DEVELOPMENT OF NOVEL MICRO-EMBOSSING METHODS AND MICROFLUIDIC DESIGNS FOR BIOMEDICAL APPLICATIONS

DISSERTATION

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By

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The goal of this study is to develop novel microfabrication methods and microfluidic devices for BioMEMS applications. The emphasis is on the development of new hot embossing techniques, the design of microfluidic functions and biocompatible packaging methods for polymeric microfluidic chips.

First, two unconventional hot embossing techniques were developed: laser assisted and sacrificial template based hot embossing. In laser assisted embossing, localized micro patterning can be achieved on polymer surfaces with a cycle time of less than 1 minute due to the localized heating, which is comparable with that of micro injection molding. The sacrificial template based hot embossing solved the de-molding issue involved in conventional hot embossing especially for high aspect ratio microstructures. Embossing of microstructures with aspect ratio of 6 was demonstrated successfully and the possibility of laser assisted embossing in conjunction with sacrificial template embossing was investigated.

A fishbone microvalve was designed based on the concept of super-hydrophobicity such that the valve function remains after protein blocking, a required step in some enzyme-linked immuno-sorbent assays (ELISA) applications to prevent non-specific binding. Compared with another type of super-hydrophobic microvalve
developed based on the micro-/nano structure formation by chemical synthesis, the fishbone valve can be easily incorporated into the microfluidic designs. Polymer compact-disk (CD) microfluidic platform integrated with different fluidic features was designed and fabricated. We have demonstrated successfully that flow sequencing can be achieved on a CD-like microfluidic platform.

For packaging microfluidic platforms, a new interstitial bonding technique has been developed, which bonds the polymer-based microfluidic platforms without introducing any alien materials into microchannels. This method can easily bond biochips with complex flow patterns, but in a relatively smaller size. A multi-channel DNA sequencing chip was demonstrated experimentally. Another bonding method, CO$_2$ assisted bonding, was also demonstrated for bonding a 5-inch CD platform. By applying a thin PLGA interlayer, the CD platform can be bonded at low temperature and low pressure to achieve a hermetic bonding. ELISA tests showed that both bonding methods have no or little effect on the activity of preloaded proteins, which is essential for microfluidic designs that requires preloading of some regents such as proteins, antibody/antigen and cells.
Dedicated to my wife
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CHAPTER 1

INTRODUCTION

This Thesis covers two major parts: polymer micro-embossing and polymer based microfluidic biochips. They are briefly introduced in the following sections.

1.1 Microfabrication

Over the past two decades, fabrication techniques of polymer based micro- and nano-structures have been widely explored for bio- and chemical-MEMS (micro-electro-mechanical-system) applications. A variety of methods are available to manufacture micro-features including lithography, soft lithography, micro-injection molding, and hot embossing. While advances have been made with all of these methods, they remain as slow batch processes. Lithography and soft lithography techniques are multi-step batch processes that have relatively long cycle times and are expensive. Micro-injection molding is better suited for mass production although it requires longer cycle times than in conventional injection molding. Also, molds for micro-injection molding are very expensive. Among them, the hot embossing process provides several advantages such as
relatively low cost for the embossing tools, the simplicity of the process, the high replication accuracy for small features, and the relatively high throughput. A schematic of the hot embossing is shown in Figure 1.1. It can be operated in both cyclic and continuous modes. The basic principle is that a polymer substrate is first heated above its softening temperature, usually glass temperature \( T_g \) for amorphous polymers. A mold (or master) fabricated by either CNC-machining or lithographic methods with subsequent electroplating or casting procedure is then pressed against the substrate, allowing the pattern to be fully transferred onto the substrate (embossing). After a certain time of contact between the mold and the substrate, the system is cooled down below \( T_g \), followed by separating the mold and the substrate (de-embossing).

![Figure 1.1 Schematic of hot embossing process](image)

The hot embossing processes have been applied in the industry for many years and the fundamental understanding of the relationships among material properties,
processing conditions and part quality has been widely investigated [Y.-J. Juang, 2001, Part I and Part II]. Typically, polymers are processed near the glass transition temperature under the isothermal conditions in the conventional hot embossing process. However, the conventional hot embossing process has some inherent drawbacks such as the long cycle time because of the isothermal process, in which the whole substrate must be heated above its glass transition temperature before embossing and cooled down after embossing. Another common issue is de-embossing, during which damage to the mold and/or the substrate is a major mode of failure. This is the common issue for both hot embossing and micro-injection molding. Various methods have been tried to solve this problem, including using molds with positive draft angles, and surface modification of the mold, but only limited success was achieved, especially for high aspect ratio microstructures.

