

**DESIGN AND FABRICATION OF MICROFLUIDIC
DEVICE THAT ALLOWS INVESTIGATION OF
DISTANCE DEPENDENT INTERACTIONS OF
TWO DIFFERENT CELL TYPES**

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**by
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ABSTRACT

DESIGN AND FABRICATION OF MICROFLUIDIC DEVICE THAT ALLOWS INVESTIGATION OF DISTANCE DEPENDENT INTERACTIONS OF TWO DIFFERENT CELL TYPES

The main studies of in this thesis, the mold and a microfluidic device are achieved by using SU-8 photoresist and PDMS polymer. Firstly, molds are obtained which are thickness ranging from 30 to 400 μm by using SU-8 photoresist with UV lithography technique and this molding will use for shaping polydimethylsiloxane (PDMS) polymer. Finally, PDMS molds combined with the glass surface to create a three dimensional reservoirs.

Microfluidic device that allows investigation of distance dependent interactions, two factors are positioned at certain distances from each other and the microfluidic device is allowed to investigation of distance dependent interaction of two factors. There is an alternating width channel between two channels which have each of two factors. These three channels are separated from each other by colonnades, not by walls, therefore physical, chemical and biological interactions are possible between the factors. Necessary physical, chemical, and biological conditioning can be provided by the reservoirs which are neighbor of channels including factors.

Microfluidic chip has a lot of advantages that are small liquid volumes (pL- μL), precise spatial & temporal control, successfully mimic the physiological context, high-throughput analysis, low fabrication costs; portable and safer therefore it facilitates us to refine our methods of analysis and development in cell biology investigations and determining the content of chemical samples.

ÖZET

İKİ FARKLI HÜCRE ÇEŞİDİNİN BİRBİRLERİNE UZAKLIKLARINA BAĞLI ETKİLEŞİMLERİNİN İNCELENMESİNE İMKAN VEREN MİKROAKIŞKAN CİHAZIN TASARIMI VE ÜRETİMİ

Tez kapsamında yapılacak temel çalışmalar, kalıp oluşturmak ve polimer kullanarak mikroakışkan çip elde etmek. Öncelikle UV litografi tekniği ile SU-8 fotoresisti kullanılarak kalınlıkları 30-400µm arasında değişen farklı kalıplar oluşturmak ve bu kalıpları polidimetilsiloksan (PDMS) polimerinin şekillendirilmesi için kullanmak. Sonrasında PDMS kalıplar cam yüzey ile birleştirilerek üç boyutlu haznelere yaratılacak.

Uzaklığa bağlı etkileşimlerin incelenmesini sağlayan mikroakışkan cihazda, iki etken birbirlerine belirli uzaklıklarda konumlandırılarak, aralarındaki uzaklığa bağlı etkileşimlerin incelenmesi sağlanır. Herbir etkenin bulunduğu iki kanal arasında genişliği değişen bir kanal mevcuttur. Bu üç kanal birbirlerinden duvarlarla değil sütun dizileriyle ayrıldıkları için etkenler arası fiziksel, kimyasal ve biyolojik iletişim mümkündür. Etkenlerin bulunduğu kanallara komşu haznelere ile gerekli fiziksel, kimyasal ve biyolojik koşullandırmalar sağlanabilir.

Mikroakışkan çip, yapılması gereken kimyasal ya da biyolojik ve benzeri analizlerde zamansal kontrol, az malzeme kullanımı, az atık oluşumu, taşınabilirlik, çok çıktılı inceleme, düşük maliyet ve fizyolojik ortamı taklit etme gibi özellikleri sebebiyle hücre biyolojisi araştırmalarında ve kimyasal örneklerin içeriğini belirlemede, analiz yöntemlerini hassaslaştırmamızı ve geliştirmemizi kolaylaştırıyor.

To my family...

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CHAPTER 1

INTRODUCTION

1.1. Microfluidic

The study of fluidics at the microscale for applications is defined as microfluidic. Those applications are cell culture, drug delivery and sample handling (Westwood, et al. 2007). As it is written above, microfluidics specialized for the applications at the microscale; so that these devices are manufactured with features in micrometer scale that can process fluids with volumes in nanolitres. By using conventional microelectronics processes which are etching, deposition and photolithography are used on silicon or glass substrates to obtain microfluidics. On the other hand, polymers are chosen as the material for microfluidics because of their some properties such as their costs, transparency, biocompatibility and ease of fabrication (Belanger 2001). Silicon is patterned through photolithography and etching. However, polymers can be patterned not only through photolithography and etching, but also through development and simple micromoulding (Xia and Whitesides 1998). As a result, silicon and polymers were easily used in the fabrication technologies. SU-8 and polydimethylsiloxane (PDMS) are examples of popular polymer materials for fabrication of microfluidics.

The main advantages of microfluidics;

- ✓ Small liquid volumes (pL- μ L)
- ✓ Precise spatial & temporal control
- ✓ Successfully mimic the physiological context
- ✓ High-throughput analysis
- ✓ Low fabrication costs
- ✓ Portable
- ✓ Safer

1.2. Photolithography

Photolithography, also termed optical lithography or UV lithography, basically consists in transferring geometric shapes from an optical mask to the surface of a silicon wafer by using UV light. The term Photolithography is a composed name that derives from the Greek words “photo” (light), “lithos” (stone) and “graphein” (to write).

The term photolithography can be broken into two components; photo and lithography. The photo component is derived from the use of light to pattern the desired features for the integrated circuit. Lithography was first invented in 1798 by Alois Senefelder when he discovered that he could pattern images on stone using a greasy mixture, water and ink. The first example of lithography is not radically different than photolithographic techniques used in the manufacturing of integrated circuits today. (Meggs 1983).

Photolithography was first invented in 1855 by Alphonse Louis Poitevin, when he discovered that if a solution of potassium dichromate and albumin is let to dry on a lithographic stone and then exposed under a photographic negative, the parts exposed to light become insoluble and ink only adhere to those parts. Photolithography shares some fundamental principles with photography in that the pattern is created in the resist layer by exposing it to light, either directly or indirectly with a projected image using an optical mask. Subsequent stages in the process have more in common with etching processes than to lithographic printing (Parr 1998).

1.3. Soft Lithography

Soft lithography was first introduced by Whitesides, et al. This technique refers to a family of techniques using a patterned elastomer as the stamp, mold, or mask to generate micropatterns and microstructures. The term “soft” comes from the usage of the flexible organic molecules and materials for a replica such as PDMS (Zhao, et al. 1997)

Soft lithography is one of the primary fabrication techniques to obtain microfluidic devices. This technique includes replication of structural properties on a rigid master mold in a silicone substrate such as PMMA (polymethylmethacrylate) or PDMS (polydimethylsiloxane).

The soft lithography method has the advantages of low cost, easy of learning, straight forward applying, and accessibility to a wide range of users (Whitesides 1998). Soft lithography includes various microfabrication methods using a patterned master: micro-contact printing (μ CP) (Kumar 1993), replica molding (REM) (Xia et al. 1996), micro-transfer molding (μ TM) (Zhao 1996), micro-molding in capillaries (MIMIC), solvent-assisted micro-molding (Kim 1995), cast molding (Haverkorn, et al. 1982), embossing (Lehmann et al. 1983), and injection molding (Hoyer 1996).

There are several advantages of soft lithography. One of the advantages is that the clean room is not required for soft lithography. In addition to this, the soft lithography method can be applied in any laboratory without the need for expensive equipment. Structures width size ranges as small as a few microns can be fabricated with good quality. Microfluidic devices can be patterned flat or rough surfaces and they are easy to release from the mold without causing any damage to the device or master. Additionally, a large number of devices can be made from a single master depending on the size of the master and devices.

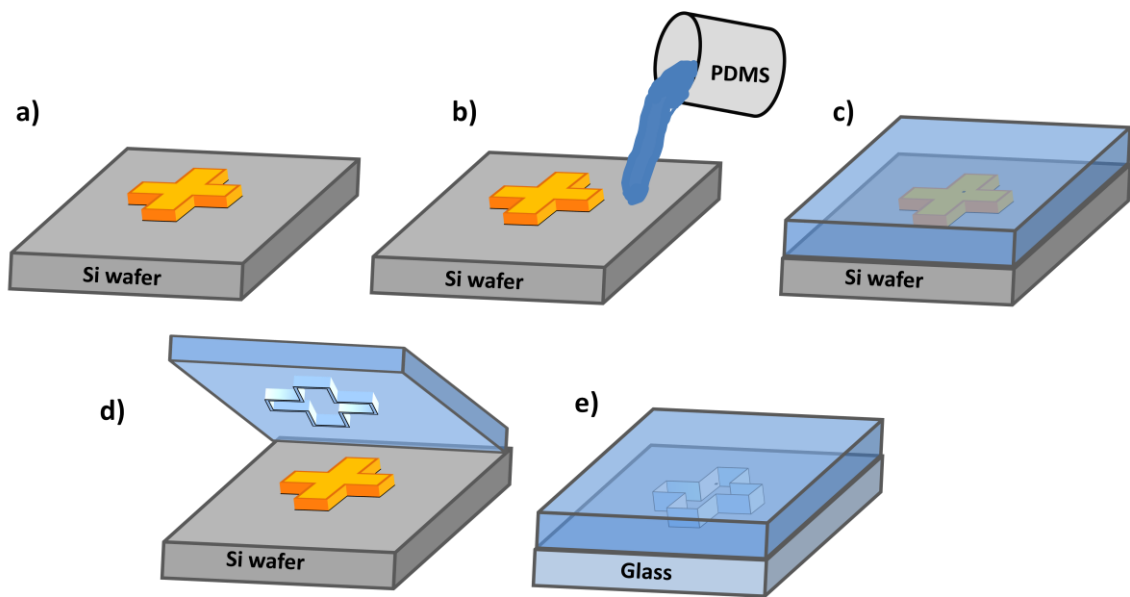


Figure 1.1. Overview of Soft Lithography.

Schematic diagram of a PDMS micro-device process: (a) a master with desired pattern, (b) pouring PDMS, (c) The PDMS are waited on the bench (at RT) for

polymerization, (d) peeling off PDMS replica, and (e) bonding with glass substrate after surface treatment for adhesion.

1.4. Mask Design

Initiation of soft lithography begins with printing design on a mask which include opaque and transparent areas are attuned to patterns. In order to obtain main production these patterns are passed on the photoresist. SU-8 is chosen as photoresist which have negative photoresist properties that means as UV light is exposed to the negative photoresist, cross links are occurred and this cause solidifying the substance which provides to decreasing the engraving ratio when it is compared to non-cross-linked substance. For that reason, transparent parts of mask on photoresist will not dissociate and this provide to channel forming on the pattern

1.4.1. Designing the Layout

Making any microfluidic apparatus design is feasible with very low costs thanks to Corel Draw program usage. Design is executed and then it is impressed on a mask. When printing is applied, both laser printer with using transparent medium and electron beam source can be performed on mask directly.

In soft lithography technique, mainly two different mask usage is possible which are chrome masks and laser printed transparency masks. Usage of chrome masks provide to high resolution and small microfluidic channel design as size which is between 10 μm to 500 nm. It is important that printed mask resolution affect the channel size of main production, in briefly channel length and widths are affected from only printed mask. On the other hand, depths of channel structure can be changed through spin of the covered thickness of photoresist.

1.4.1.1. Chrome Mask

The using of a chrome mask can be necessary for specific applications, such as sub-micrometer to a few micrometers or the manufacturing of small rectangular posts for enhancing turbulent mixing in high Reynolds number flow (Nguyen 2005),

produced mini rectangular posts can be used. It is necessary that adhesion of plating and growing cells or structural strengthening, as points of adhesion for seeding and growing cells (Tan, et al. 2004). Chrome mask has high costs and difficult fabrication process.

1.4.1.2. Laser Printed Transparency Mask

Usage of mercantile or “off the shelf” laser printers are widespread method to manufactured masks in soft lithography implementations. Monochrome laser printer is used when designed masks have been printed on normal transparency film. Limited factor of laser printers is the dpi that stands for dots per inch realizable by the printer. 20,000 dpi as resolution can be obtained with the help of commercial laser plotters which supplies about 12.5 μm as the least feature extent. By reason of beginning and continuance costs of printer, generally the design is outsourced.

Lower resolution printers can be used bound up with execution. However, these printers must be applied carefully. For instance, with 2400 dpi resolution, transparency mask may be printed by using a laser printer and these may be used to fabricate constructions for becoming them greater than a width of nearly 500 μm but as the needed property sizes (i.e. channel width) is reducing. Laser printer's dust particles are becoming comparatively great when matched with channel width. In the extraordinary situation, when the constructions are at a scale called resolvable by printer, specific parts of the channel will be banned.

