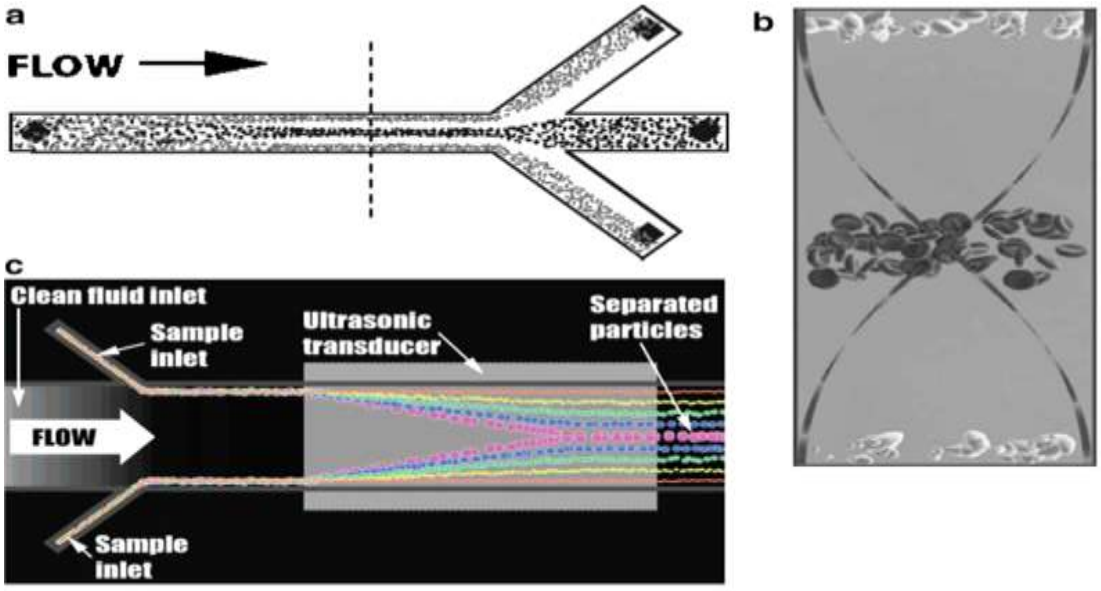


Figure 4. Acoustic separation. The acoustic radiation force may be manipulated for density-based, equilibrium separation (a, b) From Laurell et al. Chem Soc Rev 2007. or size-based kinetic separation (c). From Petersson et al. Anal Chem 2007.

Passive separation techniques:

1.pillar and weir structures

Microfluidic or microstructure filter (pillar and wire structures) technique is attractive for size and deformability-based cell sorting. This technique allows us to precisely adjust the filter pore size to the required needs [14]. However, these filters face many challenges including heterogeneity of cell sizes within a population, clogging and fouling.

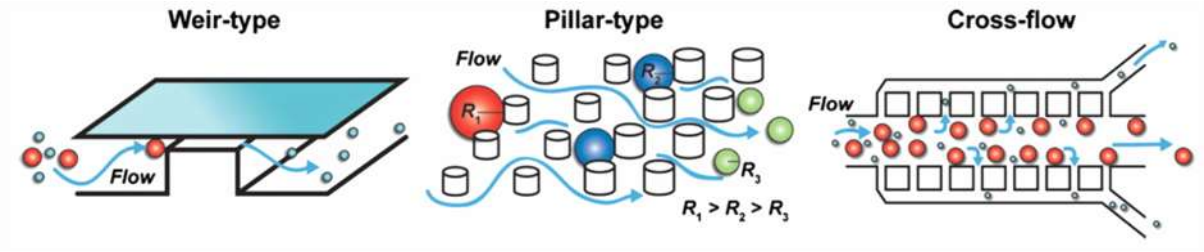


Figure 5. Schematic illustration for weir, pillar and cross-flow microfluidic filters. From Gossett et al. Anal Bioanal Chem 2010.

2. Hydrodynamic filtration

Hydrodynamic filtration is another passive technique for size-based separation in microfluidic device. This technique presumes that at a low Reynolds number the center of a particle will follow fluid streamlines. In this technique particle laden flow is pumped through a channel having multiple side branching outlets. This side channels drain the liquid from the main channel continuously, thereby aligning all particles along the sidewalls of the main microchannel. The difference in size positions the smaller particles to closer to the sidewall than larger particles, thus resulting in them being filtered out earlier than the larger sized particles. As the separation mechanism is based purely on flow profile and not microchannel geometry, channels significantly larger than the cells diameter can be employed, minimizing clogging and further increasing throughput.

By portioning the flow in a clever way i.e. controlling the flow rate through one or more inlets, channel geometry, and configuration of outlets) several methods make possible to dictate size-based cell sorting and separation [15].

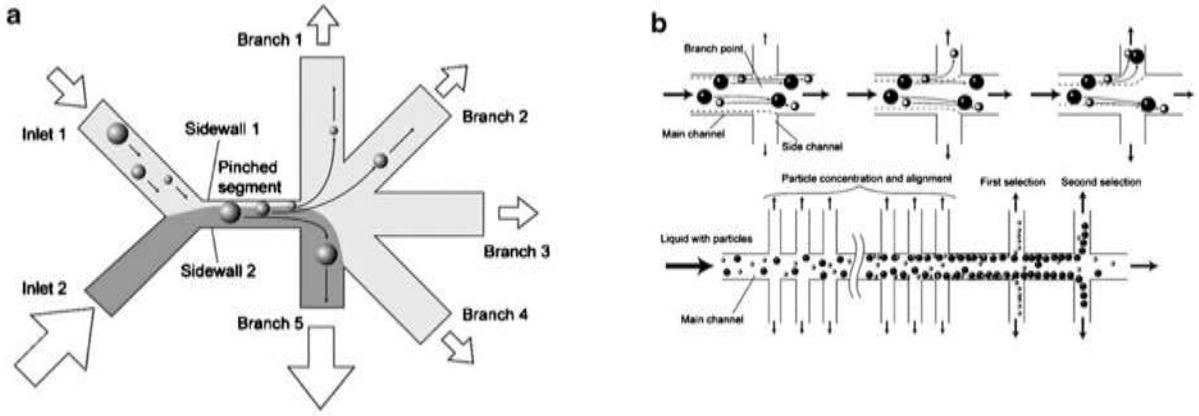


Figure 6. Hydrodynamic methods of separation. A) Pinched flow microfluidic fractionation. From Takagi et at. Lab Chip 2005. B) Hydrodynamic microfluidic filtration. From Yamada et al. Lab Chip 2005.

4. Inertial forces

Inertial lift forces inherent to cell motion in microchannel flow can be exploited to precisely manipulate the cell position in these flows. Lateral migration of cells in microchannels occurs due to superposition of two inertial forces. These two forces equilibrate the cells at distinct positions inside the microfluidic channel cross-section based on their size relative to relative to the microchannel dimensions, thus achieving separation. Inertial separation technique is based on asymmetric sheath flow and proper channel geometry. It generates a soft inertial force on the sample fluid in the curved and focused sample flow segment to deflect larger particles away while the smaller ones are kept on or near the original flow streamline.

The working principle of this separation is to devoid of filter interfaces or external force fields, avoiding clogging and making it easy to setup. Balanced transverse force components concentrate and divert particle streams according to the designed size cutoff. This spiral flow filtration concept can address size- and mass-based separation of microparticles, including biological agents [6].

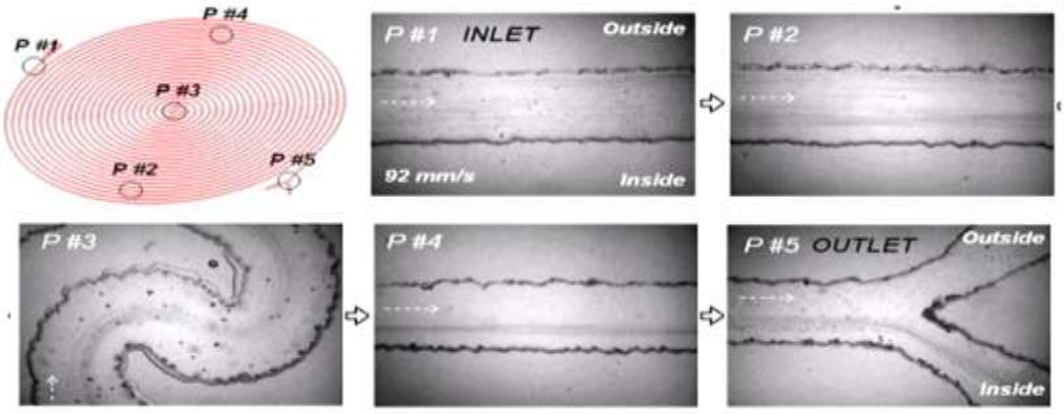


Figure 7. Example of an inertial microfluidic technique. From Seo et al. Appl Phys Lett 2007.

5. biomimetic separation

In order to achieve desired fractionation of blood components, biomimetic microfluidic separation techniques emulate hemodynamic phenomena involving the intrinsic properties of blood and the microvasculature. These phenomena have been observed and replicated in microfluidic systems and include leukocyte margination, plasma skimming, and the bifurcation law, also known as Zweifach-Fung effect. A number of techniques have been developed to take advantage of these effects [16] [17].