In this study, we report on a fast heating method to achieve the reduced cycle time comparable to that micro-injection molding by rapid heating to soften or melt the polymer on the surface while pressing it against a mold to form the micro-features. This approach capitalizes on the advantages of hot embossing while reducing the cycle time to offer an opportunity for continuous manufacturing. We have also developed a sacrificial template based micro-embossing technique, using the water soluble material to solve the de-molding issue by dissolving the template in an environmentally benign solvent. We also carried out systematic experiments and compared the part quality under various processing conditions. In addition, FEM simulation was conducted to describe the flow behavior in hot embossing process. Through such quantitative analysis, we tried to link both fast surface heating and sacrificial template technique in the hot embossing process.
1.2 Microfluidics

The demand for high-precision miniature devices and efficient processing technologies for micro-/nano-fabrication has been growing rapidly. Emerging markets include chemical and medical devices (e.g. gene-chips, hearing aids, drug delivery systems, bio-sensors, fuel cells) [Freemantle, 1999]; telecommunication components; optical components (e.g. diffraction gratings, miniature lens and mirrors); automotive crash, acceleration and distance sensors; camera and watch components [Snyder, 1999]; and mechanical devices (e.g. printer heads, micro heat exchangers).

The major technical challenges in making these microsystems include: design and implementation of necessary microfluidic functions; integration of these functions with complete automation; and development of cost-effective manufacturing technology [Madou 2001]. Microfluidics is the manipulation of fluids in channels having at least two dimensions at the micron scale. It is a core technology in a number of miniaturized systems developed for chemical, biological, and medical applications [Freemantle, 1999].

Major microfluidic components include sample introduction or loading (and in some cases, sample preparation); propulsion of fluids (such as samples to be analyzed, reagents, and wash and calibration fluids) through micron-sized channels; valving; fluid mixing and isolation as desired; small volume sample metering; sample splitting and washing; and temperature control of the fluids. A wide range of microfluidic components such as micropumps, microvalves, micromixers, flow sensor, etc., have been demonstrated. The main challenge in making miniaturized systems is the integration of different microfluidic components to perform certain functions at high speed and high throughput. Integrated microfluidic systems have the potential for applications such as
microreaction technology, on-chip flow-through-PCR (polymerase chain reaction), bio-
separation, clinical diagnostics, drug discovery and delivery, lab-on-a-chip technology,
air bag triggers, and ink jet nozzles [McDonald, 2000].

A microfluidic platform has been designed on a compact-disk (CD) for medical
diagnostics, which includes functions such as pumping, valving, sample/reagent loading,
mixing, metering, and separation. The fluid propulsion is based on centrifugal force,
which is achieved through rotationally induced hydrostatic pressure. A passive capillary
valve, which is based on a pressure barrier that develops when the cross-section of the
capillary expands abruptly, was used to control the fluid flow [Lai, 2002]. However, in
enzyme-linked immuno-sorbent assay (ELISA) applications, all the reservoirs and
channel surfaces need to be blocked to prevent non-specific binding for increased testing
accuracy. After protein blocking, these capillary valves lost their function due to the
change of the surface property.

We have developed a fishbone microvalve based on the concept of super-
hydrophobicity, which can solve the issue related to protein blocking. Various methods
to achieve a super-hydrophobic surface and the influence of protein on the surface
properties were investigated. We successfully demonstrated that, flow sequencing can be
achieved on a CD-like microfluidic platform after protein blocking by integrating the
necessary microfluidic functions such as centrifuge pumping and fishbone valving.

In most BioMEMS applications, biocompatibility is one of the main requirements
for a fabrication process due to the presence of proteins and even cells on the device. For
example, protein or antibody needs to be pre-loaded onto the channel surface before
bonding in ELISA applications. Current bonding methods usually involve high temperature, electric voltage, organic solvent, or contamination. They would de-nature pre-loaded proteins. It is essential to develop a packaging technique for microfluidic platforms compatible with pre-loaded proteins/cells. Two new methods, interstitial bonding and CO\textsubscript{2} assisted bonding, were developed to bond the polymer-based microfluidic platforms without denaturing the preloaded proteins and contaminating the microfluidic channels.

1.3 Outline

Chapter 2 contains a comprehensive literature review of microfabrication and microfluidics. Chapter 3 contains experimental and simulation of the laser assisted micro-embossing process. In Chapter 4, sacrificial template and surface heating based micro-embossing is presented. Chapter 5 describes the CO\textsubscript{2} bonding method and protein-proof fishbone microvalving design for microfluidic chips and their application in a CD-ELISA platform. Chapter 6 is the conclusions and recommendations.

In Appendix A, the study of super-hydrophobicity, which can be applied in a passive micro-valving design, is presented. In Appendix B, the interstitial bonding method is described and a case study in bonding a multi-channel DNA separation chip as well as experiments of DNA separation is reported. In Appendix C, the protocols for designing, manufacturing, and testing of a CD-ELISA chip are presented.
CHAPTER 2