1.5. SI Wafer Substrate

SI Wafer Substrate is regularly used in the electronics fabrication industry, where surface smoothness and flatness is tightly controlled. Consequence of this, the surface roughness is very small and the substrate is uniform. Due to the fact that wafers and SU-8 photoresist can easily adhesion each others. In addition, no necessary cleaning the wafers, because silicon wafers are fabricated directly for clean room facility use. Despite these advantages, the main disadvantage is that SI wafer substrates are more expensive than glass substrates. In addition, silicon wafers have larger surface areas. Thus, more photoresist is required for spincoating.

1.6. Spin Coating

The SI wafer and the SU-8 photoresist are used in the spincoating process. Photoresist is a toxic substance, and should be handled with care. In the spincoating process, the spincoater plays a very critical role to achieve the desired thickness and quality of the coating. Thus, this section discusses spincoater parameters and SU-8 properties.

1.6.1. Spincoater Parameters

The thickness of photoresist is determined by three main parameters: viscosity depends on type of SU-8, spin time (ramp-dwell) and spin speed (revolution per minutes-rpm). The grade of SU-8 depends on viscosity. Such as, SU-8 2002 is more viscous than others. That's why; a higher SU-8 grade number corresponds to a lower solvent content. Spin time; ramp is reach time and dwell is wait time between two spin speed. Lower spin speeds and shorter spin times also created thicker photoresists films.

Once a desired thickness is chosen, a grade of SU-8 must be chosen according to the desired thickness. In general, the grades of SU-8 photoresist are depending on their viscosity. In addition to this, spin speeds between 1000 rpm and 3000 rpm are ideal for every SU-8 types, when spin speed is slower than 1000 rpm, there will be uniformity problems (Figure 1.2 and Figure 1.3).

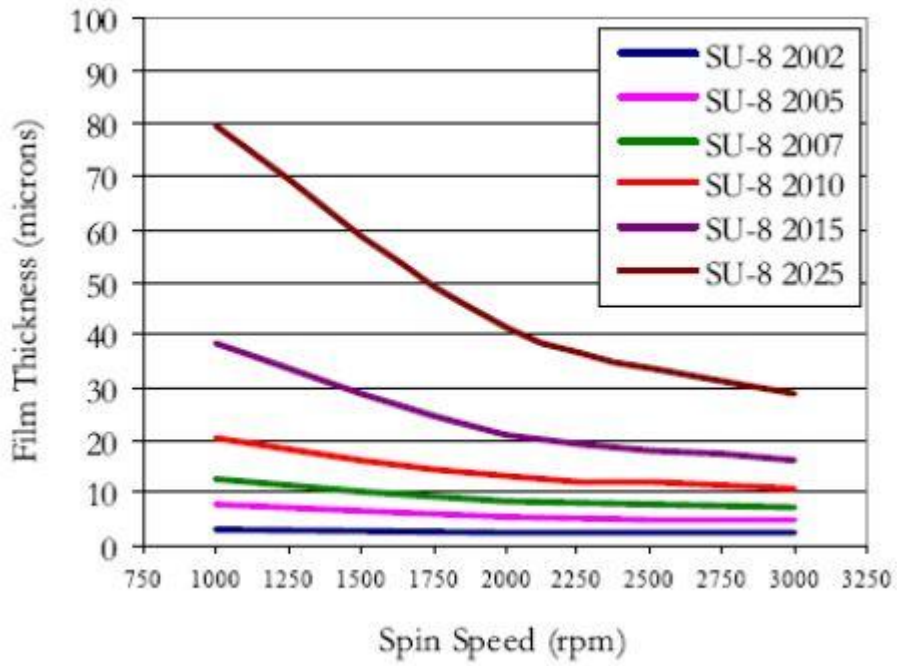


Figure 1.2. Graph of Film Thickness versus Spin Speed for SU-8 2000 (Source: MicroChem, Inc., 2001).

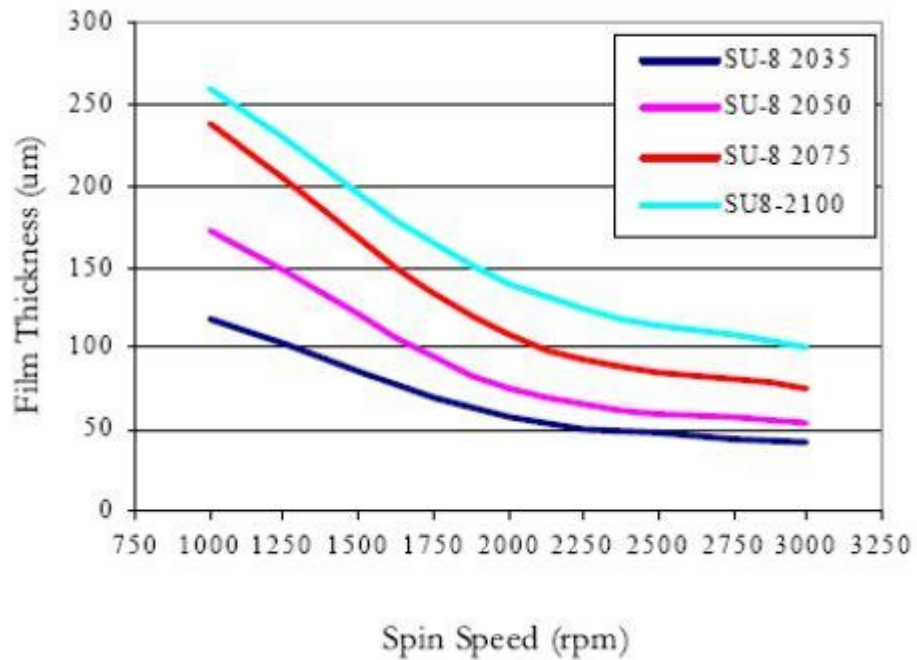


Figure 1.3. Graph of Film Thickness versus Spin Speed for Higher Grades of SU-8 2000 (Source: MicroChem, Inc., 2001).

1.6.2. SU-8 Photoresist Properties

SU-8 is an epoxy-based negative photoresist developed and patented by IBM and licensed for sale by MicroChem, Inc. Using spin-coating techniques, SU-8 can be deposited to thicknesses from less than 1 micron to more than 500 microns and patterned with extremely high aspect ratios and straight sidewalls using near UV (350-400 nm) and electron beam imaging (i-line, 365 nm, is recommended). SU-8 also has high optical transparency and good chemical and thermal resistance. Basic material properties of SU-8 are listed in Table 1.1. (MicroChem, Inc., 2001).

	Value
No table of figures entries found.	
Young's modulus E (postbake at 95 °C)	4.02 GPa
Young's modulus E (hardbake at 200 °C)	4.95±0.42 GPa
Biaxial modulus of elasticity $E/(1-\nu)$	5.18±0.89 GPa
Film stress (postbake at 95 °C)	16–19 MPa
Maximum stress (hardbake at 200 °C)	34 MPa
Friction coefficient μ_F (postbake at 95 °C)	0.19
Glass temperature T_g (unexposed)	~50 °C
Glass temperature T_g (fully crosslinked)	>200 °C
Degradation temperature T_d (fully crosslinked)	~380 °C
Thermal expansion coefficient α (postbake at 95 °C)	52±5.1 ppm/K
Polymer shrinkage	7.5%

Table 1.1. Physical properties of SU-8 photoresist
(Source: MicroChem, Inc., 2001).

In general, there are two kinds of photoresists: positive and negative photoresist. For positive photoresists, exposed areas become more soluble in the developer; they are removed by development. Correspondingly, the mask design will have transparent and opaque areas corresponding to unwanted and wanted areas on the photoresist, respectively. For negative photoresist, exposed areas become less soluble in the developer; they remain after development. In this case, the unhardened resist is developed and washed away.

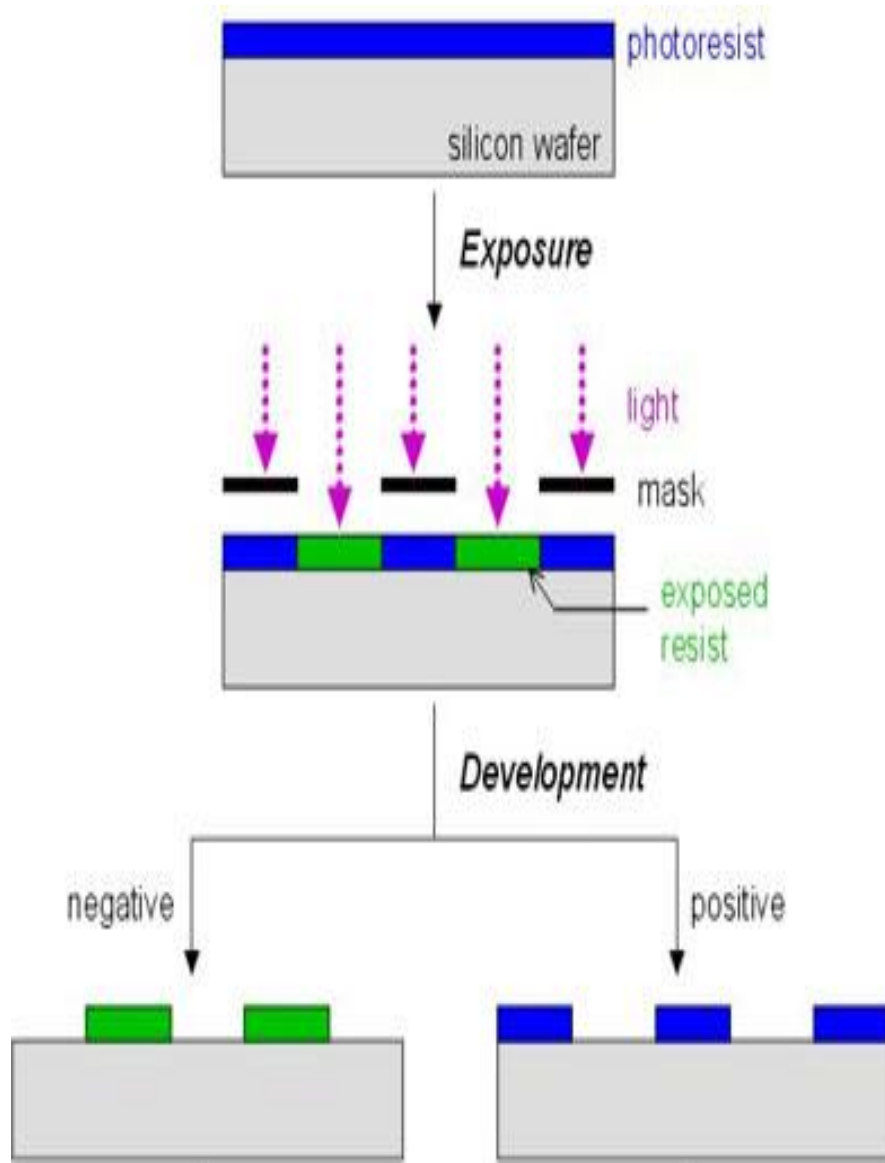


Figure 1.4. Different pattern obtained starting from a positive or negative photoresist.

As SU-8 is exposed to UV light, crosslinks are formed in this polymer substance. Actually, this process is not simple. Clearly, photoresist have certain compounds in it go through a reaction which contains dissociation and making an acid as first production of reaction. Then crosslinking process is initiated with the help of acid reacting, also acid provides to combine of multiple chains. Thermal effect is necessary for driving crosslink process throughout post exposure bake step. Finally, almost 8 monomers are come together and formed stable network in substance (Figure 1.4) (Teh, et al. 2005).