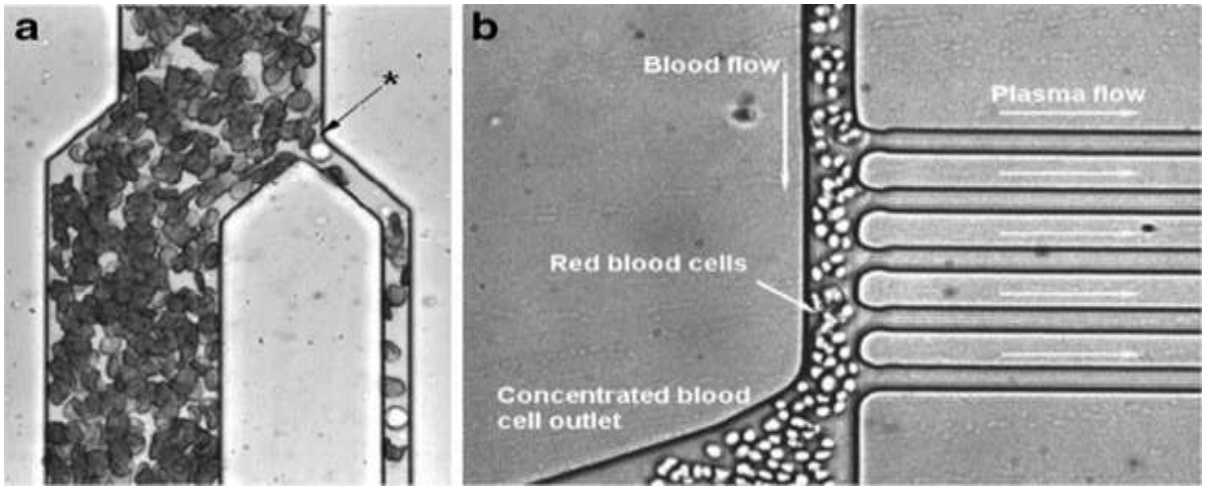


Figure 8. Example of two microfluidic devices that mimic the microvasculature. a) Leukocyte margination is used to isolate leukocytes (*) as they are typically found in the near-wall regions of flow. From Shevkoplyas et al. Anal Chem 2005. b) Bifurcation law is manipulated to remove cell-free plasma from blood. From Yang et al. Lab Chip 2006.

2.2.3. Cell-cell communication analysis

Bacteria communicate with one another using chemical signal molecules. As in higher organisms, the information supplied by these molecules is critical for synchronizing the activities of large groups of cells. In bacteria, chemical communication involves producing, releasing, detecting, and responding to small hormone-like molecules termed autoinducers. This process, termed quorum sensing, allows bacteria to monitor the environment for other bacteria and alter behavior on a population-wide scale in response to changes in the number and/ or species present in a community. Most quorum sensing-controlled processes are unproductive when undertaken by an individual bacterium acting alone but become beneficial when carried out simultaneously by large number of cells. Thus, quorum sensing confuses the distinction between prokaryotes and eukaryotes because it enables bacteria to act as multicellular organisms [18].

QUORUM SENSING

Quorum-sensing bacteria produce and release chemical signal molecules termed autoinducers whose external concentration increases as a function of increasing cell-population density. Bacteria detect the accumulation of a minimal threshold stimulatory concentration of these autoinducers and alter gene expression, and therefore behavior, in response. Using these signal-response systems, bacteria synchronize particular behavior on a population-wide scale and thus function as multicellular organisms [19].

2.3. Porous monoliths (hydrogels)

Hydrogels, or hydrophilic polymers, were first discovered by Wichterle and Lim in 1960[20]. Since then, there have been significant advancements in hydrogel research. Several types of hydrogels have been widely explored for their synthesis and applications, such as poly (hydroxyethyl methacrylate) (PHEMA), poly (ethylene glycol) (PEG) and derivatives. Porous materials have wide applications in the areas of catalysis, chemical sensing, filtration, separation and so on. They have attracted much attention since they were demonstrated as separation media for high-performance membrane chromatography in 1990 [21]. In particular, hydrogel have been widely studied and used in biomedical applications [22] [23] such as contact lenses [24] and for drug delivery [25]. The recently developed microfluidic technology has further enlarged the application areas of porous polymers for efficient mixing as well as for filtration and separation. In this research, we use three types of hydrogels as a filtering membrane in microfluidic devices.

2.3.1. PEG-DA

Poly (ethylene glycol) diacrylate is one of the derivatives of poly (ethylene glycol) and one of the most promising photosensitive hydrogels with numerous advantages such as good biocompatibility, cell viability and high mass transfer efficiency which make it suitable material in biological applications. Using photolithography, PEG-DA hydrogels can generate precise microstructure pattern.

PEG-DA is selected as the material for the diffusion barrier (filter) because of its low protein adsorption and its UV crosslinking capabilities. As a strongly hydrophilic polymer, PEG-DA is very resistant to protein and has been used to minimize mammalian and bacterial cell adhesion.

The unique capability for precise spatial control of photo crosslinking allow fast, low-cost prototyping to modify microfluidic structures.

2.3.2. HEMA-EDMA

HEMA is hydrophilic in nature due to the presence of hydroxyl groups. Hydrophilicity or hydrophobicity of polymers is important in microfluidic application, as it determines the interaction between the porous polymer and the fluids which will react with it. (HEMA) hydrogel is suitable candidate as a filtering membrane due to its good biocompatibility, photoinitiated polymerization and having different pore size and porosity by changing some parameters like porogen type, temperature and UV intensity [26].

The precursor solution contains monomers, cross linkers, photo-initiator and solvent which is needed to synthesize the hydrogels. porosity and pore size are variable by changing the volume and composition of solvent in the precursor solution.

The preparation of porous polymers is based on free radical polymerization in the presence of a porogenic solvent. UV-light is used to initiate free radical polymerization. Photoinitiated polymerization process is very suitable for microfluidic development, the porous structure can be formed in situ in a defined position of a flow channel by using a photo-mask.

2.3.3. BMA-EDMA

photoinitiated polymerization of butyl methacrylate and ethylene glycol dimethacrylate monomers have open pore size and median pore size 15nm-3 µm. Controlling porosity and pore size is important for filtration applications and it's possible with changing some parameters such as porogenic solvent type and concentration, UV intensity, initiator concentration. The prepolymer solution included Acrylic monomers; butyl methacrylate (BMA) and ethylene glycol dimethacrylate (EDMA), UV sensitive initiator, 2-dimethoxy-2-phenylacetophenone, and porogen solvent, methanol and decanol. The polymerization of BMA/EDMA is as same as Poly (HEMA) hydrogels [27].

Chapter 3. Materials and methods

3.1. Materials

3.1.1 Polydimethylsiloxane (PDMS)

PDMS is a thermally curable elastomeric polymer widely used for the fabrication and prototyping of microfluidic chips. It is a mineral-organic polymer with the structure containing a siloxane backbone (Si-O) and organic methyl groups (CH_3). Apart from microfluidics, it is used as a food additive, in shampoos, and as an anti-foaming agent in beverages or in lubricating oils.

The use of PDMS elastomer for miniaturized bioassays has numerous advantages over silicon and glass. PDMS as a material is inexpensive, flexible, and optically transparent down to 230 nm and therefore compatible with many optical methods for detection). It is compatible with biological studies because it is permeable to water, nontoxic to cells, and permeable to nonpolar gasses like oxygen. A major advantage of PDMS over glass and silicon is the ease with which it can be fabricated and bonded to other surfaces. For the development of bioassays, where many designs may need to be tested, the ease of rapid prototyping in PDMS is a critical advantage.

The polymerization is described by the example of elastomer kit Sylgard 184 from Dow corning Inc: the two components, base polymer and curing agent, are mixed with 10:1 ratio (the mixing ratio depending on the intended Young's modulus of the elastomer which can be tuned between 0.1-10 MPa).

3.1.2. SU-8 photoresist

SU-8 is a negative photoresist mainly used as a structural resist due to its good mechanical and optical characteristics. The amount of resin solvent determines

the viscosity of the material and obtainable layer depth. The thickness of the SU-8 layer is also variable by changing the spinning speed.

Cured SU-8 has excellent chemical and thermal resistance thanks to the high degree of crosslinking. The company "Microchem" is producing several different products with some differences in chemical composition. The number of the resist refers to both the series and the thickness range achievable. In this thesis, for fabrication of mold, SU-8 50 (Microchem – Newton, MA) was used.

3.2. Microfluidic chip design and fabrication

Fabrication of PDMS microfluidic is performed in two steps: SU-8 master mold fabrication using photolithography on a silicon wafer and PDMS replication molding. The design of the microstructures for SU-8 lithography is made in a computer-aided design (AutoCAD) program. The CAD-generated patterns are printed on transparencies and it is then used as a photomask in UV-photolithography to generate a master. Figure 9 shows the overal mask design which include 14 chips with two different pattern shows separately in figures 10 & 11. The width of the anchor (filter) is different in chips ranging from 15-35µm.

Dimensions of the traps (100*100 µm) was selected according to the previous cell culturing experiments. Depth of traps and anchors are 7.5 µm and 4.5 µm respectively. Traps length were around 110 µm with the width 15, 20, 25, and 35 µm in different chips. Channel's dimensions were 250µm wide and 35µm depth.

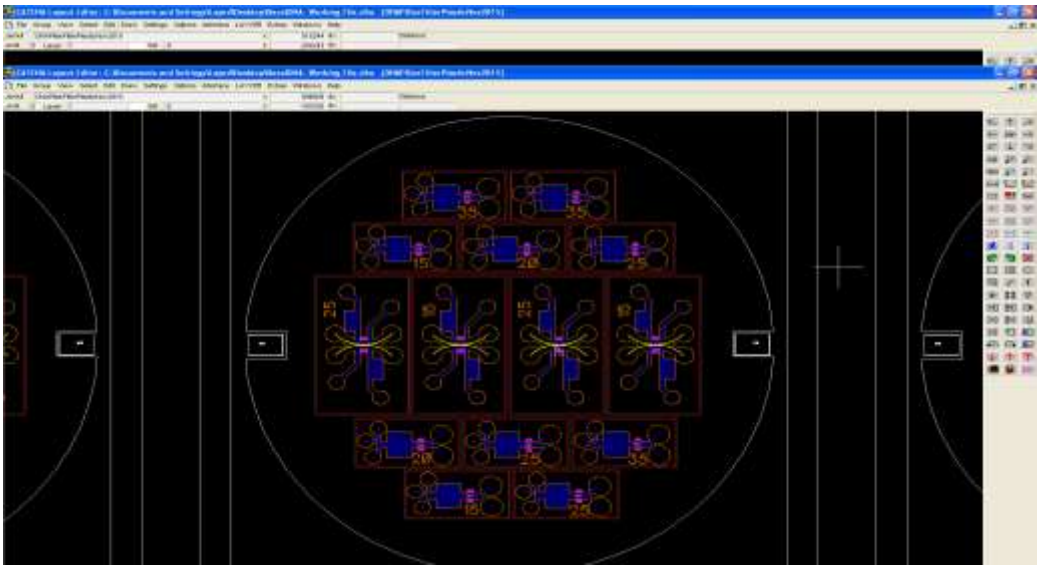


Figure 9. Wafer layout of 14 chips- 4 large chips for 4-way communication experiment and 10 small chips for 2-way communication experiment