LITERATURE REVIEW

Over the past two decades, the development of miniature devices consisting of one or many micromachined components and structures, i.e. MEMS (micro electromechanical system) has been growing rapidly. This is because the application of MEMS devices can be found in many areas such as optical components (e.g. sensing devices, wave guides, fiber connections, mirrors), mechanical/magnetic sensors and actuators, microfluidic devices (e.g. flow sensors, valves, pumps, mixers, channels), chemical- and medical-devices (e.g. gene-chips, hearing aids, drug delivery systems, bio-sensors, fuel cells) [Freemantle, 1999], genotyping, DNA sequencing, and so on. Most of these devices are currently built on single crystal and polycrystalline silicon (Si) materials because micro-fabrication methods for these materials have been extensively developed for the micro-electronics industry over the last four decades. However, for many applications (particularly in the biomedical field), these materials and the associated production methods are too expensive, or else the material properties often induce problems like lacking optimal clarity, having low impact strength and poor bio-
compatibility. Currently, fabrication of miniature devices favors non-silicon materials, in particular polymers because polymeric materials offer a wide range of physical and chemical properties. They also have the advantages of low cost and good processibility for mass production. According to the Yole Développement magazine for MEMS, Nanotechnology, Optics, Bio&Microfluidic Chips and Semiconductors [Oct., 2004], polymer represents about 12% of micromachined materials for MEMS manufacturing in more than 360 MEMS companies in the world. Most polymer microfluidics components and services are commercialized by American companies (Tecan, Micronics, Gyros, ….) and in 2007, Yole Développement estimates that polymer microcomponent could reach $1.5 Billion.

2.1 Polymer replication

Various approaches have been tried to replicate the micro-machined features on polymers. Reaction casting, hot embossing, and injections molding are the successful methods to produce polymeric microdevices. Lee and coworkers [Lee et al., 2001] have summarized the advantages and disadvantages of these three molding methods from various aspects as shown in Table 2-1.

A general description regarding these three techniques can also be found in the literatures [Gale, 1997; Becker and Gartner, 2000]. The difference in processing conditions between the traditional injection molding process and the modified injection molding process for microfabrication was discussed and summarized by Piotter et al. [1997]. Studies have been conducted to understand the relationship between processing conditions such as mold temperature, injection speeds, etc. and part quality [Piotter et al.,
1997; Wimberger-Friedl, 1999; Despa et al., 1998]. For the casting process, a tremendous amount of work and technique development has been done by Whitesides’ group [1995,6,7,8,9]. The issues of mold sticking and part quality have been addressed by another research group by using different castable polymers [Chiang et al., 1999].

<table>
<thead>
<tr>
<th></th>
<th>Liquid Resin Molding</th>
<th>Injection Molding</th>
<th>Hot Embossing</th>
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<tbody>
<tr>
<td>Mold Inserts</td>
<td>Any molds</td>
<td>Metal molds (Silicon molds suitable for prototyping)</td>
<td>Metal and silicon molds (Plastic molds suitable for prototyping)</td>
</tr>
<tr>
<td>Feature Size</td>
<td>No limit</td>
<td>Good for small features with low aspect ratio, or large features with high aspect ratio Good for 3D features</td>
<td>Good for small features Difficult for high aspect ratios Difficult for multiple depth Planar features only</td>
</tr>
<tr>
<td>Materials</td>
<td>Liquid resins (thermosets provide high chemical resistance)</td>
<td>Mainly low molecular weight thermoplastics</td>
<td>Low and high molecular weight thermoplastics</td>
</tr>
<tr>
<td>Processing</td>
<td>Simple (except for RIM) Easy mold filling Closed mold process Long cycle time (hr) Mold release problem for some resins</td>
<td>Short cycle time (sec -min) Closed mold process High automation</td>
<td>Simple Medium cycle time (min) Potential for continuous production Open mold process</td>
</tr>
<tr>
<td>Replication Accuracy</td>
<td>Less dimensional control (polymerization shrinkage)</td>
<td>Excellent dimensional control</td>
<td>Less dimensional control</td>
</tr>
<tr>
<td>Part Quality</td>
<td>Low molded-in stresses Contamination (resin residue)</td>
<td>High stress on mold insert High molded-in stresses</td>
<td>High molded-in stresses</td>
</tr>
<tr>
<td>Cost</td>
<td>Low tooling cost (except for RIM) For prototyping and low volume production</td>
<td>High tooling cost For large volume production</td>
<td>Low tooling cost For low and medium volume production</td>
</tr>
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</table>

Table 2.1 Comparison between molding methods [Lee, 2001]
2.1.1 Reactive casting

Micromolding based on low viscosity liquid resins (instead of high viscosity polymer melts) is a very attractive approach, since mold inserts made by photolithography techniques are limited to soft metals (e.g., nickel), silicon, quartz, and plastics. During liquid resin molding, the low viscosity reactive polymer components are mixed shortly before injection into the mold cavity, and polymerization takes place during the molding process. Both reaction injection molding (RIM) and transfer molding, two techniques widely used in conventional processing of thermoset resins, are options for mass production [Lee et al., 2001]. For new design of microfluidic devices, casting is an attractive method for rapid prototyping. Whitesides and his group at Harvard University [Qin et al., 1998; Xia and Whitesides, 1998] combined a photolithography technique with PDMS molding for microfabrication. The PDMS resin was cast onto a photoresist mold produced by photolithography on a silicon wafer and cured at elevated temperatures. The polymer replica of the master containing a negative relief of features could be easily peeled away from the silicon wafer and either used as the microdevice directly [Xia, 1996, 1997], or as a master for micro-contact printing [Xia., 1996], micromolding in capillaries [Xia, 1998], or micro-transfer molding [Kim, 1995]. This method, called soft lithography, has also been used by other researchers [Effenhauser, 1997] because of its simplicity. The long cycle time (several hours) and limitation to only PDMS rubber, however, make it difficult to use for mass production in most large scale BioMEMS applications. Nevertheless, PDMS molding has been found many applications [Bogdanski, 2004; Choi 2006; Hulme 2006]
2.1.2 Injection molding

Injection molding is based on heating a thermoplastic material until it melts, thermostating the parts of the mold, injecting the melt with a controlled injection pressure into the mold cavity, and cooling the molded polymer. Injection molding is probably the most widely used technique in macroscopic production of polymer parts.