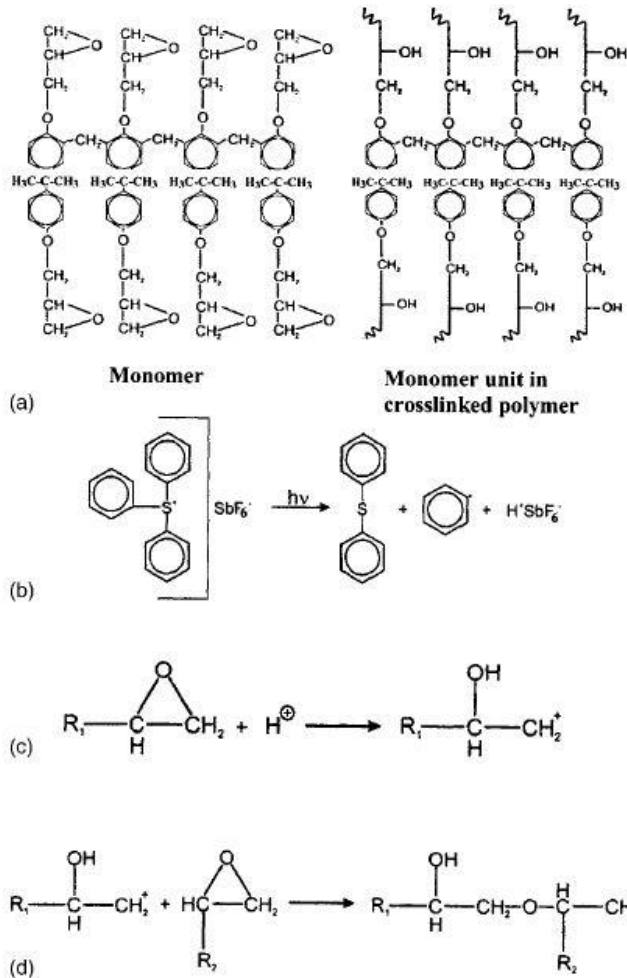


Figure 1.5. (a) Molecular structure of EPON SU-8: The monomer (left) and the monomer unit in the crosslinked polymer (right). (b) Generation of acid upon UV exposure as a result of protolysis of triaryl sulfonium hexafluoro antimonium, the cationic photo initiator. (c) Initiation of polymerization (crosslinking) via opening of the epoxy group. (d) Chain propagation of the crosslinking process. (Source: Teh, et al. 2005).

When SU-8 is compared to other photoresists, it has the capability of obtaining high aspect ratios as benefit which provides furthest height of structure is 2 mm, accurate side wall after chemical processing and thickness standardization even substrate accumulation. Besides benefits of SU-8, there are also some disadvantages, one of them is when post back temperature is adjusted at 95°C, shrinkage ratio of SU-8 reach to 7.5 % which inhibits reflowing after crosslinking. In addition to that, when SU-8 is heated which can cause the crosslinks and polymer solidification increasing, and substance have strength to developing solution so this is not preferred situation.

1.6.3. SU-8 Handling

The SU-8 photoresist is a toxic material and special handling equipment is required. SU-8 is a flammable material, and must be kept in a cool and dark environment, since crosslinking can occur in sunlight or heat. Fume hood is required when working with the SU-8 photoresist. This may be caused irritation of the skin, eyes, and respiratory tract; therefore proper handling must be followed. When handling SU-8, rubber gloves and safety goggles are required (MicroChem, Inc., 2001).

1.7. Soft Bake

Soft bake plays a very critical role in photo-imaging because the photoresist coating becomes photosensitive only after this step. Bake time is dependent on resist thickness. Soft bake temperatures and times for a two step process were provided by the supplier and proved sufficient for use in the laboratory (MicroChem, Inc., 2001). Initially, a short bake at 65 °C ramps the temperature of the substrate and photoresist, allowing the solvent to evaporate out of the film at a steady rate. This improves the coating fidelity and adhesion to the substrate, while also reducing the edge bead (MicroChem, Inc., 2001). A subsequent longer bake at 95 °C continues the evaporation and condensation process.

Product Name	Thickness (µms)	Pre-bake (@ 65° C)	Softbake (@ 95° C)
	35	2	5
SU-8 2035	55	3	6
	110	5	20
	50	3	6
SU-8 2050	75	3	9
	165	5	30
	75	3	9
SU-8 2075	110	5	20
	225	5	45
	100	5	20
SU-8 2100	140	5	35
	260	7	60

Table 1.2. Thickness and soft bake durations for various SU-8 2000 (Source: MicroChem, Inc., 2001).

1.8. UV Exposure

After soft baking, the next step in producing the master mold is to place the mask and substrate into a mask aligner and expose the resist to UV light.

1.8.1. UV Exposure Methods

Main types of exposure methods are represented in figure 1.6 like contact, proximity and projection.

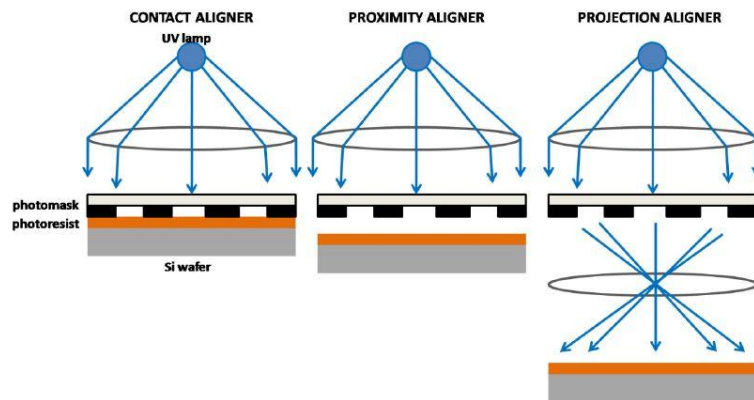


Figure 1.6. Principal mask alignment methods.

In contact printing, there is a physical contact with resist-coated silicon wafer and glass photomask. Till touching the wafer and mask, wafer is stuck in a vacuum. When touching the wafer and mask, UV light exposes on the photoresist. In contact printing, there is a feasible high resolution, owing to touching the resist and mask (e.g. from positive resist 1 micron property in 0.5 microns) But, there are some disadvantage of contact printing is that debris and the mask can be damaged.

The proximity exposure has a small gap as maintained between wafer and mask in the course of exposure like 10 to 25 microns wide on the contrary to contact printing. Damaging the mask can be lowered by this minimizing gap (not absolutely removed). Approximately 2 to 4 micron resolution is possible with this method.

In projection printing one advantage is that mask completely will not damage. The image of the mask's pattern is designed into centimeters away from the resist-coated wafer. You can get high resolution by imaging of a small divided of the mask.

Through the surface of the wafer's scanned or paced is the small image field. Pace the mask image through the wafer surface are called step-and-repeat systems. In projection printers, step-and-repeat systems have a roughly 1 micron resolution (Scotten 2008).

1.8.2. SU-8 Exposure Dose

All kinds of SU-8 photoresists are fabricated to be utilized in the near UV spectrum (350-400nm wavelengths). Generally, 365nm wavelengths are used to expose SU-8, which works well than other values. The photons are observed and cause a reaction in the material, if they having the required wavelengths, producing acid which is used to initiate crosslinking during post exposure bake. When wavelengths are above 400nm, the photons are not absorbed and no reactions occur. However, when wavelengths are below 350nm, the photoresist becomes very absorbent to photons. Figure 1.7 shows absorbance versus UV wavelengths for different resist thickness.

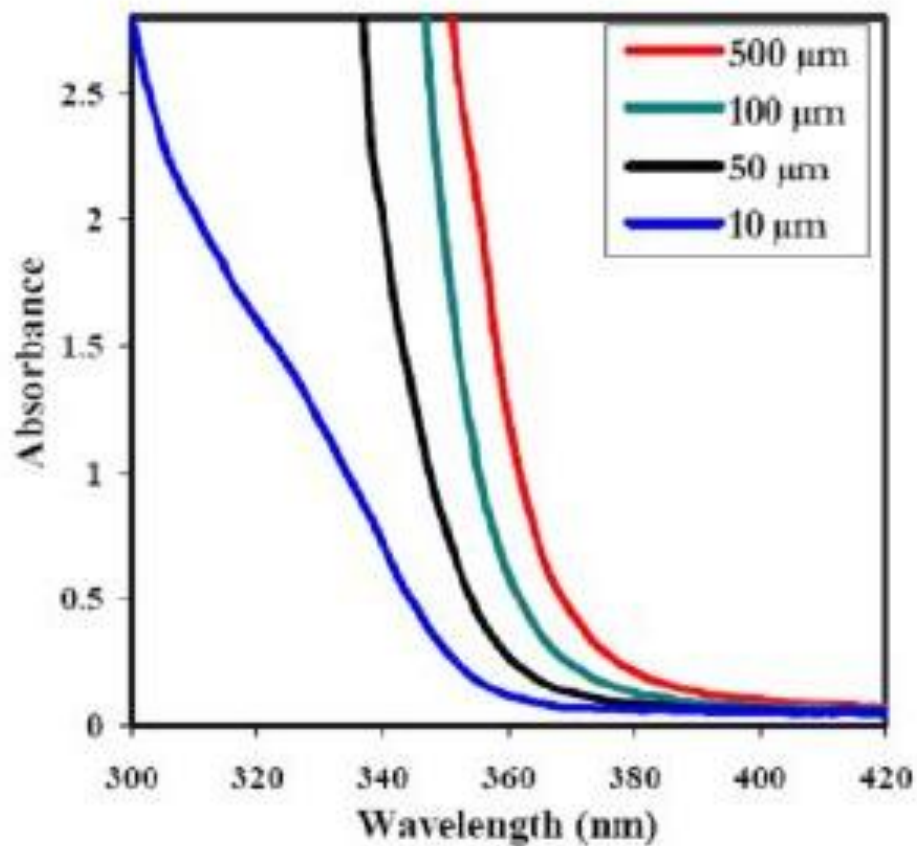


Figure 1.7. Absorbance versus UV Wavelengths for Different Resist Thickness (Source: MicroChem, Inc., 2001).

The wavelengths under 350nm, generally a filter is used to intercept over absorption of photons. Consequently, the exposure dose is an important parameter, which should be controlled to ensure appropriate exposure and crosslinking. In addition to this, when UV light passes through the filter and mask, UV light reduces energy, such that the exposure dose must compensate for this loss. Also, the substrate types are important to the amount of UV light required, such as glass will reflect less of the UV back into the photoresist compared to silicone. If desired thickness is more, the expose energy should be increased. Thus, the ideal UV energy required are by the machine to expose the resist, accounting for all the losses. Figure 1.8 shows film thickness versus exposure dose for reflective and non-reflective surfaces is presented.

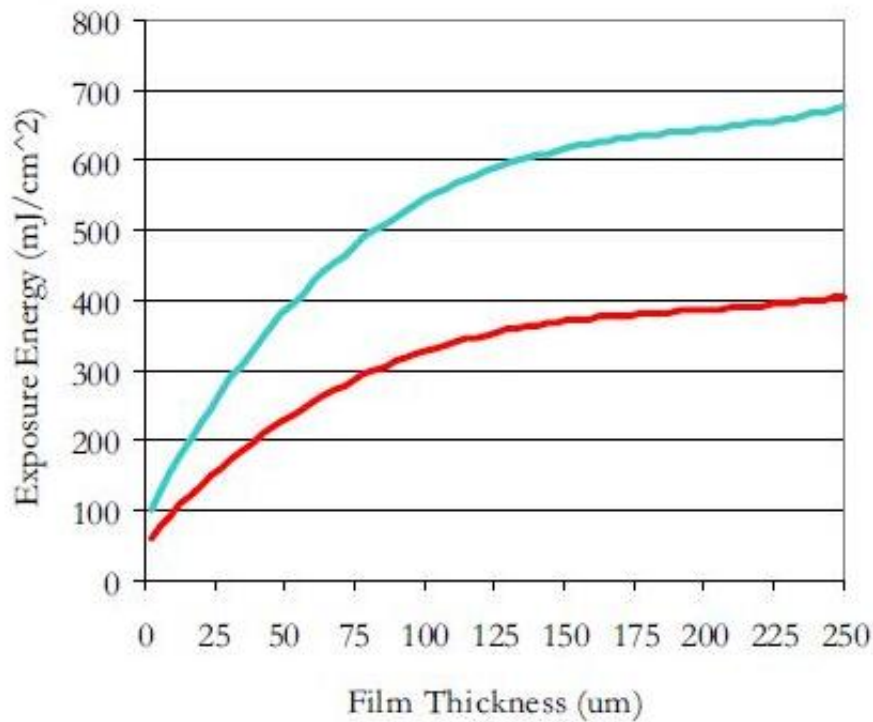


Figure 1.8. Exposure Energy Required versus Film Thickness for SU-8 2000 for reflective and non-reflective surfaces (Source: MicroChem, Inc., 2001).

When the photoresist film are exposed with correct UV light, resist becomes less or more acidic and the other important to ensure that the correct area is exposed. The wavelength of UV light is very important to expose correctly. Figure 1.9.a shows the result of to short exposure is a semi exposed film where some additional film will be remaining after development. But figure 1.9.b shows the result of over exposure is exposed areas under the mask. These effects are present at all exposures, but during short exposure, the parasitic effect is very small.