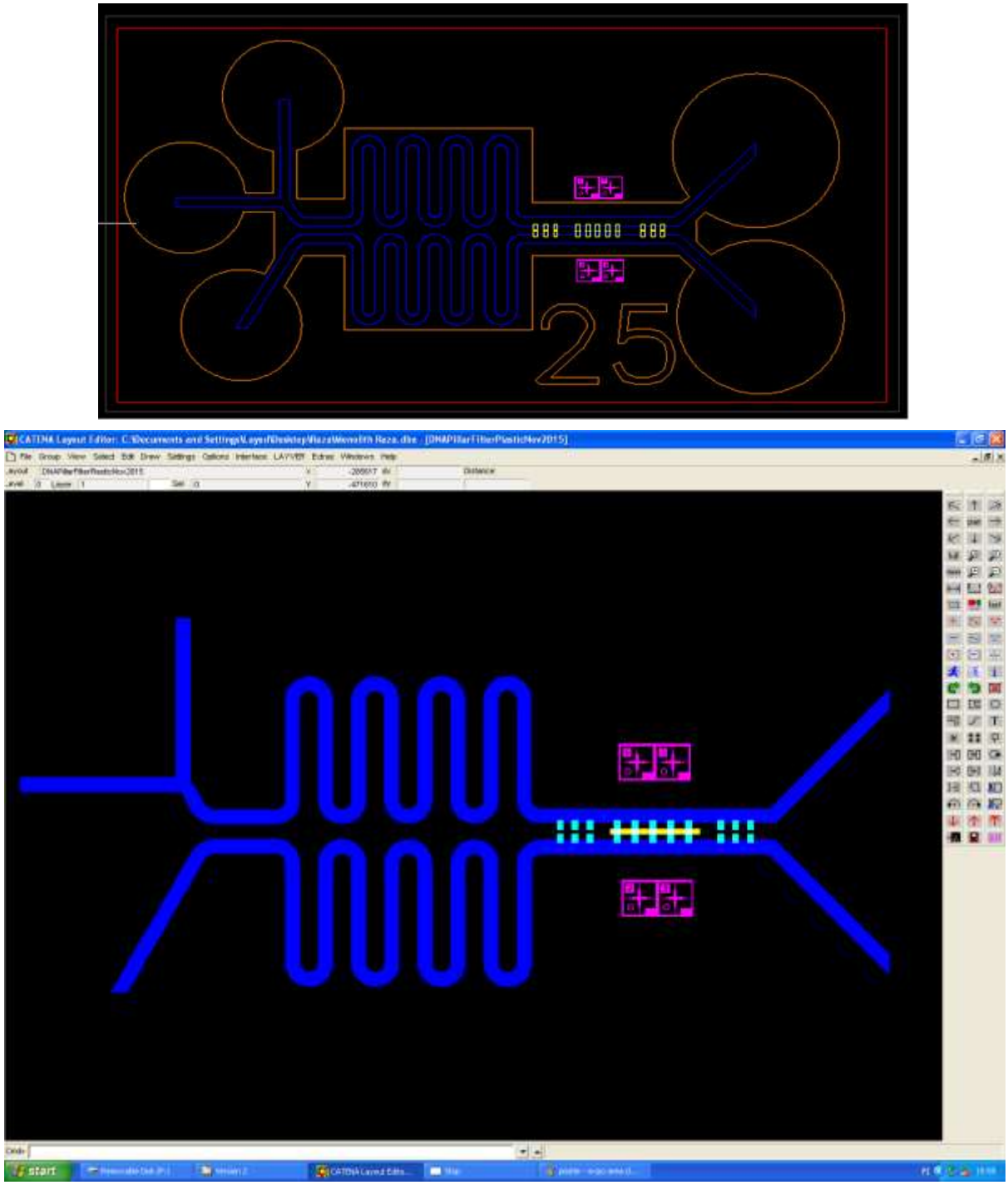
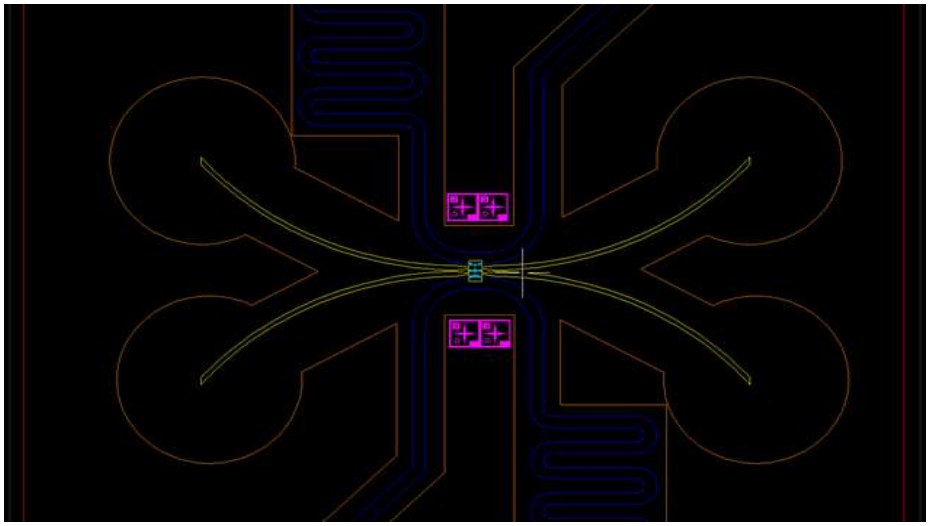


Figure 10. two-way communication microfluidic mask design (Top figure) and hydrogel layout (bottom figure, yellow part)



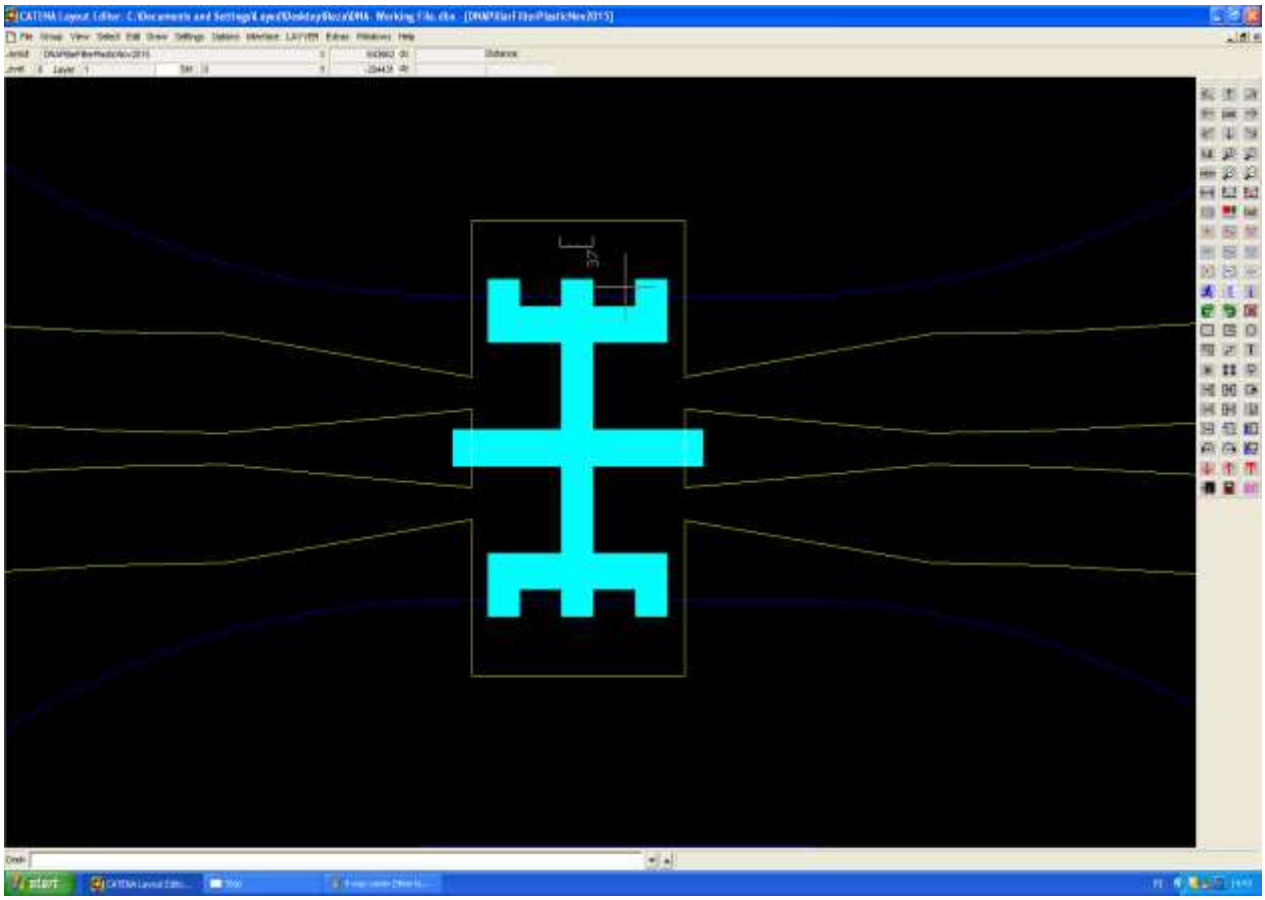


Figure 11. four-way communication microfluidic mask design (Top figure) and hydrogel layout (bottom figure)

The PDMS-GLASS microfluidic design used in this study is depicted in the figure 12. It is comprised of two parallel channels with independent access ports. Cells are captured hydrodynamically in side traps and growth phase will follow. When two different cells types are loaded into adjacent traps from opposite channels, the communication will commence and can be followed via fluorescent signal.