Injection molding of parts with small features and low-aspect ratios (like CDs) has been widely applied. Currently, the main challenge is to extend this technique to the fabrication of components with smaller feature size but larger aspect ratio, needed in many medical and bio-chemical applications. In recent years, some research work has been initiated in Europe. Ehrfeld and his co-workers at IMM (Institut fur Mikrotechnik) in Mainz, Germany [Dunke et al., 1995; Ehrfeld et al., 1995], used precision injection molding machines, similar to those commonly used for the fabrication of CDs, to mold MEMS-components based on mold inserts made by LIGA. Another group at the Institut fur Materailforschung in Karlsruhe, Germany [Fahrenberg et al., 1995; Ruprecht et al., 1995; Goll et al., 1997; Piotter et al., 1999], used CNC-machined and laser ablated metal molds in microinjection molding. Wimberger-Friedl in the Netherlands [1999] fabricated sub-μm grating optical elements by injection molding. The mold inserts were made by E-beam lithography together with nickel electroplating, and by RIE in SiO$_2$ (fused quartz). In the United States, Edwards et al. [2000] used SU-8 molds and Kelly [1999] used LIGA-produced nickel molds for injection molding to make devices such as micro heat exchangers. In general, these studies showed that the molds need to fill rapidly in order to prevent early freezing. A mold temperature above the ‘no-flow’ temperature can guarantee a complete filling. Shape deviation and damage of the fragile mold walls occur.
quite easily, possibly due to shrinkage of the polymer or defective filling and release. Since the mold cavity is filled at a mold temperature that exceeds the melting point or the glass transition temperature $T_g$ of the polymer, the mold needs to be cooled down to obtain a sufficient strength before part ejection. In addition, conventional venting of the cavity is not feasible due to the presence of microfeatures in the mold inserts. Therefore, prior evacuation of the mold cavity is needed. As a result, the cycle time is five minutes or longer, including the time needed for evacuation, heating and cooling of the mold. Molding of microfeatures with large aspect ratios or the use of materials with a higher viscosity leads to even longer cycle times. Shen et al [2004] and Liou et al [2006] investigated the main factors that influence the part quality by micro injection molding. According to Liou’s study, the most important factors include mold temperature, injection pressure and polymer. Higher mold temperature will facilitate the mold filling, however, excessively high temperature will reduce the productivity, increase the cost, and degrade the polymer. So for PMMA, a mold temperature of 120-150ºC was suggested.

In recent years, the injection compression molding is gaining more and more interests in manufacturing micro/nano structures. Injection compression molding basically combines conventional injection molding and hot embossing. An extruded polymer melt is filled into the mold cavity when it is open, then the mold is closed for the polymer melt to fill the micro/nano structures. The critical factors that influence the part quality in this process is different from micro injection molding. Wu et al [2006] studied the injection compression molding of diffraction gratings. According to their study, the compression speed was the most critical factor other than the mold temperature.
2.1.3 Hot embossing

Hot embossing (or relief imprinting) [Ramos et al., 1996; Becker et al., 1999] provides several advantages compared to injection molding, such as relatively low costs for embossing tools, a simple process, and a high replication accuracy for small features. The basic principle of embossing is that the polymer substrate is first heated above its glass transition temperature, $T_g$ (or softening temperature). A mold (or master) is then pressed against the substrate, fully transferring the pattern onto it (embossing). After a certain time of contact between the mold and the substrate, the system is cooled down below $T_g$ (or softening temperature), followed by separating the mold and the substrate (de-embossing). Replication of micro- and nano-size structures has been successfully achieved [Becker et al., 1999; Kopp et al., 1997; Chou et al. 1996, Schift et al., 1999; Jaszewski et al., 1998; Casey et al., 1997 &1999; Gottschalch et al., 1999; Chang, 2005]. Adding an anti-adhesive film to reduce the interaction between the mold and the replica during embossing has also been studied [Jaszewski et al., 1997 & 1999]. Instead of the conventional nickel molds, the possibility of using silicon molds has been demonstrated due to their excellent surface quality and easy mold release [Becker and Heim, 1999; Lin et al., 1996]. Also, the use of a plastic mold in the embossing process was recently illustrated [Casey et al., 1999]. This can be achieved in either a cyclic or continuous process [Lee et al., 2001]. In a cyclic process, a metal master is placed in a hydraulic press. A heated polymer sheet is stamped by applying the appropriate force, thus replicating the structure from the master to the polymer. This constitutes a low-cost method for making prototypes. For mass production, a continuous process is preferred. A polymer sheet stretches through a temperature chamber and several masters, mounted on
a conveyor belt to continuously produce parts. The process also may incorporate a lamination station to enclose certain features. Another example of continuous embossing is the achievement of surface nanostructures [Schift 2006]