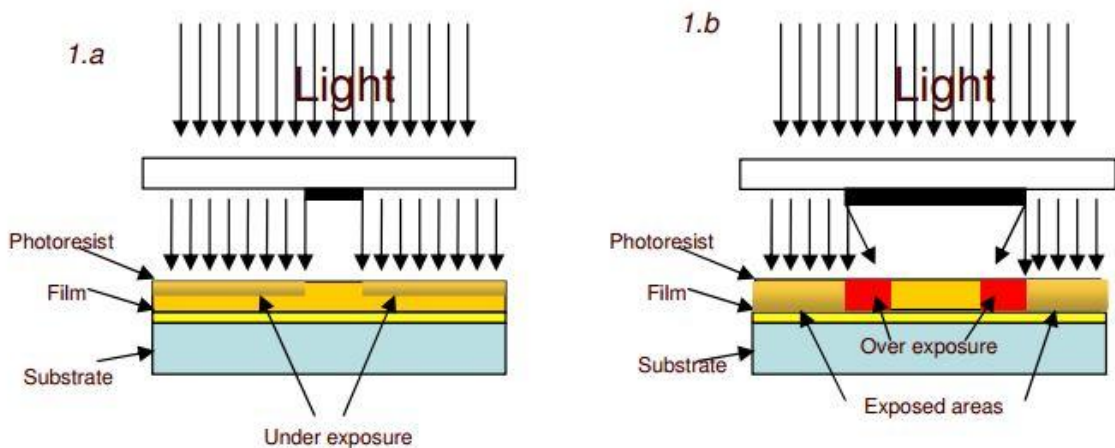


Figure 1.9. a) the result of short exposure is a semi exposed film where some additional film will be remaining after development. b) The result of over exposure is exposed areas under the mask.

1.9. Post Exposure Bake

This heating step is often necessary in order to harden the photoresist and improve its adhesion to the wafer surface. The substrates are baked to accelerate the cross-linking reaction in the exposed photoresist. Inadequate post exposure bake times, therefore, will result in poorly cross-linked resist structures, which can cause the deterioration or failure of the transferred design during developing. Besides ensuring adequate cross-linking of the photoresist, care must be taken to minimize stresses that are induced during the post-exposure bake. In extreme cases, stresses in the film can cause photoresist cracking and bowing of flexible substrates. Stress can be minimized by reducing thermal gradients during the heat-up to 95 °C for the post exposure bake and by avoiding rapid cooling after the bake (Despont et al. 1997). Performing the post-exposure bake at excessive temperatures has been found to cause flowing of the exposed photoresist structure, causing structural deformations (Liu et al. 2004). Bake time is dependent on resist thickness. Approximate post-exposure bake times and temperatures for a two-step bake process are provided by the supplier and were found to be sufficient (MicroChem, Inc., 2001).

Product Name	Thickness (µms)	PEB 1 @65° C	PEB 2 @95° C
	35	1	3
SU-8 2035	55	1	5
	110	1	10
	50	1	5
SU-8 2050	75	1	7
	165	1	12
	75	1	7
SU-8 2075	110	1	10
	225	1	15
	100	1	10
SU-8 2100	140	1	15
	260	1	15

Table 1.3. Thickness and post exposure bake durations for various SU-8 2000 (Source: MicroChem, Inc., 2001).

1.10. Development

After the PEB process, the last part of soft lithography is the developing process which is performed to remove the un-crosslinked photoresist. Usually the developer used is propylene glycol methyl ether acetate (PGMEA) or known commercially as SU-8 Developer. The time required for the rinse depends on thickness of the resist which means that thicker resists needs longer time for successful development. An approximation of the development time and thickness at room temperature for several common SU-8 grades is provided (Table 1.4).

Product Name	Thickness (µm)	Development (mins)
SU-8 2010	10	2
	13	3
	20	3
SU-8 2025	25	4
	41	5
	75	7
SU-8 2050	50	6
	75	7
	165	12
SU-8 2075	75	7
	110	10
	225	12

Table 1.4. Thickness and development time for various SU-8 2000 resist (Source: MicroChem, Inc., 2001).

1.10.1. Precipitate Test

After development, doing precipitate test has a very important role to test whether development was successful or not. The substrate is rinsed with isopropyl alcohol which reacts with uncrosslinked photoresist to form milky whites, effectively acting as a detector for insufficient development. If precipitates form, the development process should be repeated for another minute. The substrate is rinsed once more with PGMEA.

1.10.2. PGMEA Handling

SU-8 developer is relatively toxic to handle. Potential health effects for inhalation at high concentrations include irritation of the respiratory tract, and causes nausea, vomiting, numbness, and other symptoms affecting the central nervous system. It is also readily absorbed through the skin, and harmful if swallowed. Breathing products during ingestion or vomiting may cause lung damage or death. PGMEA is easily combustible, and must be stored in cool and dry places. It produces a vapor that spreads along the ground and may collect in low, confined areas. For handling purposes, goggles and laminate gloves should be worn.

1.11. General Properties of PDMS

PDMS is composed of two components, base polymer (silicone elastomer) and a curing agent. The most widely used PDMS is Sylgard 184 from Dow Corning Corporation (Dow Corninig Corp. 2010). The base polymer is composed of dimethylsiloxane oligomers ($[\text{SiO}(\text{CH}_3)_2]_n$) with vinyl-terminated end groups ($-\text{CH}=\text{CH}_2$), platinum (Pt) catalyst, and silica filler (dimethylvinylated and trimethylated silica).

The curing agent contains a cross-linking agent (dimethyl methylhydrogen siloxane) and an inhibitor (tetramethyl tetravinyl cyclotetra siloxane) (Lee, et al. 2003). Mixing the vinyl and the silicon hydride groups causes the cross-linking in the presence of the catalyst to form a Si-C bond building ($-\text{CH}_2-\text{CH}_2-$) linkage between PDMS

chains (Campbell et al., 1999). The cross-linking reaction is accelerated as the temperature increases. Figure 1.10 illustrates the crosslinking of PDMS.

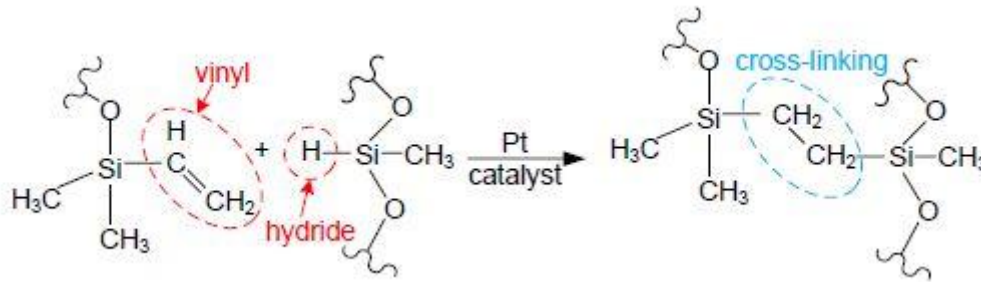


Figure 1.10. Schematic diagram of PDMS cross-linking (Source: Campbell et al. 1999).

Conventionally, a mixture of 10:1 (base polymer: curing agent) composition of PDMS is broadly used. However, 4:1 PDMS gives a higher (-CH₂-CH₂-) cross-linking since the ratio contains a higher fraction of curing agent (Ye, et al. 2006).

1.11.1. PDMS Handling Information

PDMS and PDMS curing agent are trustworthy materials for handling purposes. There are not required gloves or respirators to normal handling of the PDMS and curing agent. Of course, eye protection should always be worn, and washing hands after handling is an enough safety measure (Dow Corning Corp. 2010).

1.12. Surface Modification via Plasma Treatment

After peeling the PDMS, the molds are cut and trimmed to size. The surface containing the microchannels is treated with plasma.

1.12.1 Plasma Cleaner

Use of the plasma cleaner has an importance to treat the PDMS surface and surface cleaning, increasing surface charge and supporting irreversible bonding of the surface are the three fundamental objectives achieved by using the plasma cleaner. In the treatment of plasma, the ionized oxygen atoms are significantly used as the primary

ingredient which permits for cleaning and modifications of the material. In spite of that amount of oxygen in atmospheric air is enough for the ionization process and surface treatments when PDMS is used.

1.12.1.1. Surface Cleaning

While the ability of the plasma cleaner to remove organic contaminants from sample is an advantage, it is the limit that is the removing only for organic molecules that can easily react with oxygen. When any surface is bombarded with ionized oxygen, the oxygen atoms react with the hydrocarbon chains in organic contaminants and form CO₂ and CH₄ that are discarded from the plasma cleaner system.

1.12.1.2. Increasing Surface Charge

For microfluidic applications, obtain a substrate which has a consistent and net surface charge that can promote electro kinetic effects like EOF. Native (non-treated) PDMS which has a small negative surface charge shows hydrophobic behavior to water. Besides the magnitude of the surface charge is increased by operating surface modification with an oxygen plasma. In comparison, (Vickers, et al. 2006) noted that an increase in EOF mobility from $4.1 \times 10^{-4} \text{ cm}^2/\text{Vs}$ to $6.8 \times 10^{-4} \text{ cm}^2 \text{ V/s}$. This increasing on surface charge is going to thanks to the bombardment of oxygen ions. There is an oxygen atom substitutes into and effectively “kicks-out” a carbon atom from an individual “mer” unit of the polymer PDMS which are methyl groups (O-Si-CH₃). After bombardment, methyl groups effectively change silanol groups (Si-OH) while realizing the reaction. While the zeta potential charge is increasing, they also react with a polar medium like water and they promptly dissociate to SiO⁻ and H⁺ ions that attract to water. Finally at end of the process, the surface is changing from an initial hydrophobic structure to a hydrophilic one (McDonald et al., 2000) and (Efimenko, et al. 2002).

1.12.1.3. Irreversible Bonding

The flexibility of surface properties of PDMS to change allows for irreversible bonding and it is third advantage of plasma treatment. Bonding is very essential since the micro-channels are often enclosed by PDMS. Nonetheless, the replica molding process can construct only three sides of a channel as a maximum. Besides, the maximum pressure that these channels can resist is 5 psi. In the regions of bonding through plasma treatment, the pressures of 30 to 50 psi can be maintained. The silanol groups created by the plasma treatment provide chemical compositions needed for strong bonding. As an example, the two surface-treated PDMS substrates are reacted with each other, strong Si-O-Si bonds are formed and H₂O is released as a product. Additionally it can easily bond with different materials that contain silicon and one of OH, COOH, or ketone groups (McDonald et al., 2000).

Good adhesion requires strong interfacial forces via chemical compatibility and/or chemical bonding. Oxygen plasma surface treatment can assist the creation of a chemically active functional group, hydroxyl (-OH), to improve interfacial adhesion between PDMS and glass. This chemical reaction yields Si-O-Si bonds after loss of a water molecule (Bhattacharya, et al. 2005), as illustrated in Figure 1.11. (Malecha, et al. 2009)

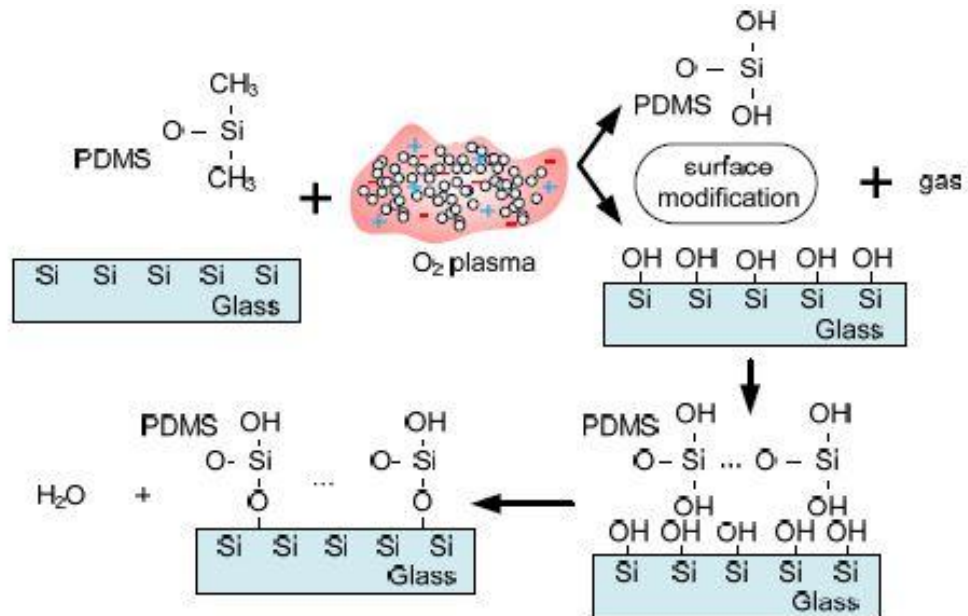


Figure 1.11. Schematic view of the surface modification of PDMS and glass by O₂ plasma and their bonding (Source: Tang et al. 2006).