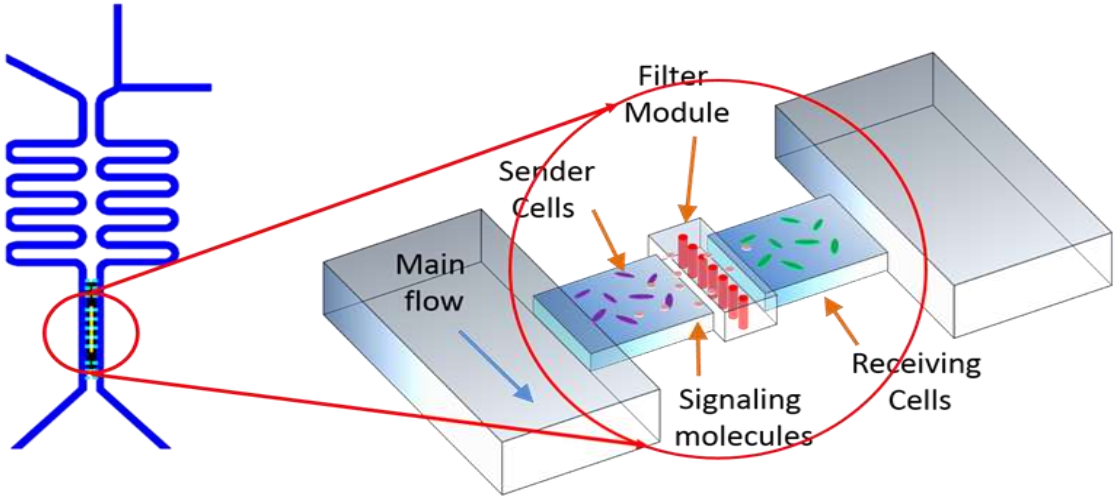


Figure 12. left: Fluidic circuit overall design, right: 3D rendering of three layers PDMS chip and functional components.

3.2.1. Fabrication of SU-8 master mold

To perform PDMS soft lithography, fabrication of a master mold is needed. The most common and used molds are certainly the epoxy resin SU-8 molds.

The fabrication of the master mold involved a silicon-SU-8 process with three layers. The SU-8 layers were spin coated on top of single side polished silicon wafer with a crystal orientation of <100>. Traps and anchors layers were spun by SU8-50 at 4300 and 4000 rpm, sequentially (the spinning speed is decided regarding to the thickness of the layer, higher spinning speed will result in thinner layers). The range of thicknesses achievable is set by the resist composition with more viscous solution resulting in thicker layers.

Each individual layer involving a soft bake and PEB of 90 ºC for 2 min. i-line (365nm) exposure of 3.5 and 4s (lamp power of ~30 mW, MA6/BA6 SÜSS MicroTec, Micronova-Espoo) were used for layers respectively. The channel

layer was created by spinning SU8-50 at 7750 rpm, 5 min at 95 ºC soft bake, exposure of 7.5 sec and PEB of 95 ºC for 5 min. The resulting thicknesses of SU8 layers after development were measured by Dektak profilometer using a vertical stylus, and were found to be 4.5µm, 7.5µm and 35µm respectively for traps, anchors and channel layers. In the last step, a low surface energy film of fluorocarbon polymer (CHF3) was deposited on the master mold using RIE apparatus for ease of cured PDMS peel-off.

3.2.2. Replica molding and assembly of chips

The procedure of producing the PDMS structure from the silicon master, called replica molding, can be carried out under normal laboratory conditions without an expensive clean room, and can replicate certain types of features with dimensions down to 10 nm. By means of a SU-8 mold with desired structure, mass production of PDMS microfluidic chips is possible (i.e. many PDMS replicas can be made from a single master).

Liquid PDMS and crosslinking agent mixed together with the ratio of 10:1 and poured onto the master mold (silicon wafer). Afterwards, it was put into the vacuum system for 30 min to get rid of air bubbles. Then it was cured at 65°C in an oven for 2 hours. Cured PDMS was peeled off from the master, producing the final replica bearing the designed microstructures, then cut off as desired. outlets and inlets are punched using a puncher with the size of 3mm and 1.5mm, respectively. Microchips were cleaned by isopropanol in the ultrasonic cleaner for 30-45 min. after drying by the air, we got the clean microchips. Then the PDMS channels were sealed on glass.

PDMS chips was sonicated in ethanol or isopropyl alcohol for 10-15 min and rinsed with DI water and allowed to dry overnight at room temperature. Cover glasses were cleaned with acetone and isopropyl alcohol respectively and dried with nitrogen gas.

PDMS can seal to glass irreversibly if both surfaces are Si-based materials and have been oxidized by plasma before contact (a process that forms a covalent O-Si-O bond). PDMS chip with the microchannel side on top and cover glass, have been oxidized by microwave plasma machine (Tepla 400). The plasma was under oxygen at 500 mL/min with the power at 60 W for 1 min. Immediately after

removal from the plasma cleaner, the substrates were brought into conformal contact and an irreversible seal formed spontaneously and thus formed an enclosed microchannel. 10-30 min post-bake at 65°C in the oven was used to strengthen and accelerate the covalent bonding.

The fabrication of PDMS microfluidic chips is described briefly in the figure 13.

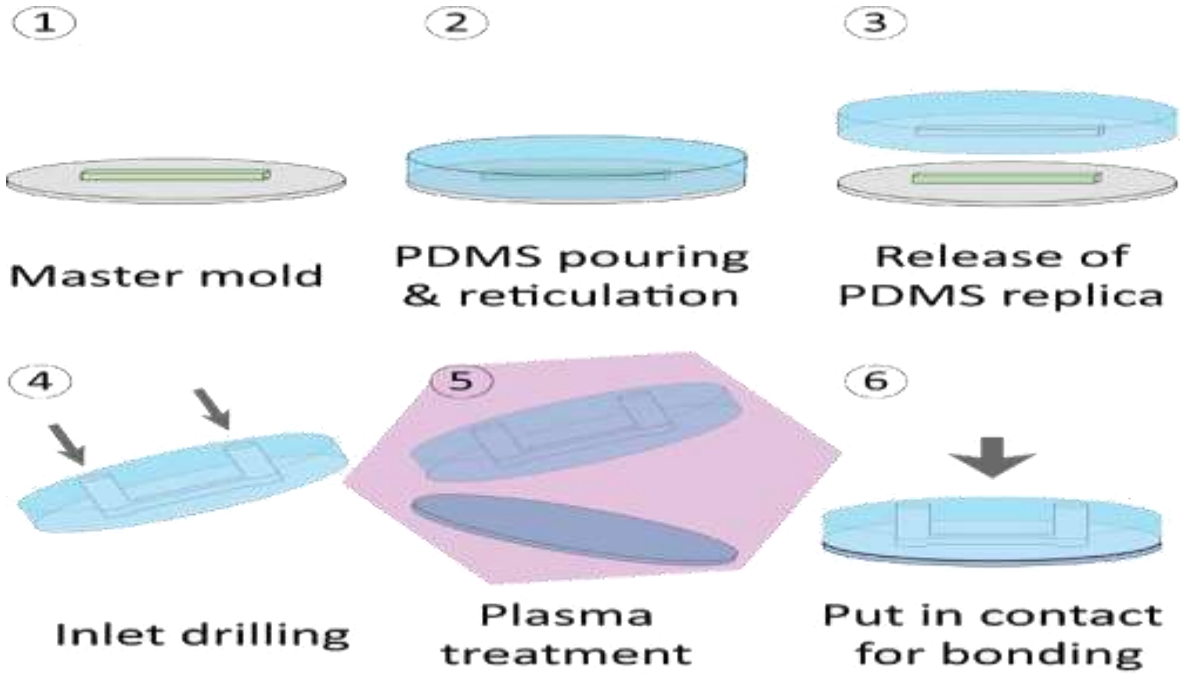


Figure 13. fabrication of a microfluidic chip by soft-lithography methods

3.3. Protocols of making porous monoliths

Porous monolith materials used in this project as a filtering membrane have different protocols to prepare and different UV exposure time nedded for each material to polymerize. Selected membrane materials are biocompatible and photoactive hydrogels including Poly (ethylene glycol) diacrylate (PEG-DA), 2-hydroxyethyl methacrylate-co-ethylene dimethacrylate (HEMA-EDMA), and butyl methacrylate-co-ethylene glycol dimethacrylate (BMA-EDMA). Some of the protocoles repeated from the previous literatures, but some parameters have been modified. In the following all the protocoles describe. All the chemicals were obtained from "Sigma Aldrich Chemical Co" and were used as received.

PEG-DA

The protol of making PEG-DA is based on previous literatures[28] [29] , but some parameters has been changed. Three recipe that we have used is as follows:

1. Polymer solution was prepared by mixing 50% PEG-DA (MW 575, Sigma), 20% deionized water and 30% ethanol; 0.1 % photoinitiator (Irgacure 2959, Sigma) was added into the solution right befor injection into the microfluidic

chip. Ethanol was used to promote PEG-DA dissolution and to decrease solution viscosity.[30] [31]

2. polymer solution consisting 50% (w/w) PEG-DA (MW 575, Sigma), 0.5% (w/w) photoinitiator (2-hydroxy-2-methylpropiophenone) in deionized water.

3. 1% photoinitiator (2,2-dimethoxy-2-phenylacetophenone) based on PEG-DA concentration with 50% PEG-DA solution mixed together and kept it into the oven at 60°C for 15 min, until all DMPA has dissolved.

After all the DMPA has disolved, the solution mixed to make a uniform solution and cooled down to the room temperature. Then 50% deionized water added and stired to become uniform.

UV exposure time needed for polymerization of PEG-DA is 6 sec (Carl-Süss MA-6 mask aligner, 365 nm, 30 mw/Cm^2).

HEMA-EDMA

HEMA-EDMA are used to create micro and nanoporous structures. Regarding to the previous literatures, the pore size of the nanoporous HEMA-EDMA is less than 200 nm which is suitable for our work.