Processing parameters include thermal cycle, compression force and compression speed. The temperature difference between embossing and de-embossing determines the thermal cycle time, typically from 25°C to 40°C. In principle one could, after hot embossing, cool down the whole device to room temperature before de-embossing or, at the other extreme, one could de-emboss just below or at the glass-transition temperature. A compromise is needed: the quality of the replication may not be good if one tries to remove the master when the polymer is still soft, while cooling all the way down to room-temperature takes too long. A narrower small temperature cycle leads to smaller induced thermal stresses. Such a narrower temperature cycle also reduces replication errors due to different thermal expansion coefficients of the tool and substrate. By actively heating and cooling the upper and lower bosses, a cycle time of about 5 minutes can be achieved.

In summery, the hot embossing process provides several advantages over the other two processes because of its simplicity, relatively low tooling cost, high replication accuracy, and relatively high throughput. Depending on the press used, the hot embossing process can be divided into several groups, e.g. flat bed, reciprocating, rotary, and LIGA press. LIGA is a German acronym for Lithographie (lithography), Galvanoformung (electroplating), Abformung (molding). Generally speaking, rotary hot embossing is a well-established technology with high speeds and it is a continuous process. However, its resolution and aspect ratio are inferior to those of other types of
hot embossing techniques. On the other hand, flat bed, reciprocating and LIGA press hot embossing can only be done in a batch fashion.

Beside those three methods mentioned above, several new techniques were investigated such as infrared heating, laser assisted embossing, ultrasound embossing [Grewell, 2003; Lu, 2004 and 2005; Liu, 2005; Seunarine, 2006] and electrical resistive heating [Yao, 2002; Kimerling, 2005].

The proper operating conditions were mentioned when applying the hot embossing process for fabricating the microstructures, especially those with high aspect ratio [Heckele, 1998; Becker, and Heim, 2000]. In order to minimize the process cycle time, thermally induced stresses in the materials, and replication errors due to the thermal expansion coefficients of tool and substrate, the embossing temperature is set slightly above \( T_g \), while the de-embossing temperature is slightly below \( T_g \) (i.e. the operating temperature range is near \( T_g \)).

However, because of the near \( T_g \) processing, the polymer behaves more like a solid and cannot relax rapidly. This will result in larger compression force required to emboss the material, which leads to higher flow-induced stresses. Furthermore, higher compression force may cause the damage or wear of the mold insert more quickly. Vacuum is necessary to prevent air bubble formation owing to the entrapment of air inside small cavities and to increase the lifetime of the mold insert. Slow embossing speed is preferred for fabricating microstructures with freestanding columns or high aspect ratio because these types of structures are sensitive to lateral forces. Undercut structure cannot be constructed by hot embossing since the mold insert needs to be removed after processing. Release agents, although helping with de-embossing, are not
good for fabrication of microfluidic devices since the polymer substrate may be contaminated or the autofluorescence of the polymer will tend to increase.

2.2 Mold materials and operation parameters

Many research groups have been fabricating micro-/nano structures by means of hot embossing due to its various applications mentioned previously. Rode and Hillerich [1999] embossed metallic materials (Al, Cu, and so on) to produce grooves for self-aligned positioning of microoptical components such as optical fibers, microlenses, glass rods with mirrors or filters, etc. The results showed that the lateral shape deviation remains below 6 µm for the vertical depth less than 300 µm and the shape is sufficient for self-aligned positioning. Furthermore, since a tool-steel mold was used, it may be expected to last for thousands of embossing cycles without reconditioning. Pan et al. [1999] used a nickel mold insert to emboss an array of microlenses (80 µm in diameter and 200 µm in depth) on a polycarbonate film (500 µm). Processing conditions such as embossing pressure and temperature were discussed in order to produce the microlenses. They found that, at pressure equal to 0.6 MPa, microlenses could be produced at 170°C embossing temperature while, below this temperature, the structures on the mold insert could not be fully transferred onto the substrate. On the other hand, at 170°C, a small amount of increase of pressure will not affect the dimension of the final product too much as long as the minimum pressure requirement is reached. Embossing time is another factor mentioned which may affect the refractive index of polycarbonate after embossing. Locascio et al. [1999] used a wire-imprinting technique to make a channel with 25 µm in diameter on three different plastic materials to study electroosmotic mobility. Optimal
conditions were summarized for fabrication and sealing of the plastic device. Lee et al. [2000] used a quartz template as a mold insert to emboss PMMA to fabricate a micro capillary electrophoresis device for DNA separation. Good reproducibility of channel was reported with relative standard deviation of channel profile less than 1%. Becker et. al. from Jenoptik Mikrotechnik have explored the feasibility of applying the hot embossing process to produce Miniaturized Total Analytical Systems (µ-TAS) and high aspect ratio structures [1998; 1999a, b; 2000]. The following summarizes their recommended processing conditions:

1. The thermal cycle (which is the temperature range from embossing to de-embossing temperature) should be 25 to 40°C in order to minimize the thermally induced stresses.
2. The embossing pressure is around 0.5 to 2 kN per cm².
3. Automated de-embossing is crucial and required if the structures have vertical walls and are with high aspect ratio.
4. For embossing high aspect ratio structures, the surface of side walls of the mold needs to be maintained as smooth as possible in order to minimize the friction force between the mold and polymer substrate. 80 nm RMS is an empirical limit for making structures with an aspect ratio higher than 0.5. Also, a small draft angle shown in Figure 2.3 will ease this constraint. In addition, the thermal expansion coefficients of the mold and the polymer need to be taken into consideration due to the additional force caused by differences in shrinkage of mold and polymer.
5. Mold release agent or plasticizer is not desirable if fabricating a biochemical or biomedical device due to the sample contamination or an increased fluorescence background.

Other issues have also been addressed. For example, in addition to traditional tool-making materials such as the computer numerical control (CNC)-machined tool steel, and electroplated nickel, silicon [Lin et al., 1996; Becker, 1999], glass [Niino, 2004] thermoplastic [Casey, 1999] and thermosetting polymer [Iu, 2005; Koerner, 2005] molds have also been fabricated. The advantages of using silicon molds can be summarized as follows:

1. suitable material properties in terms of tensile strength, hardness, thermal conductivity, etc.
2. variety of fabrication methods for different features
3. flat and even surface which is good for mold release

The use of thermoplastic molds reduces the cost and increases the speed of mold making. Electron beam lithography was performed to directly pattern on PMMA. The basic idea is to select two different polymers with different glass transition temperatures with higher $T_g$ polymer being the mold material.

Applying a thin anti-adhesive film on the mold was investigated by Jaszewski et al. [1997; 1999]. They found that the plasma-deposited film has better anti-adhesive properties than the sputtered one. Furthermore, the film would lose its anti-adhesive properties owing to multiple embossings, longer embossing time, and higher embossing temperature. The higher embossing temperature also destroyed the bonding between the
film and the mold. They concluded that the PTFE-like films could have both adhesion to a metal shim and anti-adhesion to the embossed thermoplastics.

### 2.3 Polymeric substrate materials

Polymer issues were studied by Gottschalch et al. [1999]. They embossed PMMA with different molecular weights under 100 bars at two different temperatures, i.e. 50 and 90°C above \( T_g \). They stated that, at a temperature 90°C above \( T_g \), the flow is sufficient to transfer large and isolated features even into the polymer with the highest molecular weight they used. The lack of parallelism may lead to uneven distribution of pressure, which results in local inhomogeneities.

Schulz et al [2004] studied the influence of molecular weight of polystyrene (PS) on hot embossing. They discussed the shear rate effect and recovery that may result in the local non-uniformity. It was found that a higher molecular weight favors imprinting at lower temperatures and a medium value of \( M_w \) with \( M_w/M_n=2 \) was suggested.

The film embossing process has been investigated by Haber and Kamal [1992]. Both tubular blown film embossing and batch embossing process were studied and low density polyethylene (LDPE) was used. Since the analysis of the process requires the study of various aspects relating to the characterization of the microstructures before and after the embossing, they started with flat film analysis, followed by the heat transfer analysis and stress analysis in which the contact and indentation problems were addressed. Finally, the embossed film was analyzed in terms of mechanical properties, physical dimensions, morphology, and pattern quality. For the flat film analysis, the orientation of crystalline and amorphous phases was examined. They found that the
orientation of the crystalline phase was mostly in the machine direction but might occur in some other directions due to reorientation. The orientation of amorphous phase was in the transverse direction in the film plane. There existed a shift of maximum value of tensile modulus from the machine direction to the transverse direction while a shift of maximum value of ultimate tensile strength and elongation from the transverse direction to the machine direction as the blowup ratio increased. Also, the gloss data showed the combination of crystallization-induced and die-flow-induced surface roughness on the film. For heat transfer analysis, energy equations for preheat roll, free-moving film, radiation heater system, and embossing roll were considered. In preheat roll region, mainly the conduction and the convection were involved. For the free-moving film region, the convection was considered. The radiation heater system involved radiation and convection, and the embossing roll involved mainly conduction. Through the heat transfer model, the film surface temperature was estimated as a function of plastic film thickness, film velocity, roll temperatures and radiation heater temperature set point. As to the stress analysis, the contact and indentation issues were taken into consideration. The contact issue (contact of two rolls with one roll having an elastic cover) was treated using Hannah’s equation. For the indentation issue, both Harding and Sneddon’s equation and Dhaliwal and Rau’s methodology were used and compared. Yielding of the plastic film during embossing was also discussed. Only at the edge of the cylindrical punch did the stress exceed the critical yield value of LDPE and plastic deformation occurred. The embossing pressure and the rubber layer on the backup roll are important factors in determining the dimension and stress distribution in the nip region. The embossed film analysis was conducted by measuring several quantities such as embossed
film thickness, bulk thickness, shrinkage, a-axis orientation, crystallinity, infrared dichroic ratio, sonic modulus, tensile properties, pattern quality and texture, and surface gloss. They concluded that the thermal treatment of the plastic film played the most significant factor in determining the embossed film quality, e.g. higher film temperature yielded higher pattern quality but increased the degree of lateral shrinkage, higher temperature shifted the properties away from the initial values determined during the film blowing process, etc.