1.12.2. PDMS Bonding

UV/ozone treatment removes organic residues on surfaces and renders them hydrophilic to activate the PDMS and glass slide surfaces. After 5 minutes, the surfaces become hydrophilic as can be seen different contact angles.

PDMS and glass surface are successfully bonded to each other's by using a UV/Ozone treatment. This part should be made immediately because the PDMS surface will return to its hydrophobic state after time.

CHAPTER 2

EXPERIMENTAL

The experimental approach consists of two parts: Photolithography and PDMS molding. UV lithography was carried out in a Class 1000 clean room at the Applied Quantum Research Center at Izmir Institute of Technology. PDMS molding was completed on bench in standard laboratory conditions.

2.1. Materials and Equipments

Laboratory equipment includes a spin coater (G3P-8 Desk-Top Precision Spin Coating System, Specialty Coating Systems, Indianapolis, IN), digital hot plates (WiseStir® MSH-20D, DAIHAN Scientific, Korea), UV mask aligner (OAI Hybralign Series 200, San Jose, CA), vacuum desiccator (PlusMED, 7E-A, Danyang, P.R.C), sonicator (WiseClean, WUC-A03H, DAIHAN Scientific, Korea), UV Ozone procleaner (BIOFORCE NANOSCIENCES, UV/Ozone ProCleaner™ 220, U.S.A), optical microscope (OLYMPUS CORPORATION-CKX41SF, Tokyo, JAPAN) , dry oven (NÜVE STERILIZER, FN 032, TURKEY) and glass slides (76×26×1mm, Paul Marienfeld, GERMANY).

Design was drawn a photo mask by Corel Draw program. The mask design was printed on the acetate paper by ÇözümTanıtım.

The photoresist and developer were used NANO™ SU-8-2075 and SU-8 developer provided by Microchem (Newton, MA). The SU-8 photoresist is an octa-functional epoxy resin and the SU-8 developer is propylene glycol methyl ether acetate (PGMEA). The substrates were used 100mm diameter silicon wafers (500 ± 25 microns, thickness) provided by University WAFER. The PDMS and PDMS curing agent were used 184 SILICONE ELASTOMER KIT and provided by SYLGARD® (Dow Corning, Midland, MI, U.S.A). Demolding agent (Triton-X, DH₂O, EtOH; 1:9:40), isopropyl alcohol (IPA), ultra pure water (UPH₂O) deionized water (dH₂O) and ethanol 70%EtOH in sterile deionized water.

2.2. Photolithography

The steps involved in the photolithographic process are photoresist application; photoresist (SU-8) coating, soft baking, SU-8 exposure, post exposure baking and SU-8 development (Figure 2.1.).

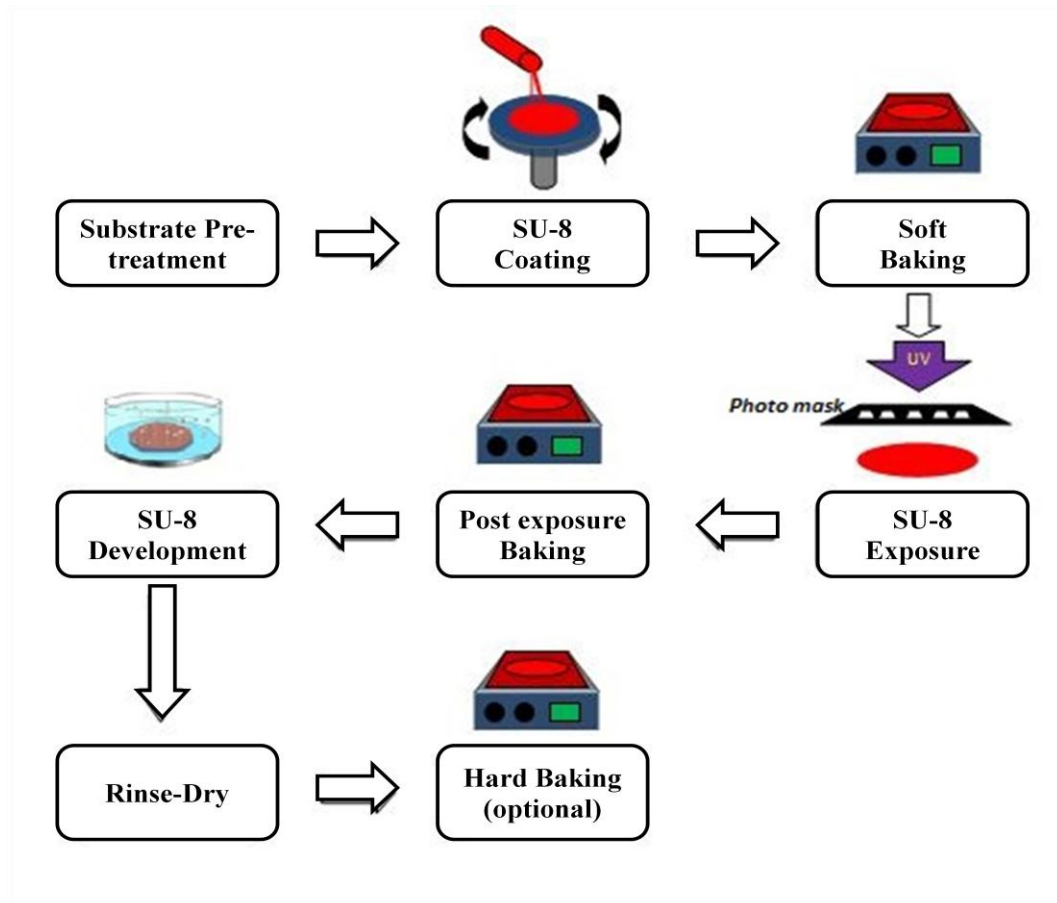


Figure 2.1. Overview of Soft Lithography.

Once a desired thickness is chosen, a grade of SU-8 must be chosen according to the desired thickness and poured onto the wafer through a spin coater. The wafer is soft baked firstly at 65°C, then at 95°C, and then cooled down. After cooling in room temperature for 15 minutes, it is exposed to UV radiation underneath a laser-printed mask transparency. Afterwards, the wafers are post exposure baked at 65°C, then at 95°C, and then cooled down to room temperature (RT). Next, the substrate with the photoresist is developed in SU-8 developer, rinsed, and dried. All parts are performed in clean room.

2.2.1. Photomask

Masks were drawn using Corel Draw. The files were converted to PDFs and high resolution (3600 dpi) print-outs were ordered from, zumtanitim, Ankara, Turkey.

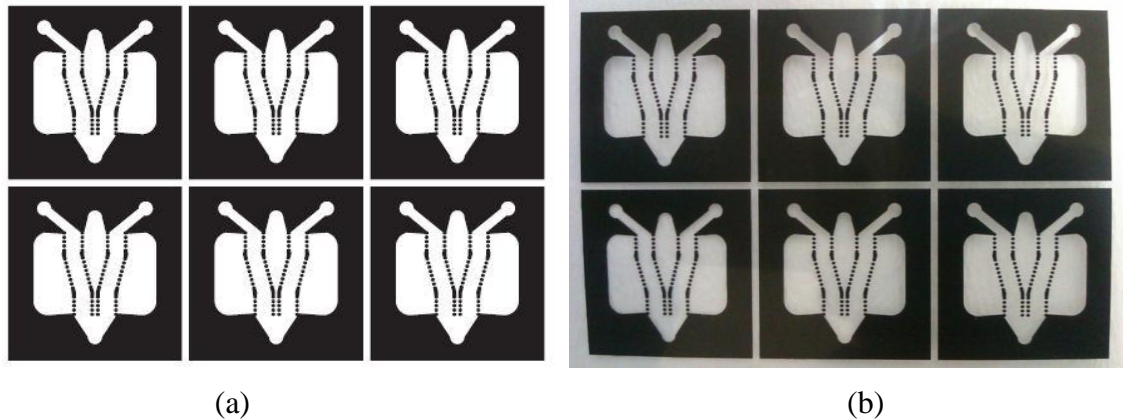


Figure 2.2. The photomask (a) design drawn with Corel Draw and (b) printed on acetate paper.

2.2.2. SU-8 Coating

The hotplate was brought to 65°C. A clean silicon wafer was placed on hot plate and ~ 4 ml SU-8 2075 was poured onto silicon wafer, which was kept on the hotplate for 5 minutes before being placed on the wafer holder of the spinner at room temperature (RT). The silicon wafer coated with SU-8 was spun at 1000 or 3000 rpm for 30 – 65 seconds.

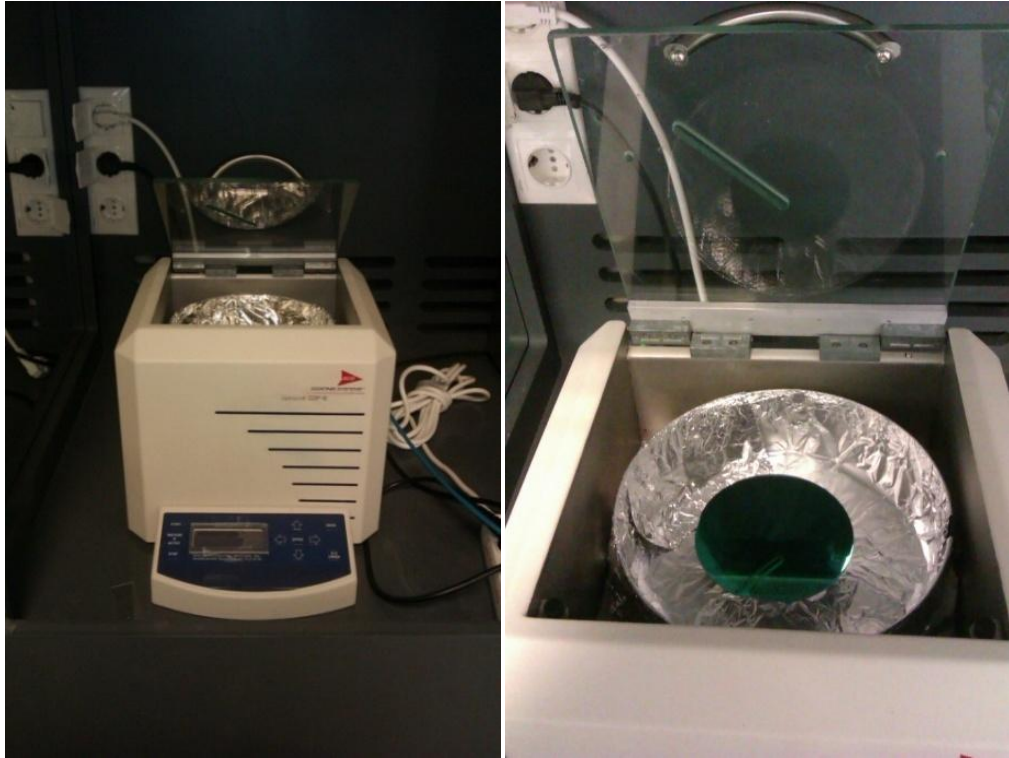


Figure 2.3. The picture of spinner used for photoresist coating.

2.2.3. Soft Bake

The sample was soft baked first at 65°C for 10 – 20 minutes then at 95°C for 30-180 minutes. The sample was left to cool on the turned-off hotplate for the rest of the day. The next day, Heater was set at 95°C and the SU-8 covered wafer was put on it, if any wrinkle was seen, sample was baked 10 minutes more. After 10 minutes sample was left on bench (at RT) and after sample reached to RT, it was put on heater again to determine whether wrinkles formed. This step was repeated until no wrinkles were seen.