The precursor solution contains the monomer, cross linker and solvent necessary to synthesize the hydrogel. The volume and composition of solvent in the precursor solution influence the structure of the synthesized hydrogel, thus affecting its porosity and average pore size. The precursor solution is mixed according to a method described elsewhere. [32]

The precursor solution used to synthesize Nano-porous HEMA-EDMA is mixed in the following composition:

- 2-hydroxyethyl methacrylate (HEMA, 24% wt)
- Ethylene dimethacrylate (EDMA, 12% wt)
- Cyclohexanol (48%) & 1-decanol (12% wt)
- 2,2-dimethoxy-2-phenylacetophenone (DMPA, 1% wt with respect to monomers)

HEMA–EDMA is cross-linked with UV illumination and in the precursor solution, HEMA and EDMA are the monomers, DMPA is the photo-initiator, while 1-decanol and cyclohexanol are the solvents. It has been shown that changing the ratio of 1-decanol and cyclohexanol leads to different average pore sizes in the hydrogel. The membrane pore size can be modulated to allow or suppress transport of molecules of different sizes.

Polymer solution is exposed for 15 min under a UV lamp (Carl-Süss MA-6 mask aligner, 365 nm, 30 mw/Cm^2).

BMA-EDMA

Polymer solution of BMA-EDMA consisted of monomers, initiators, and progenic solvent. BMA and EDMA used as two types of acrylic monomers. EDMA is a divinyl monomer; it also acted as a cross linking agent to synthesize the cross-linked copolymers of BMA and EDMA. UV-light-sensitive compound of 2-dimethoxy-2-phenylacetophenone was used as an initiator. Methanol was used as a porogenic solvent. All the materials used were obtained from Sigma Aldrich.

The monomers BMA/EDMA with the ratio of 50/50 and 3% initiator were dissolved in 55% Methanol and mixed well in a glass vial to form the polymer solution [33]. The time for polymerization was 15 min at room temperature under a UV lamp which is done by (Carl-Süss MA-6 mask aligner, 365 nm, 30 mw/Cm^2).

3.4. Microstructure photo-patterning of porous monolith within microchannel (Filter fabrication)

Microfluidic channels were filled completely with hydrogel precursor solution. Injection of precursor solution into the chips have been done from both inlets simultaneously to prevent bubble generation in the trap and anchor region (injection is done by means of syringe pump (NE-1600, New Era pump system Inc).

After loading the gel solutions into the chips, UV lithography is utilized to pattern gel structures inside microfluidic cavities. The UV exposure is performed in clean room mask aligner from cover slip side. mask was aligned on top of the glass substrate, and then the solution below the clear areas of the mask (anchors region) were cross-linked by exposure to UV light (Carl-Süss MA-6 mask aligner, 365 nm, 30 mw/Cm^2). After exposure, uncrosslinked species are washed away using a syringe pump. Figure xx shows the UV exposure via the photomask which polymerizes the gel on designated filtering module.

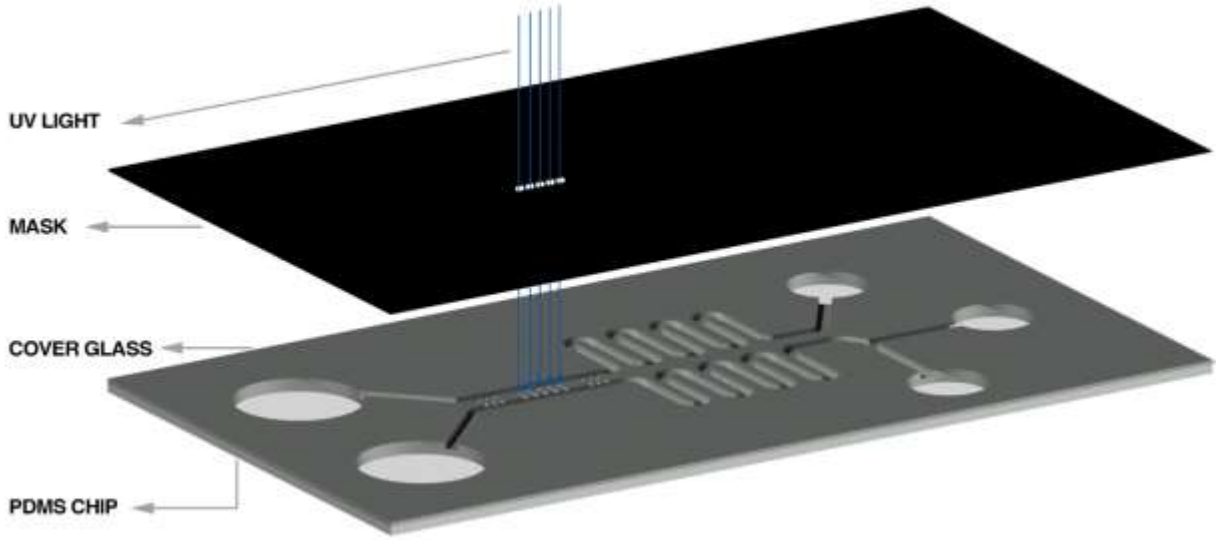


Figure XX. *Figure 12. Exposure arrangement via a dark photomask patterning hydrogel blocks after hydrogel loaded*

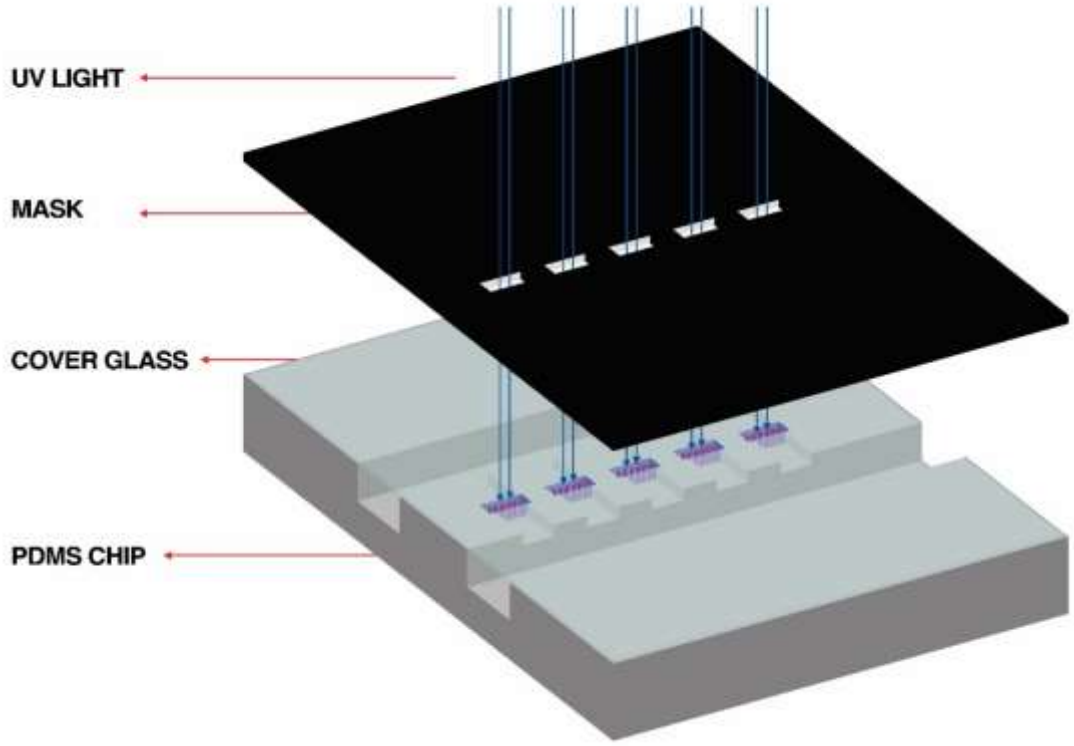


Figure 13. exposure arrangment via a dark photomak (focusing in the filtering area)

The exposure time is different for each type of hydrogel which mentioned separately for each material in protocols section.

some tips should be regarded during exposure:

- Chips should be expose to UV light as soon as they filled with hydrogels
- Chips should be kept in a dark box before and after exposure
- A dummy silicon wafer is used on the stage of mask aligner to create a flat surface for fixing the chips on it during exposure
- After exposure, each chip should be flushed with the solvent which the hydrogel is made with.

Chapter 4. Results and discussion

4.1. PDMS chips fabrication

After fabricating the SU-8 master, the whole microstructure pattern is checked with optical microscope, all the patterns were transferred successfully on the silicon wafer. Then, the resulting thicknesses of SU8 layers after development were measured by Dektak profilometer using a vertical stylus, and were found to be 4.5µm, 7.5µm and 35µm respectively for traps, anchors and channel layers which was ideal for our work.

Microfluidic devices were fabricated in PDMS using standard photolithographic techniques. A 10:1 mixture of PDMS prepolymer and curing agent were stirred and then degassed under vacuum with desiccator to completely remove the bubbles in the solution. The polymer mixture was poured onto the SU-8 master which is placed in glass petri dish and cured 2h at 65°C. After curing, the PDMS replica was peeled from the master, then inlets and outlets were punched. Figure 14 shows the assembled PDMS-GLASS microfluidic chip.

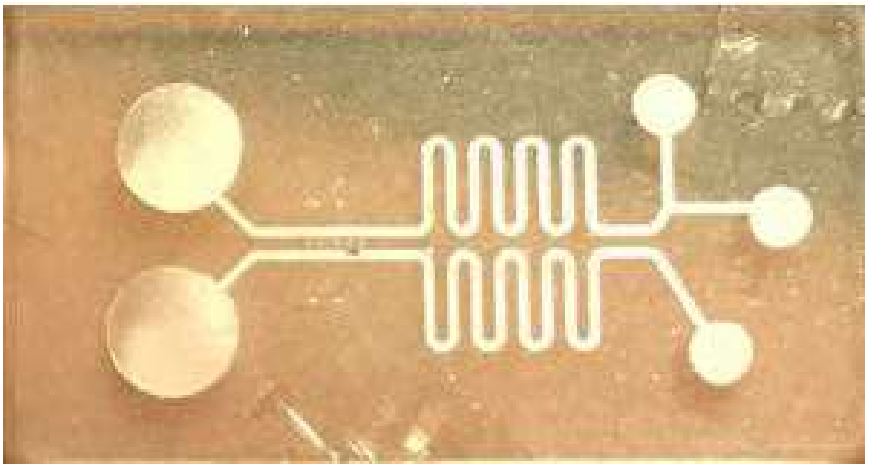


Figure 14. Assembeld PDMS-GLASS microfluidic chip

Some of the chips have been selected randomly and tested by injecting the deionized water with the syringe pump in different range of fluid flow rate to be sure that there was no leakage while pump is run.