The flow behavior of thin polymer film during hot embossing was studied by Heyderman et al. [2000]. Unlike the common hot embossing process in which the embossing temperature is approximately 20 to 40°C above T_g, they embossed a thin film of PMMA at a temperature >100°C above its T_g. Filling the microcavities and demolding were investigated. PMMA with different molecular weights was used and the embossing force was from 10 to 40 kN. Processing conditions to fill up the microcavities were provided and the embossing time for complete simple filling of stamp mold cavities can be predicted by two-dimensional squeeze flow theory. They found that the thinner the polymer film, the higher the embossing temperature and the longer the embossing time. Doubling the embossing force provided a reduction by 20°C of embossing temperature with the same embossing time. Under the same embossing force, a drop of 20°C of embossing temperature increased the embossing time from 2 to 10 minutes for complete filling the cavities. Polymers with higher molecular weight require higher embossing temperature, force or time to fill up the cavities. For the filling of cavities, two filling mechanisms were observed, i.e. simple flow of polymer from the borders and the formation of the mounds of polymer within the cavity, which were resulted from
capillary action and the compression causing buckling of the polymer. As to demolding, the reasons for distortion or damage of the molded part were discussed such as the adhesion at the surface, surface roughness of the mold, and the negative slopes of the cavity sidewalls.

The non-isothermal embossing has also been widely investigated [Juang, 2002; Lu, 2005; Yao, 2005]. In non-isothermal embossing, a heated die is translated into the cold substrate. The material near the wall is heated up, compressed downward by the mold and squeezed outward. It was found that mold cavity plays a role in the flow pattern formation. For larger cavity thickness, a wall-climbing flow pattern was observed, but not for smaller cavities.

2.4 Master fabrications

Fabrication of the mold inserts (or masters) is another important issue since they are the basic elements in the hot embossing process. They can be fabricated by a variety of techniques. For large features (> 50 µm) with tolerances and repeatability in the range of about 10 µm, traditional CNC-machining can be applied. The advantage of this technique is that a wide range of materials can be machined, especially tool steel and stainless steel, which are the common tool materials used in conventional polymer molding. Hence, their design, strength, and service life are well established. Complicated 3D structures can also be machined easily. In addition, the development time of the mold insert can be shorter since no mask fabrication or photolithography are involved. The disadvantages are that it is difficult to make sharp corners or right angles, high aspect ratio structures, very deep holes or very small structures. Also, the surface quality is
usually poor (surface roughness around several µm). Diamond-based micro-milling or micro-drilling [Warrington, 1999], and excimer or femtosecond laser-based [Momma et al., 1997] direct removal processes can reduce the surface roughness to 1 µm or less [Roberts et al., 1997]. While diamond-based methods can also make features smaller than 10 µm, they are only applicable to ‘soft’ metals such as nickel, aluminum, and copper. For smaller feature sizes (down to sub-micron), lithographic methods and silicon micromachining have to be employed [Madou, 1997]. For the lithographic methods, either a photoresist structure on a galvanic starting layer which is used directly for electroplating or a combination of silicon etching and electroplating, which is called DEEMO (deep etching, electroplating, molding), can yield a metal tool, usually nickel or nickel-cobalt. A silicon wafer serving as a mold insert can be constructed through silicon micromachining either by wet or dry etching (e.g. reactive-ion etching (RIE), advanced silicon etch (ASE) or the Bosch process). For very small features (< 1 µm) with high aspect ratios (up to 100 or higher), technologies like LIGA [Madou, 1997; Ehrfeld and Lehr, 1995; McCormick, 1997] in thick resists (like EPON SU-8) are needed to obtain the mold insert. Lithographic methods and dry etching can produce molds with excellent surface quality (surface quality <0.1 µm), and sharp corners or right angles. However, they cannot be used on conventional tool materials like steel. For non-planar, complicated structures, the mold is designed in several planar slices, which in the end need to be assembled to form the completed system [Madou, 1997; Ehrfeld and Lehr, 1995].
2.5 Nanostructure replication

2.5.1 Hot embossing lithography (HEL)

In addition to micro-structures, research has also been conducted to study the feasibility of making nano-structures. Jaszewski et al. [1998] and Schift et al. [1999] applied so called Hot Embossing Lithography (HEL) techniques to produce dots and lines with nano-scale. They have successfully replicated features with size as small as 50 nm. Basically, a nano-structured shim (mold) was used to imprint a polymer film attached to a hard substrate, followed by either a lift-off or direct etching process to produce the nano-structures. A higher embossing temperature (130 to 190°C for PMMA) and vacuum were applied in order to ensure the polymer filled the cavities. Furthermore, an ultra-thin teflon-like film was deposited onto the mold to minimize the frictional force and avoid sticking dust particles. Note that there will be a loss of aspect ratio compared to the original master mold in this process. Casey et al. [1997] developed a process which can be used to fabricate nano-structures with feature size as small as 60 nm. The embossed material is cellulose acetate and the embossing temperature and time are 135°C and 30 minutes, respectively. Due to the properties of cellulose acetate, a nano-scale environment was created by directly patterning the polymer with UV light wavelength less than 254 nm. They also concluded that the limit of embossing technique is controlled by the resolution of the master fabrication process.