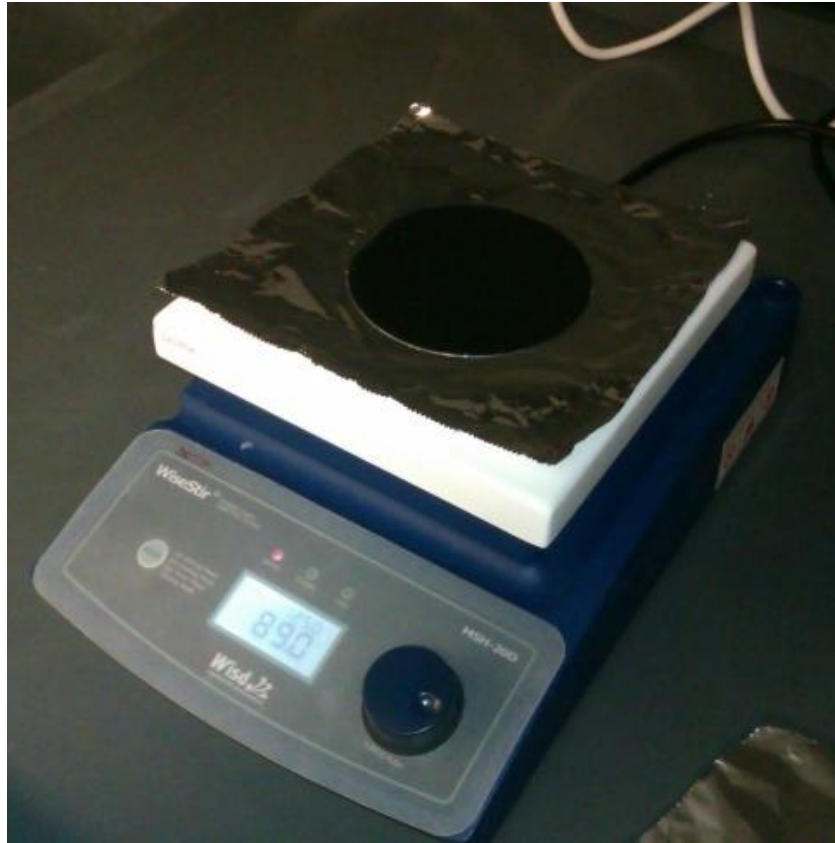


Figure 2.4. The picture of hot plate used for soft, hard baking and PDMS bonding.

2.2.4. UV Exposure

The sample was placed on the stage of the mask aligner with the acetate photomask directly in contact and the print side facing the SU-8. The sample was exposed to UV light at 500 watts for 20 – 40 seconds. After expose, sample was left on the bench for 5 minutes and placed on heater for post-exposure bake part.

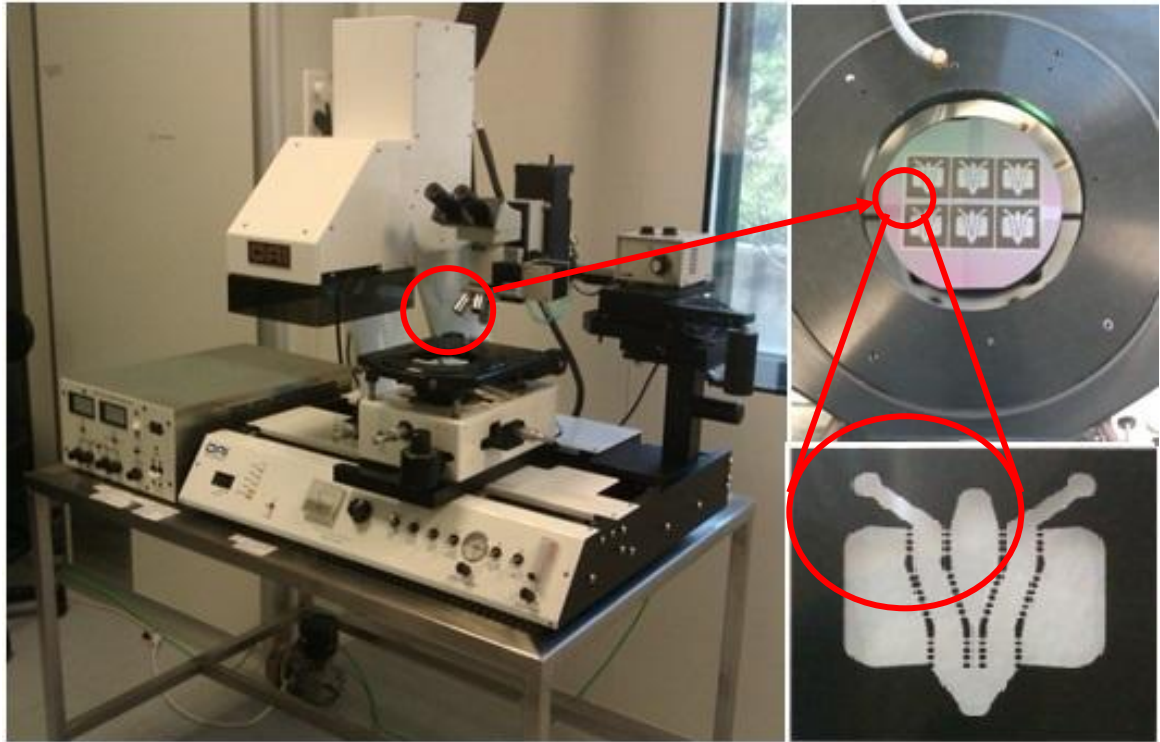


Figure 2.5. The picture of OAI mask aligner used for expose.

2.2.5. Post Bake

The wafer was post exposure baked on heater at 65°C for 3 min, and then ramped up to 95°C for 9 min. After baking, the wafer was allowed to cool down to room temperature before development.

2.2.6. Develop

First of all, the SU-8 developer and SU-8 covered wafer put in a petri dish (at RT) after putting, the wafer was waited for 5 minutes, shook for next 15 minutes. The sample was removed from the petri dish and isopropyl alcohol (IP) test was applied. This was performed to test whether all un-crosslinked photoresist was removed. The isopropyl alcohol was dropped on the wafer and the variation of the IP was observed. If color of the IP was white, the development part repeated till no white color was seen; else the wafer was rinsed for 10 times with fresh SU-8 developer and fresh isopropyl alcohol and then dried with nitrogen gas.

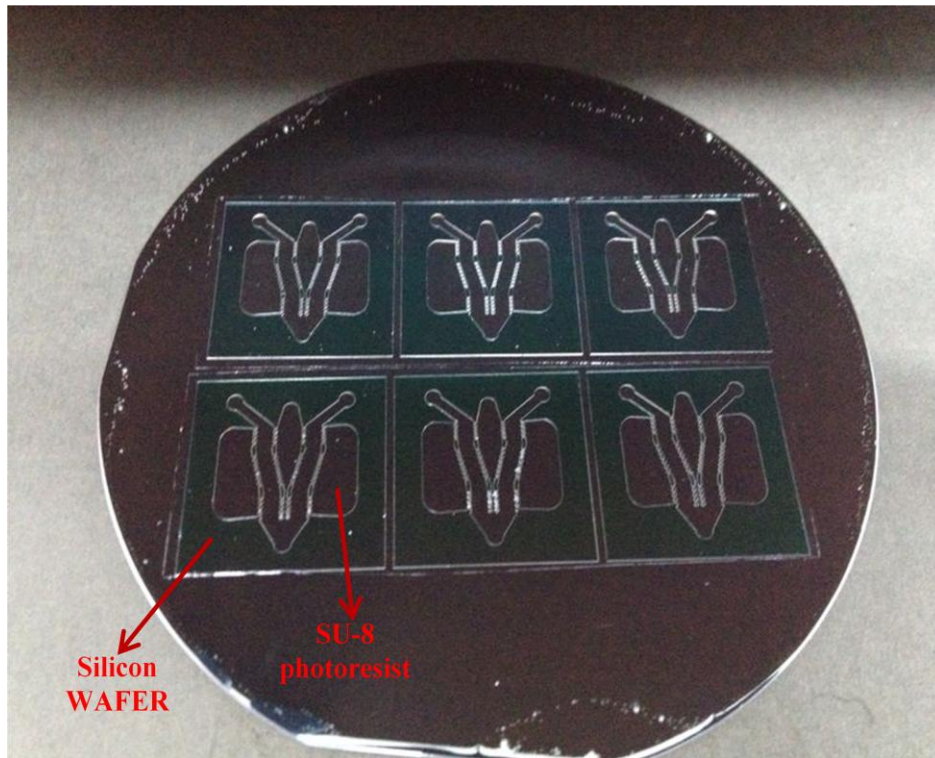


Figure 2.6. The picture of SU-8 covered wafer after development part.

2.3. Replica Molding

2.3.1. PDMS Molding

SU-8 master was wet with demolding agent. The SU-8 master was securely placed inside an aluminum foil container shaped using a 10 cm glass petri dish a piece of double sticky tape. The SU-8 master ready to be molded was then placed on a level surface to ensure that the resulting PDMS structure would have an even surface. PDMS (Sylgard 184 silicone base and curing agent) were mixed in a 1:10 ratio by weight. The PDMS mixture was degassed to remove any bubbles inside a desiccator. Then the mixture was poured on the SU-8 master which was lightly covered by aluminum foil to protect from dust. The PDMS mixture on SU-8 master was left on the bench for two days so that its polymerization would be completed.

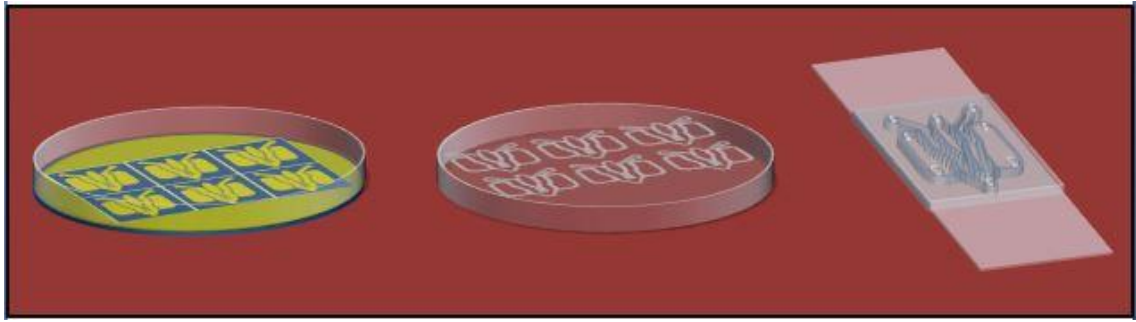


Figure 2.7. Overview of PDMS molding fabrication process for creating polydimethylsiloxane (PDMS) polymer microfluidic chips from the masters and PDMS polymer. PDMS pre-polymer in a 10 to 1 ratio of base to curing agent is mixed and poured onto the masters. Next, PDMS mold and glass is bonded.

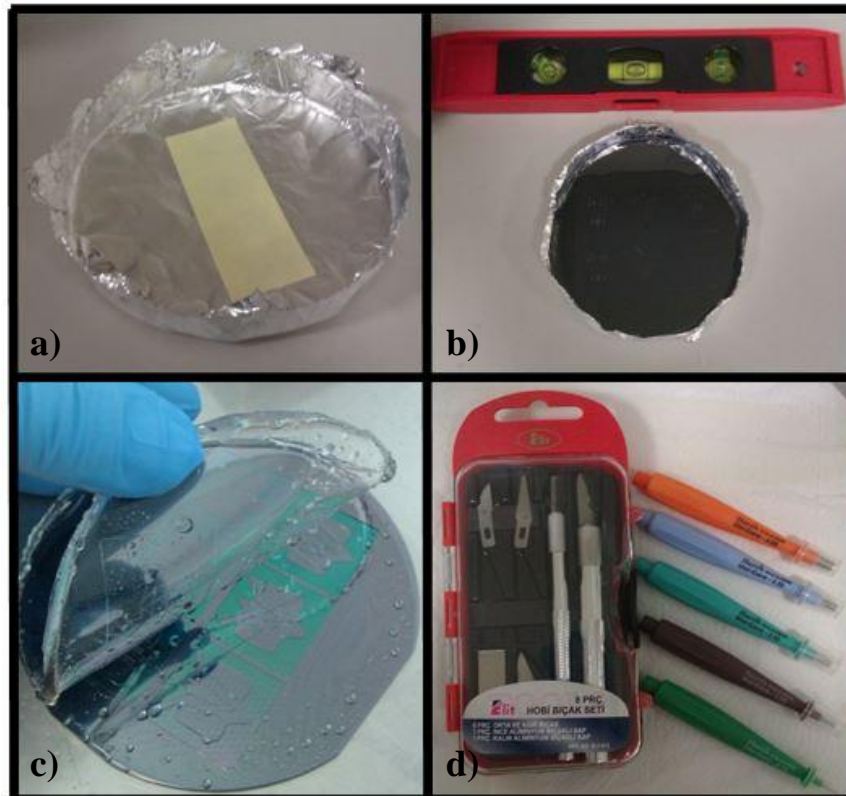


Figure 2.8. The picture of (a) aluminum foil container, (b) the PDMS mixture on SU-8 master was left on the flat bench, (c) the PDMS was peeled from the SU-8 covered wafer and (d) knives and cylindrical biopsy punches of various sizes.