4.2. chips problems and solutions

All the basic protocols worked well for fabricating the PDMS-GLASS chips, but some problems appeared in the gel fabrication step. During the UV exposure via the photomask to polymerizing the gel on designated filtering areas, we found misalignment of the mask and chip (set-off) which was related to the shrinkage of PDMS polymer after curing. When PDMS is cured at high temperature, a monomer of PDMS is cross-linked and the total volume is reduced.

Figure 15 shows the set-off during alignment which is around 50 µm. The black scale mark is on photomask and the white one is related to the chip.

Figure 15. The amount of set-off in alignment marks in two oposite sides of the chip, curing parameters: time & temperature: 2 h at 65°C & mixing ratio:1:10

The shrinkage-induced alignment registration problem arises when layers containing dense device arrays are fabricated by multiple methods and stacked. The most frequent example occurs when cast PDMS is peeled from a mold; features on the bulk slab no longer align with those fabricated by spin coating onto a master. It is well known that PDMS shrinks compared to the mold

dimensions, mainly due to curing of PDMS at elevated temperatures. Shrinkage of PDMS during curing arises mainly due to thermal contraction of the PDMS after curing, but polymer may also experience density changes during the curing step due to reconfiguration of the monomers [34].

Several approaches to this problem have been utilized, including modifying the PDMS material [35], curing samples at room temperature [36] and designing with high tolerances to misalignment. Unfortunately, PDMS is not easy to chemically modify. Designing systems with large alignment tolerances limits the potential for miniaturization, may not be desirable in many applications. One group also solved the shrinkage-induced problem of PDMS to some extent, by curing the PDMS while attached to a rigid substrate [37]. But, this method complicates the curing procedure of polymer and introduces more fabrication steps, which is undesirable since the easy fabrication process is one of PDMS's advantages.

The PDMS shrinkage due to curing temperature and mixing ratio of the liquid PDMS has been investigated. The broad range of variations are investigated, curing temperature range spanning from 8°C to 120°C, and several mixing ratios are used. To avoid shrinkage, the curing can be done at room temperature. However, this will increase the curing time from around an hour to more than two days. PDMS with the ratio 1:6 and curing temperature around 21°C has the lowest shrinkage ratio between -0.03±0.04 % [38].

Regarding to the mentioned approaches from previous works, the most convenient and simple strategy to solve the shrinkage-induced problem is the last one which we have utilized in this project. Besides the two parameters (curing temperature and mixing ratio) that we have changed to minimize the shrinkage, the flatness of the PDMS surface also influence the misalignment. So, we balanced the sample before curing to have a flat surface.

The PDMS chips have been fabricated by these strategies did not have shrinkage problems anymore or at least have negligible shrinkage ratio. Figure 16 shows the set-off in alignment marks during UV exposure. As can be seen, the misalignment is reduced to a very low amount with respect to the primary chips, it's around 5µm.

Figure16. The amount of set-off in alignment marks in two oposite sides of the chip, curing parameters:time & temperature: 65 h at 21°C & mixing ratio: 1:6

4.3. Hydrogel (filter) fabrication

Lithography steps for integration of hydrogel membranes inside microfluidic channel is performed in clean room by mask aligner (Carl-Süss MA-6 mask aligner, 365 nm, 30 mw/Cm^2), from cover slip side. After exposure, development of the chips is done by flushing the chips with deionized water (or relevant solvent of the hydrogel) using a syringe pump to wash away the un-crosslinked hydrogels. Porous monolith microstructures remained in anchor regions (blue parts). These micro-structures are in contact with the PDMS and cover glass which is shown in the Figure 17.

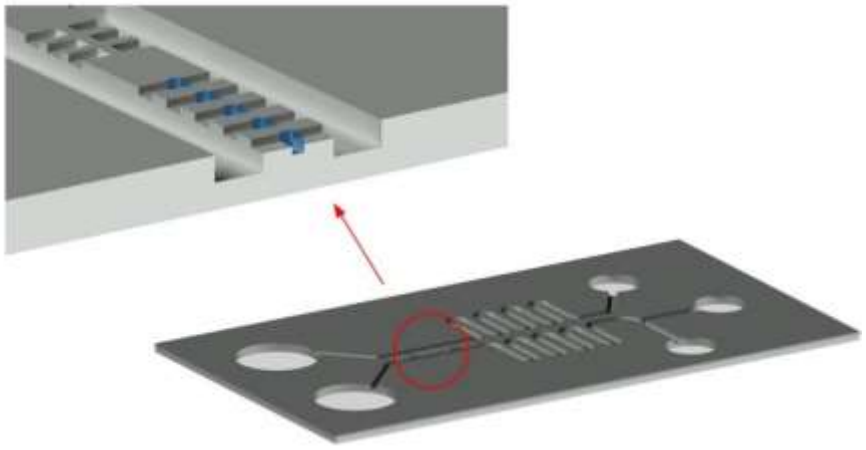


Figure 17. Microstructure filter membrane in the anchor region

4.3.1. Off-chip characterization (microscopic imaging of porous monolith)

Prior to integration of porous monolith materials inside chip, some batches are prepared out of chip to investigate the mechanical properties, elasticity and diffusion properties of these materials.

The effect of UV intensity, photoinitiator concentration and monomer ratios have been investigated and samples with suitable pore size and good mechanical properties are fabricated out of chip.

The morphology of the porous polymers was observed using Scanning Electron Microscopy (SEM, Micronova- Espoo). For the hydrogels, thin layer of gold deposited on top of the samples to render the materials conductive and made it possible to take SEM images. The hydrogel samples are kept in vacuum chamber for some hours before taking SEM images. Because, there are always some solvents left inside the crosslinked hydrogel, which can evaporate under vacuum.

Figure 18 shows the SEM images of nanoporous HEMA-EDMA which indicate the morphology and pore size of the sample.