After polymerization, the PDMS was easily peeled with EtOH and cut out with appropriately sized holes punched out using cylindrical biopsy punches of various sizes.



Figure 2.9. The picture of dessicator used for remove any bubble.

2.3.2. PDMS Cleaning

Cleaning the PDMS prerequisite is a very important part of sterilization. First, dust particles were removed by Scotch tape. Then the following steps were carried out:

- Cleaning of PDMS molds
 - ✓ 5 times rinsing with UPH₂O and EtOH
 - ✓ 10' sonication in UPH₂O
 - ✓ 5 times rinsing with UPH₂O
 - ✓ 5' sonication in EtOH
 - ✓ 1 time rinsing with EtOH
 - ✓ 5' in EtOH
 - ✓ 1 time rinsing with UPH₂O
 - ✓ Dry with nitrogen gas



Figure 2.10. The picture of sonicator used for PDMS cleaning.

2.3.3. PDMS Bonding

PDMS and clean glass slides were placed side of interest facing up inside UV/Ozone cleaner and treated for 5 minutes, immediately after which PDMS and glass were firmly pressed together. Then the assembled device was placed on a hotplate set to 100°C for 10 minutes to ensure permanent bonding. To render the interior surfaces of the device hydrophobic, the devices were incubated in an oven set to 60°C, for 24 hours.



Figure 2.11. The pictures of UV-Ozone used for PDMS bonding.



Figure 2.12. The picture of ETUV used for drying sterile cured PDMS and glass coverslips.

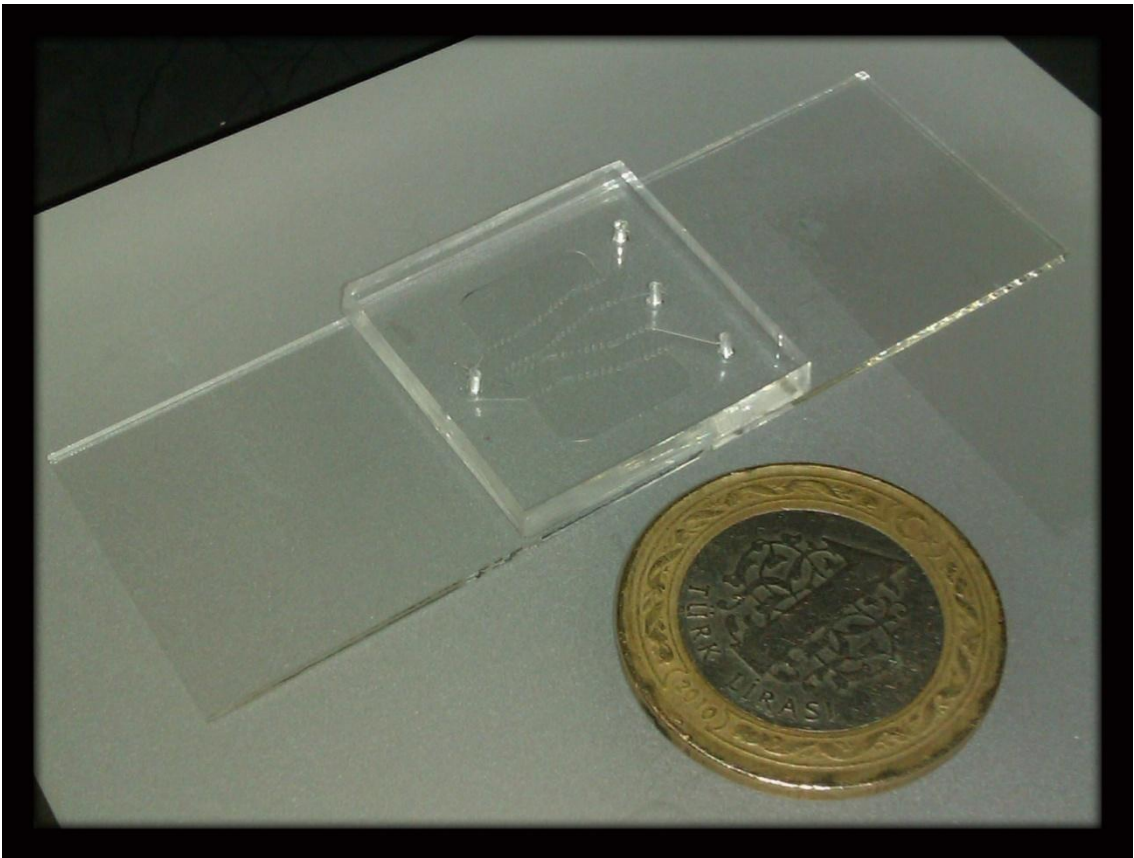
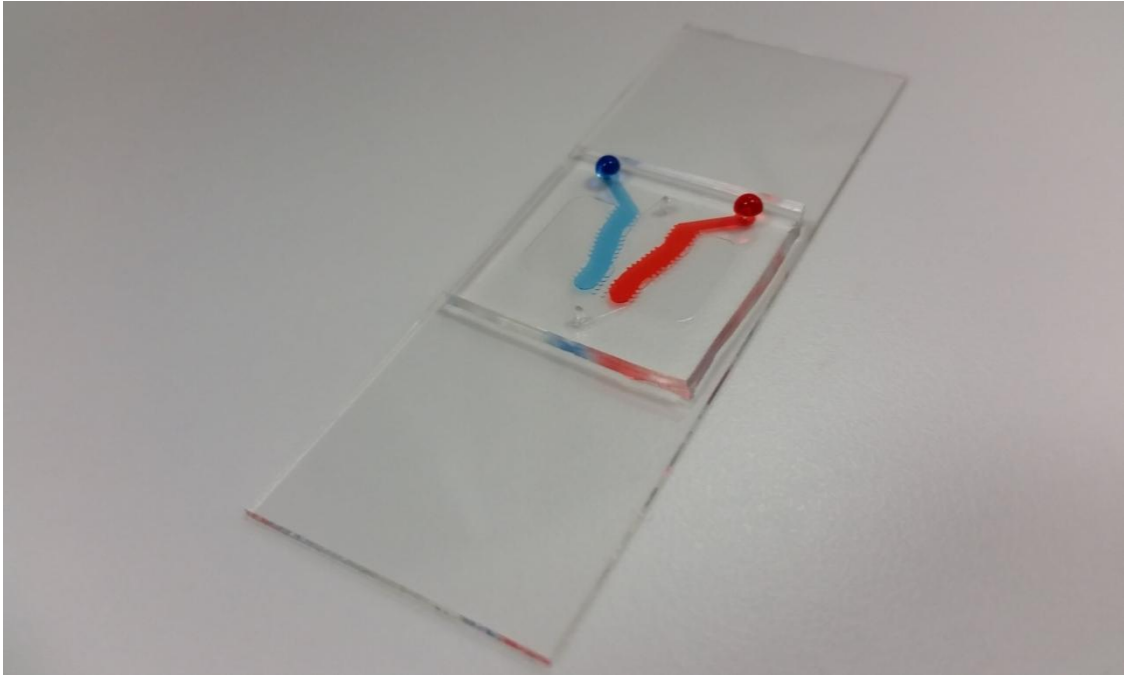


Figure 2.13. The pictures of microfluidic device.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Mask Design

At the begin of the mask design,

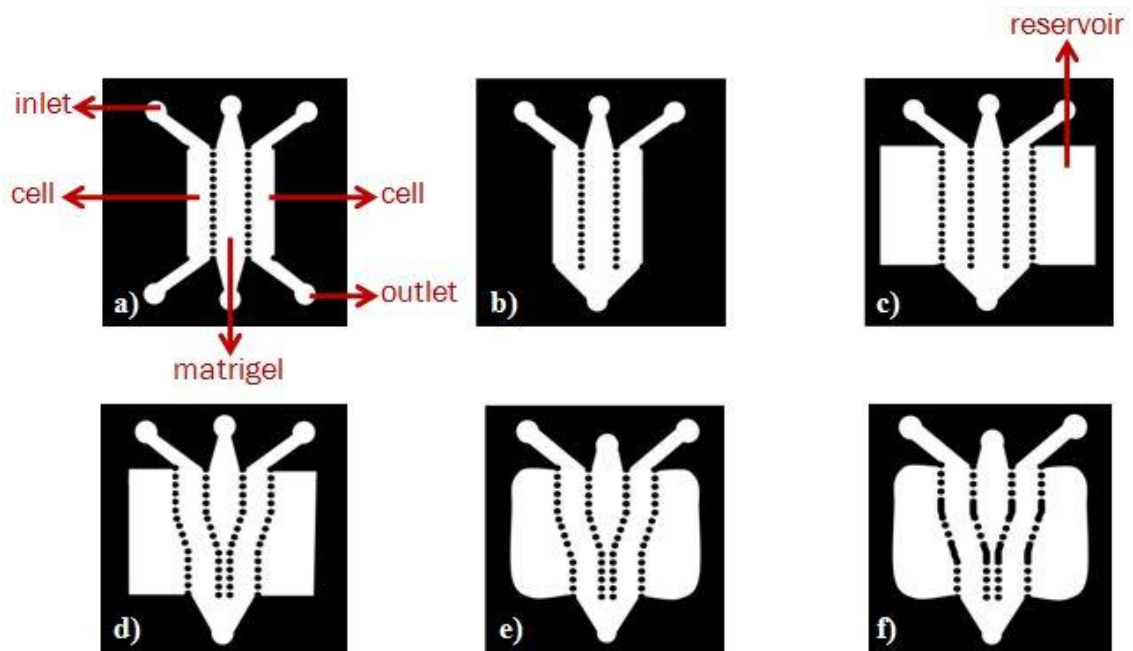


Figure 3.1. Improving our mask design.

The mask design should have contained three channels, extracellular matrix (matrigel) would be loaded in the middle of them and side channels would contain cells, every channel had an inlet and outlet (Figure 3.1.a). We have noticed that the three outlets were excessive. At the experiment, loading on the one channel was required to close the outlets of the other channels. The process converted the mask which has three inlets one outlet (Figure 3.1.b). While improving our design, we concluded that device should have reservoirs of medium near to the cell channels (Figure 3.1.c). At first glance, we were looking at the desired distance of two cells, so the mask contained only one distance. Then we enlarged the mask design that the distance changes (3mm-300 μ m) from up to down of the channel length (Figure 3.1.d). The reservoir had a

rectangular shape which would be problem during experiment because the reservoir would contain medium which is a liquid material so we have given oval shape for reservoirs to eliminate probability of occurrence of bubbles in the corners of the rectangle reservoir (Figure 3.1.e). Final changing of our design, we combined some spaced posts because pressure would be more on the corners of the spaced posts while loading the cell or matrigel, thanks to combine we optimized the pressure would be same for all channels (Figure3.1.f).

Transparent part of the mask was permanently stabilized on SU-8 master at the end of the UVL process. Because after UVL, master was transferred to PDMS mold, and post part of the PDMS mold should have elevated, post on the mask should be represented with dark color.

3.2. Substrate Types and Preparation

Choosing substrate is essential for main fabrication process, after the mask is formed. After deciding substrate, photoresist is applied in order to obtain original pattern. Glass and silicon are the most chosen substrates. When glass substrates are used, their average costs are \$0.50 for microscope slides and \$7.00 for a rectangular slide, they are cheap for using. At the same time, glass do not have formatted molecular structure, it has inequality surface which cause poor adhesion. In addition, glass usage is difficult during fabrication process because of their worse physical properties which also cause defaulting of obtained master. However, if basic fabrication is done with large dimensions which mean length and width will be bigger than 500 μm , glass substrates should be used. Because, there are more attachment points between photoresist and substrate because of large surface area and this supply more adhesion interaction and also provide enhancement of reusability.

In another respects, electronic fabrication industry use silicon substrates regularly. Surface of silicon is flat and stableness which increases the control of substrate and also roughness of silicon is smaller than glass substrate. Decreasing of roughness provide increasing adhesion between wafers and photoresist which is SU-8. Moreover, silicon wafers are produced with the help of clean room properties and this supply cleaning of this wafers is not necessary. On the other hand, costing of silicon wafers is higher than glass, their costs are about \$25.00 per wafer and they have larger surface areas which increase the requirement amount of photoresist for spincoating.