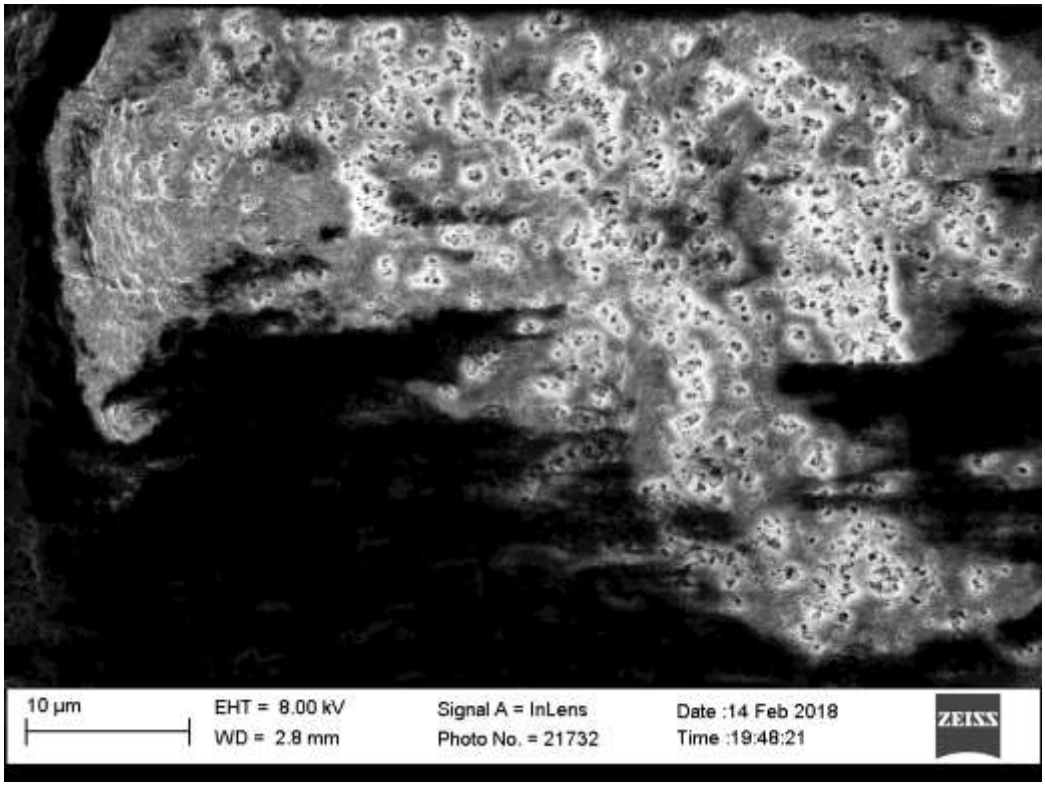


*Figure 18. SEM image of nanoporous HEMA-EDMA. Scale bar **10** µm*

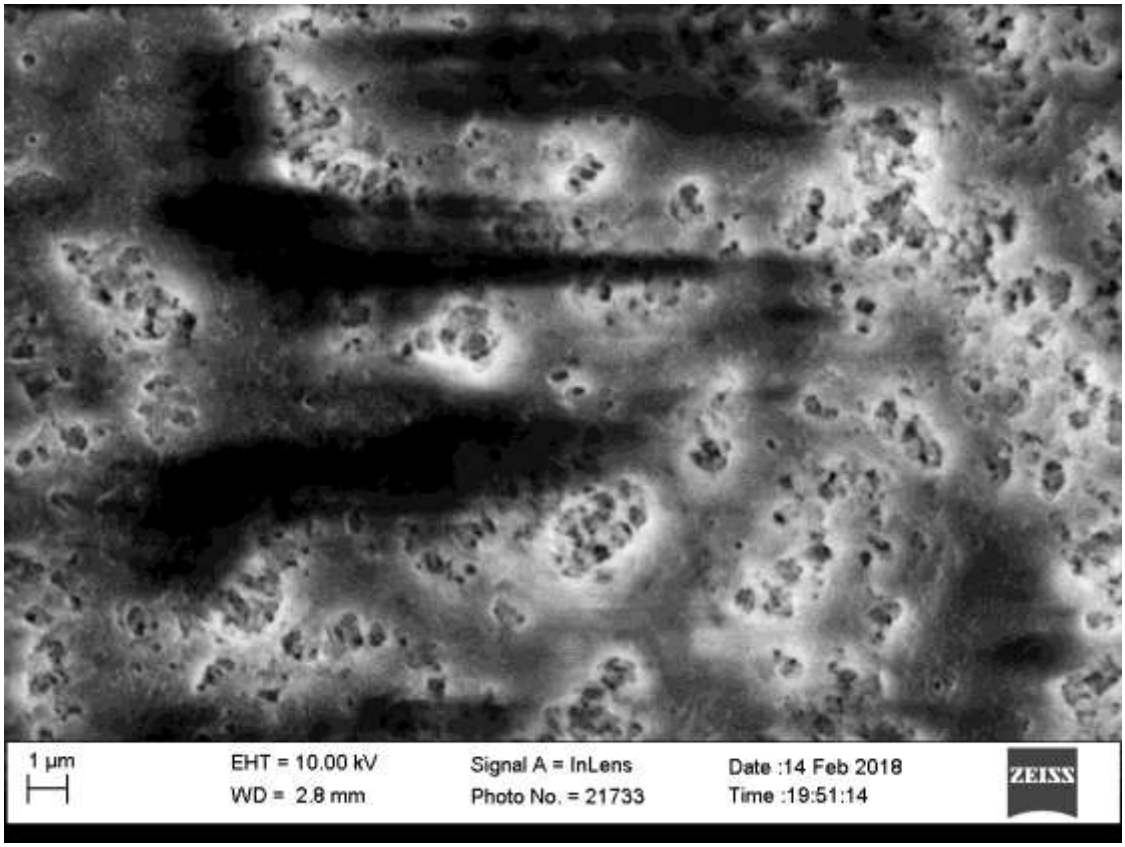


*Figure 19. SEM image of nanoporous HEMA-EDMA. Scale bar **1** µm*

4.3.2. Filtering experiments (with microbeads)

The primary filtering test for verification of immobilized hydrogels in the anchor region have been performed with 2µm polystyrene microbeads (PS). Microbeads with 2µm diameter are used as a substitute for E-coli. After wetting the microfluidic channels and applying the negative pressure to the inlets, microbeads loaded into one side of the channels (one of the outlets) are captured and accumulated inside traps before filtering modules. All the processes have been done, while the microfluidic chip is fixed on the optical microscope stage to have a vision on filtering parts. First experiment in microfluidic chip with PEG-DA as a filtering membrane was not successful and the filtering test has been failed.

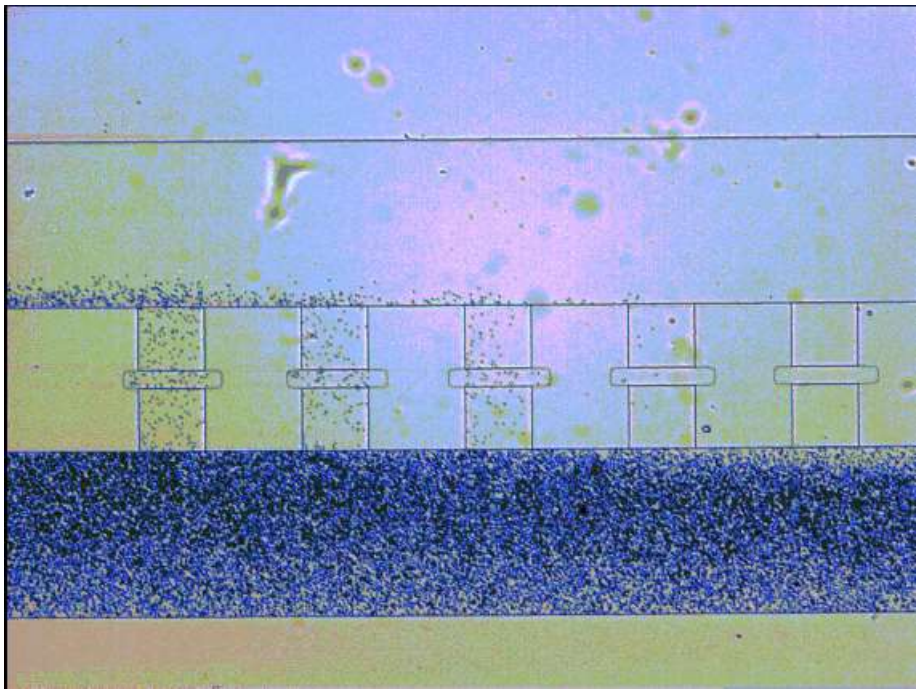


Figure 20. filtering test in microfluidic device integrated with PEG-DA

4.3.3. Filtering issues and solutions

We found that adhesion between the cross-linked hydrogel to the interior surfaces of glass and PDMS was not sufficient to prevent delamination from surfaces and movement within the channel when pressure of fluid was applied.

The hydrogel does not naturally covalent to the surface of glass and PDMS covalently. Thus, during the shrinkage and swelling associated with hydration, hydrogel microstructures can easily detach from the PDMS and glass surfaces. To solve this problem, both the glass and PDMS interior channel surfaces are functionalized with the coupling agent (methacrylate groups) in order to covalently attach hydrogels to the inner surfaces of the microfluidic device. Methacrylate groups can be covalently bonded with hydrogels (PEG-DA, HEMA-EDMA, and BMA-EDMA), if hydrogel is photo-crosslinked on the treated surfaces.

4.3.3.1. Surface functionalization

In order to increase the adhesion and stability of the filtering membranes a silanization step was added prior to gel injection.

Functionalization of device inner surfaces was carried out using two types of surface silanization solutions which extracted from previous literatures [39]; 3-(trimethoxysilyl) propyl methacrylate (TMSPMA, Sigma Aldrich) and 3-(trichlorosilyl) propyl methacrylate (TPM, Sigma Aldrich).

Immediately after PDMS/GLASS bonding, a surface silanization solution was injected into the microfluidic chip and allowed to sit for 1 hour at room temperature. TMSPMA was diluted in isopropyl alcohol at 1:20 (v/v), and 3% acetic acid was added to the solution immediately before surface treatment. 0.1% TPM was diluted in isopropyl alcohol and 0.1% acetic acid. After incubation, microfluidic channels were rinsed with isopropyl alcohol and then rinsed with water. Microfluidic channels were then emptied by vacuum and allowed to dry for some hours at room temperature.

4.3.3.2. Micro-pillar array

Besides silanization of the inner surfaces of the device, another approach to improve the adhesion and stability of the hydrogels in anchor regions is to fabricate Miro-pillar array structures in anchor region to increase the surface area of PDMS surface which secure bonding with hydrogel compared to the flat PDMS.The final layout of the chip is shown in the figure 21.

Before UV patterning of hydrogel membranes, the microfluidic chips with silanized channel surfaces were first placed in a vacuum chamber for 1 hour. Hydrogel precursor solution was immediately injected to the chip upon removal of the device from vacuum chamber. Then, the same process as before for the UV exposure was done.

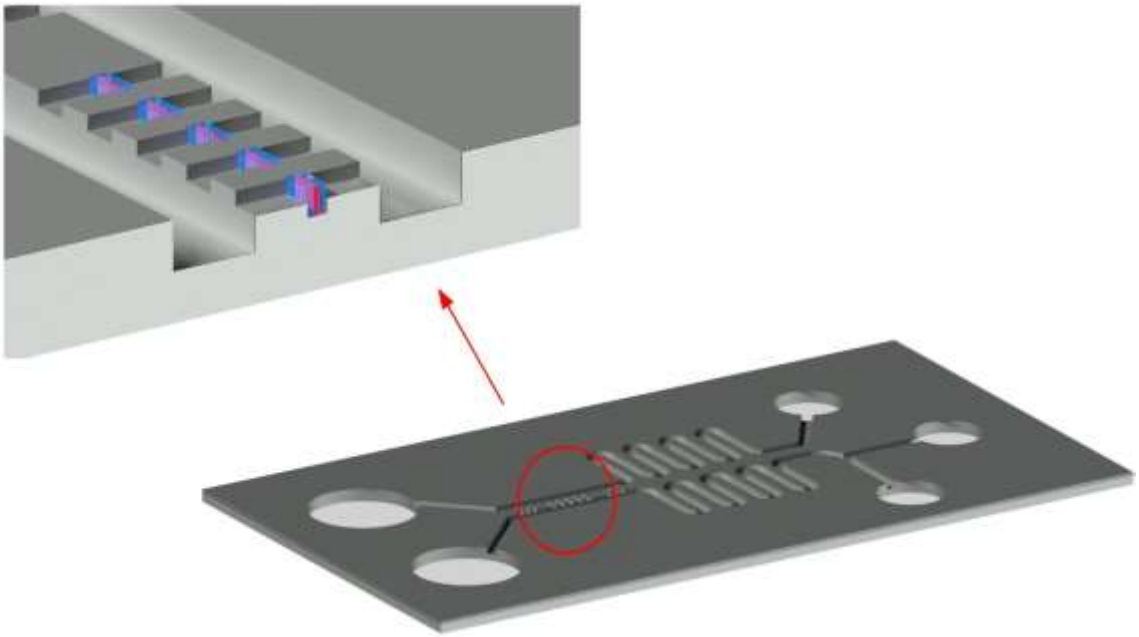


Figure 21. final layout of the microfluidic chip with Micro-pillar array in the anchor region

4.3.4. Filtering experiment 2 (microbeads)

The filtering efficiency tests for the new chips have been successfully performed with 2 µm beads at the flow rate of 0.2µl/min which is shown in figure 22. Monolith were stable during prolonged experiments with constant supply of flow. Regarding to the experiment results, both PEG-DA and HEMA-EDMA monoliths formed stable membranes with PEG-DA exhibiting more volumetric shrinkage after polymerization.

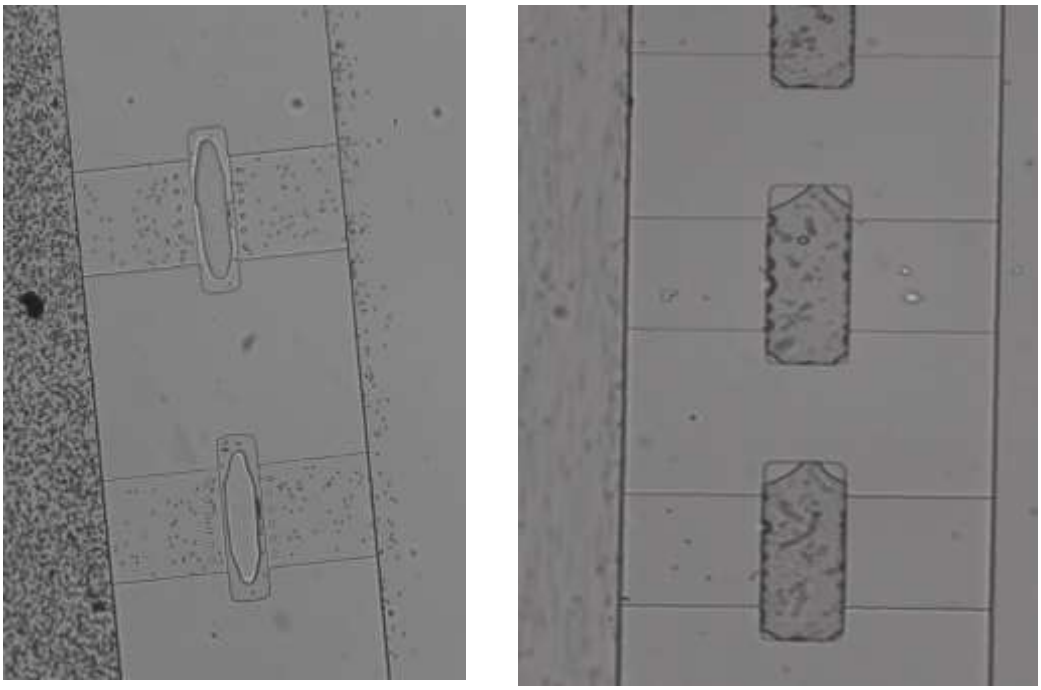


Figure 22. optical micrograph of filter membranes. Flow rate of 0.2 µl/min and 2µm polystyrene microbeads simulating E-coli cells. Left: PEG-DA, right: HEMA-EDMA

5. Conclusions & Future plans

In this work, we developed a microfluidic device with integrated porous monoliths filters for cell-cell communication studies at colonial level. Physical barrier isolated bacteria for long experimental times, while retaining chemical communications for transport of distinct signaling molecules necessary for communication.

The primary filtering efficiency tests have been successfully performed with 2μm Polystyrene microbeads. In comparison with other methods of Nano-filter fabrication such as direct 3D printing in channels with two photon polymerization technology with submicron resolution, our method renders faster and more cost effective prototyping cycles.

In the future, substituting some porous monoliths material with lower volumetric shrinkage rate may lead to have more stable filter for longer time experiments. Oxygen inhibits the crosslinking reaction at the PDMS boundary, creating a thin layer of un-crosslinked prepolymer between the hydrogel structures and the PDMS walls. So, another factor that should investigate is the polymerization of hydrogel in an inert environment.

Our future work will be development of the bacterial communication tests and culturing of cells in the fabricated microfluidics to look into inter-species chemical communications.

Chapter 6. References

[1]. E.K. Sackmann, A.L. Fulton, D.J. Beebe, The present and future role of microfluidics in biomedical research, Nature, 507 (2014) 181-189.

[2]. Eric K. Sackmann1, Anna L. Fulton2 & David J. Beebe3, The present and future role of microfluidics in biomedical research

[3]. Wei-Cheng Tian & Erin Finehout, Microfluidics for Biological Applications

[4]. P. O. Krutzik and G. P. Nolan, Nat. Methods, 2006, 3,361–368.

[5]. J. R. Rettig and A. Folch, Anal. Chem., 2005, 77, 5628–5634.

[6]. Hojin Kim,a Sanghyun Lee,a Jae-hyung Leeb and Joonwon Kim*a, Integration of a microfluidic chip with a sizebased cell bandpass filter for reliable isolation of single cells†

[7]. H. Kim, S. Lee and J. Kim, Microfluid. Nanofluid., 2012, 13, 835–844.

[8]. Ali Asgar S. Bhagat • Hansen Bow • Han Wei Hou • Swee Jin Tan • Jongyoon Han • Chwee Teck Lim, Microfluidics for cell separation

[9]. Kimura T, Sato Y, Kimura F, Iwasaka M, Ueno S (2005) Langmuir 21:830–832

[10]. Thiel A, Scheffold A, Radbruch A (1998) Immunomagnetic cell sorting—pushing the limits. Immunotechnology 4:89–96

[11]. Vahey MD, Voldman J (2008) An equilibrium method for continuous-flow cell sorting using dielectrophoresis. Anal Chem 80:3135–3143

[12]. Laurell T, Petersson F, Nilsson A (2007) Chip integrated strategies for acoustic separation and manipulation of cells and particles. Chem Soc Rev 36:492–506

[13]. Petersson F, Aberg L, Sward-Nilsson A, Laurell T (2007) Free flow acoustophoresis: microfluidic-based mode of particle and cell separation. Anal Chem 79:5117–5123

[14]. Ji H, Samper V, Chen Y, Heng C, Lim T, Yobas L (2008) Silicon-based microfilters for whole blood cell separation. Biomed Microdevices 10:251–257

[15]. Yamada M, Nakashima M, Seki M (2004) Pinched flow fractionation: continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel. Anal Chem 76:5465–5471

[16]. Shevkoplyas SS, Yoshida T, Munn LL, Bitensky MW (2005) Biomimetic autoseparation of leukocytes from whole blood in a microfluidic device. Anal Chem 77:933–937

[17]. Yang S, Undar A, Zahn JD (2006) A microfluidic device for continuous, real time blood plasma separation. Lab Chip 6:871

[18]. Christopher M.Waters and Bonnie L. Bassler, Quorum Sensing: Cell-to-Cell Communication in Bacteria

[19]. Bonnie L. Bassler1, Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

[20]. Wichterle **0,** Lim **D,** Hydrophilic gels in biologic use, *Nature.* **185:117-118, 1960**

[21]. Tennikova TB, Svec F and Belenkii BG, J Liquid chromatogr 13:63-70 (1990).

[22]. Hoffman **A.** Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews.* 64: **18-23,** 2012.

[23]. Seliktar **D.** Designing cell-compatible hydrogels for biomedical applications. *Science.* **336:1124-1128,2012.**

*[24]. Nicolson **PC,** Vogt **J.** Soft contact lens polymer: an evolution. Biomaterials. **22(24):3273-3283,** 2001.*

*[25]. Langer R, Peppas **NA.** Advances in Biomaterials, Drug Delivery, and Bionanotechnology. AIChE Journal. 49(12): **2990-3006, 2003.***

[26]. Suzhu Yu Khin Cho Cho Ma Aye Aye Mon Fern Lan Ng Yen Yong Ng, Controling porous properties of polymer monoliths synthesized by photoinitiated polymerization.

[27]. Suzhu Yu, Feng Lin Ng, Khin Cho Cho Ma, Fern Lan Ng, Jianhong Zhao, Steven Kin Kong Tong, Development of Porous Polymer Monolith by Photoinitiated Polymerization.

[28]. Jinseok Heo, K. Joseph Thomas, Gi Hun Seong, and Richard M. Crooks, A Microfluidic Bioreactor Based on Hydrogel-Entrapped E. coli: Cell Viability, Lysis, and Intracellular Enzyme Reactions.*

[29]. Hui Xu, Meghaan M. Ferreira and Sarah C. Heilshorn, Small-molecule axon-polarization studies enabled by a shear-free microfluidic gradient generator.

[30]. D. C.-W. Tan, L.-Y. L. Yung and P. Roy, Biomed. Microdevices, 2010, 12, 523–532.

[31]. 2011 IEEE/RSJ International Conference on Intelligent Robots and Systems, September 25–30, 2011, San Francisco, CA, T. Yue, M. Nakajima, M. Ito, M. Kojima and T. Fukuda, 2011, pp. 433–438.

*[32]. Zhifei Ge,a Peter R. Girguisb and Cullen R. Buie*a, Nanoporous microscale microbial incubators.*

[33]. Suzhu Yu, Feng Lin Ng, Khin Cho Cho Ma, Fern Lan Ng, Jianhong Zhao, Steven Kin Kong Tong

[34]. H. Ishida and H. Y. Low, "A Study on the Volumetric Expansion of Benzoxazine-Based Phenolic Resin," Macromolecules, vol. 30, no. 4, pp. 1099-1106, 1997.

[35]. Choi K M and Rogers J A 2003 A photocurable poly(dimethylsiloxane) chemistry designed for soft lithographic molding and printing in the nanometer regime J. Am. Chem. Soc.

[36]. Wu H K, Odom T W, Chiu D T and Whitesides G M 2003 Fabrication of complex three-dimensional microchannel systems in PDMS *J. Am. Chem. Soc.*

[37]. C. Moraes, Y. Sun and C. a. Simmons, "Solving the shrinkage-induced PDMS alignment registration issue in multilayer soft lithography," *Journal of Micromechanics and Microengineering,* vol. 19, no. 6, p. 065015, 2009.

[38]. Morten Hannibal Madsen[1,3], Nikolaj A Feidenhans'l[1,2,3], Poul-Erik Hansen[1], Jørgen Garnæs[1] and Kai Dirscherl[1], Accounting for PDMS shrinkage when replicating structures.