Optimizing Steps with Glass Substrates

In order to optimize each step of the UVL, as a substrate glass slides (25x75mm) were used before Si wafer. These experiments were performed because SU-8 2075 datasheet supplies average difference between substrates, and if we optimized steps with glass substrate, we could pass directly Si wafer substrate by applying datasheet guidelines. Beside this glass slide has two advantages at first, one of them is that glass slide price is much more less than Si wafer; second one is that glass slide supplies reproducibility due to smaller area. At once we could have more than one sample for one trial.

As a starting point we had simple design, arranged side by side rectangle channels (6x9mm). We optimized soft bake, post exposure bake and develop steps with different trials. In table 3.1, we showed applied temperatures for soft bake and post exposure bake and duration of development step. The same color indicates one experiment that has worked. As seen from the table 3.1, different settings of the procedure works well, however, we should have optimized for each sample. Because SU-8 2075 is sensitive for temperature change, we made certain that temperature changes should be done in long period. At first our main problem was cracks during development. In addition to this, some of the samples we obtained SU-8 layer as totally separated from the substrate. Because we lost most of the samples at the end of the development step, we could not conclude which step was problematic. Then, firstly we found out that temperature of developer solution should have been approximately the same with the sample. For this we kept developer solution at room temperature for half an hour before development step began (normally developer solution was stored at 4°C).

After cracks were minimized, we checked each sample under the microscope if the develop was completed. We could visualize the cracks at the edge of the design mostly, and we could determine if the exposure dose was adequate, over or under. Optimized exposure time was between 40-45 seconds, changes according to the height of the SU-8 layer. While trials were in progress, we saw that when post exposure bake duration was longer, development step duration increased. Then we determine to complete post exposure bake 5min 65°C 15min 95°C totally 20 min. Additional step after soft bake was wrinkle check. We checked wrinkle formation after soft bake. If there was any wrinkle, we baked sample at 95°C additionally 10 min. These trials showed that when layer of the SU-8 layer were getting higher, soft bake duration were getting increased. Our first trials' soft bake and post exposure bake were done in oven, but in oven surface of the SU-8 was baked, while inner SU-8 stayed as solvent and this was also the case of the cracks. From these observations, we determined to perform bake steps on heater. Before usage of the heater, we made certain that heater distributed heat uniformly through its surface.

# of wafer	1	2	3	4	5	6	7	8	9	10	11	12
Spin speed (rpm)	3000	3000	3000	3000	1000	1000	1000	1000	1000	3000	500	1000
Spin time (s)	40	65	50	40	40	40	40	60	60	50	25	30
Soft B. 65°C (m)	15	15	15	10	20	10	20	15	15	12	10	10
Soft B. 95°C (m)	180	180	180	150	70	60	70	90	30	40	30	30
Expose time (s)	40	40	40	40	35	35	25	40	40	20	40	40
Post B. 65°C (m)	5	5	5	5	5	5	5	3	3	2	3	3
Post B. 95°C (m)	15	15	15	15	15	15	15	9	9	6	9	9
Develop time (s)	20	20	20	20	20	20	20	40	20	20	20	20
Height (µm)	80	56	60	80	200	200	200	180	180	36	300	270

Table 3.1. Optimization of our microfluidics

3.3. SU-8 Coating

On the first day of UVL, coating of silicon wafer was completed. To do this happen, first we made certain that the substrate (Si wafer) was clean. In order to do that, we took out Si wafer from its specialized box just before use, and the tweezers we used while taking out the wafer, was used only for matters without SU-8. If there was a dust on the Si wafer surface, it was cleaned with dust-free napkins (kimwipe). While discarding the dust on the wafer it was important not to scratch surface of wafer. If dust was not removed, after coating step bubble formations and craters were observed. Because we used Si wafer directly from the box, we did not have any problem because of dust.

To optimize amount of SU-8 poured on Si wafer, SU-8 was poured from aliquot amber bottle. Because the viscosity of the SU-8 is high, and the flow rate was not constant from the bottle, it was hard to determine the amount. Moreover, mouth of the bottle was crystallized after some time because of the SU-8 residues. Then aliquots were prepared as falcons. This provides not only ignore crystallization, but also control the amount of casting of SU-8. In addition, small amount of aliquot supplies safe handling.

To cover the surface of the Si wafer that 4 ml of SU-8 was enough. If SU-8 was poured more than 4 ml during the spin process excess photoresist was spread out of the Si wafer. While pouring the SU-8, the bottle should be close the middle of the Si wafer to prevent the occurrence of bubbles.

Spin coater was used to arrange the thickness of the SU-8 layer on the Si wafer. For 400-300 μm 500 rpm, 300-200 μm 1000 rpm, 200-100 μm 2000 rpm, 100-30 μm 3000 rpm.

3.4. Soft Bake

3.4.1. Conventional Ovens versus Hotplates

In order to supply flowing of air or determined gas convection oven is used. During transmission of heat from the resistance coils are located in oven walls to the

wafer spontaneously. By this way, both driving and heating the solvent distant from the coating. On the other hand, difficulty of using these ovens is the organization of lamination parts or zones which parts have unequal temperature. This cause the irregularity of air temperature and decreasing heat in oven, when air touch this surfaces. This irregular heating can influence quality of film. At the same time, as batch of substrate is located in the oven to provide heating, substrate which nearest the borders can take more heat than substrates in center which situation affect heat balancing and because of this situation results can be different although substrates are in same heat situations. At that, enhancement of films is supplied from the top surface to the bottom owing to enclosing heated air. So, firstly skin layer is formed before enhancement of inside of film. If thicker coating is done, this skin layer block air passing inside of film and during curing of the film, bubbles can be formed in film because of vaporization and this affect the difficulties of adhesion and trouble of film forming.

Balanced temperature is provided by hot plate. Especially, when thin film is formed, plate temperature is stable and this provide easier to keep constant of temperature comparing to keep constant air temperature in conventional oven. With the help of this constant and balanced heat which is supplied with hot plate, enhancement and quality of film increase substantially. In addition it is east to obtain same film at another time.

Another benefit of hot plate is that it supplies faster and more influential transmission of heat that convection oven. When hot plates are used, heat is supplied bottom of substrates and forming film begins bottom of substrates, so when solvent is evaporated, air bubbles are not forms because there is not any skin layer top of the substrate. In order to balanced heating and shorter processing time, hot plates are used instead of convenient ovens.

Heating Times

Soft bake plays a very critical role in photo-imaging because the photoresist coating becomes photosensitive only after this step. Over soft bake will degrade the photosensitivity of resists by either reducing the developer solubility or actually destroying a portion of the sensitizer. Under soft bake will prevent light from reaching the sensitizer.

3.5. Post Bake

This part was very critical role before development because post-exposure bake increases the crosslinking degree in the irradiated areas and stabilizes them against the action of solvents during the posterior development step.

3.6. Develop

The last part of soft lithography was the develop process which was performed to remove the un-crosslinked photoresist.

3.7. PDMS Molding

3.7.1. Beyond PDMS

PDMS molding is perfect ways to cheaply and rapidly produce microfluidics, both suffer from certain limitations. There is an important disadvantage about microfluidic devices fabrication, which is the limit forced over microstructures shapes. Also, if structures have high aspect ratio which means that the low Young modulus of PDMS will usually curve or slump under their own weight (Zhao, et al. 1997). The following Figure 3.2 shows about this behavior. Considering that fact, it would be extremely useful to research also some other material or technique able to supplement PDMS replica molding.

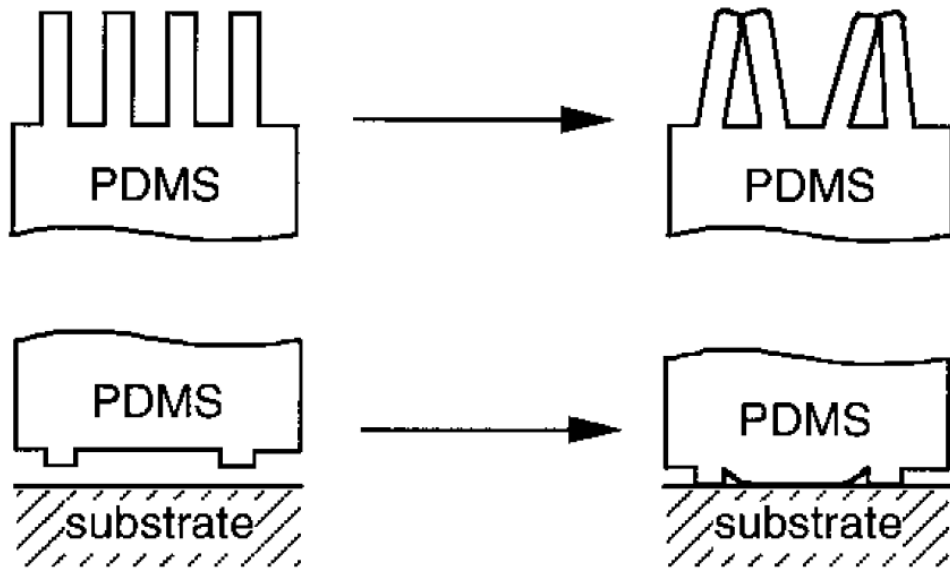


Figure 3.2. Examples of how PDMS replicas featuring structures with high aspect ratio will usually collapse due to the elastomeric nature of this material. (Source:Zhao, et al. 1997)

Pdms sagging depends on the aspect ratio which is h/d ; h is the depth of the structure and d symbolizes the width of the microchannel. These ratio of the structures directly affects the channel density and depth permitted for a fluidic chip. Due to the softness of the material, sagging can occur which effectively constricts the microchannel (Figure 3.3).

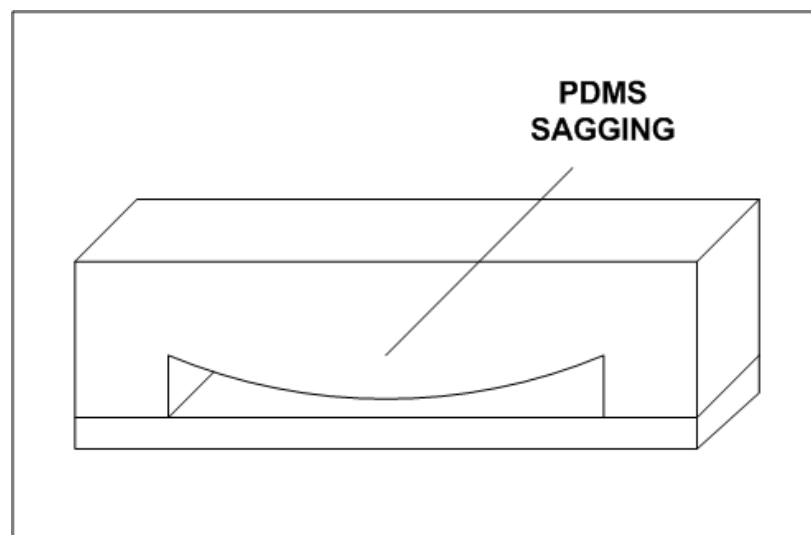


Figure 3.3. Typical cross-section of PDMS/glass where sagging has occurred. Due to PDMS flexibility, sagging can occur, restricting and blocking segments of the channel.

CHAPTER 4

CONCLUSION

In this thesis, microfluidic devices were fabricated by using two methods; photolithography and replica molding. And then fabricated devices were tested in the cell culture experiment.

In the photolithography method, once a desired thickness was chosen, a grade of SU-8 must be chosen according to the desired thickness and poured onto the wafer through a spin coater. The wafer was soft baked firstly at 65°C, then at 95°C, and then cooled down. After cooling in room temperature for 15 minutes, it was exposed to UV radiation underneath a laser-printed mask transparency. Afterwards, the wafer was post exposure baked at 65°C, then at 95°C, and then cooled down to room temperature (RT). Next, the substrate with the photoresist was developed in SU-8 developer, rinsed, and dried. All parts were performed in clean room.

In the replica molding method, PDMS and PDMS curing agent were mixed at 10:1 ratio then mixture was poured on the substrate and it was left on the bench at room temperature for the polymerization of PDMS. After that, PDMS and glass were bonded each other's and microfluidic device was fabricated successfully.

In the cell culture experiment, interaction between macrophage and cancer cell were examined in the microfluidic chip and device was tested successfully.

Consequently, novel technology did not allow investigation of distance dependent interactions (DDI) of two factors. However, our microfluidic device is allowed to investigation of DDI of two factors. There is an alternating width channel between two channels which have each of two factors. These three channels are separated from each other by colonnades, not by walls, therefore physical, chemical and biological interactions are possible between the factors. Finally DDI chip is ready for commercialization.

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