Chou et al. from Princeton University is another research group which has been making nano-structure by means of the hot embossing lithography process [1996a, b; 1997; 1998a, b; 1999]. Structures with feature size ranging from 10 to 100 nm were fabricated using PMMA and light-emitting materials. Both flat bed and roller types of
embossing have been studied. They called it as nanoimprint lithography (NIL). The flat bed type is similar to the HEL process mentioned previously. Potential applications of these nano-structures include silicon quantum dots, wire and ring transistors, and a nano compact disk (CD) with 400 Gbits/in² storage density (near three orders of magnitude higher than current CD). The following summarizes their work:

1. Mold release agent was added into the PMMA resist to reduce the adhesion between the polymer and mold.
2. Typical imprinting temperature and pressure in their study were 140 to 180°C and 600 to 1900 psi, respectively.
3. Vacuum was applied during imprinting.
4. Good process repeatability, mold durability and structure uniformity over large area were observed.
5. For the roller nanoimprint lithography, the scan speed of roller was from 0.5 to 1.5 cm/min, and the pressure was changed from 300 to 4800 psi. In the cylinder mold method, platform temperature is 50°C and the roller temperature is 170-200°C, while the flat mold method involved 70°C for the platform and 170-200°C for the roller in order to produce the best result.

Other polymers such as cellulose acetate and standard optical resist S1805 were also employed in NIL at a lower imprint temperature. Uniformity is as important as high resolution for NIL but it seems difficult to achieve desirable results for both at the same time. The flow behavior, the shrinkage and etching resistance can be different in different regions. So far, the resolution of 90 nm can be uniformly generated over the entire 4-in wafer. Just like devices produced by hot embossing, the aspect ratio of NIL features
cannot be high. A little high aspect ratio can be obtained by multilayer (bilayer or trilayer) pattern. Using the same technique, NIL can even transfer patterns to non-planar surface. In order to manufacture NIL nanostructures, continuous process can be applied, using either a bend mold or a smooth roller.

In addition, Chou and his co-workers demonstrated that when a patterned mask is moved close to several hundred nanometers above a viscous polymer liquid (above T_g for PMMA films), periodic pillar array or concentric ring would be generated spontaneously [Chou, 1997]. They explained this phenomenon as the results of the interplay between the attractive interaction and hydrodynamic instability. They called this lithographically induced self-assembly process (LISA) as a new pattern transfer technique. Using polystyrene as the material, Schaffer et al [2001] demonstrated that the resolution of LISA process could be raised to the 100 nm range with the aid of an electric field.

Other applications of NIL include chemical patterning in nanometer region [Park, 2003], and preparation of micropatterned cell substrates [Charest, 2004].

2.5.2 Soft lithography

Using the patterned elastomer (usually PDMS) as the mold, Whitesides’ group at Harvard University developed a series of pattern transfer approaches, including microcontact lithography (µCP) [Xia,1996], micromolding in capillaries (MIMIC) [Kim,1995], solvent-assisted micromolding (SAMIM) [Kim,1997], and microtransfer molding (µTM) [Zhao,1996]. Soft lithography is the collective name for all these lithographic techniques. Compared to hot embossing and nanoimprint lithography, soft lithography employed solvents to soften polymer material, instead of heating material.
Surface properties are very important for pattern formation in soft lithography. It offers a simple process with high-resolution (~$10^1$-$10^2$ nm) and some of them can be used for non-planar substrates, unusual materials, and large area patterning.

For example, microcontact printing (µCP) enhanced the development of self-assembly membranes (SAMs). It is simple process that may offer low cost, fast mass transfer of micro structures. In µCP, the PDMS stamp is wetted with some kind of ink and then is brought into contact with the substrate surface. The ink is a chemical that can form SAMs on the substrate. Since the chemical is only transferred in the patterned area by the stamp, the SAMs are generated with the same pattern on the substrate. These SAMs patterns can either serve as resist protecting the underlying substrate in wet etching or as templates to selectively deposit other materials.

2.6 Chip packaging

The need for bonding arises from either the packaging requirements or the assembly of MEMS devices. Bonding between silicon and silicon or other materials (e.g. glass, metal, etc.) can be achieved by different methods such as anodic bonding, fusion bonding, eutectic bonding, and adhesive bonding [Schmidt 1998].

Adhesive bonding has been in widespread use for over 30 years. Adhesives can be made electrically/thermally conducting (e.g. silver loaded epoxy) or electrically isolating. Typical adhesive materials include epoxy thermoset resins, acrylic thermoplastic resins, and silicone resins. High pressure (>1 MPa) is usually necessary for adhesive bonding. An eutectic bond is formed by heating two (or more) materials (e.g. Au and Si) in a joint such that they diffuse together to form an alloy composition that melts at a lower
temperature than the base materials (e.g. a Au-Si eutectic melts at 363°C). For fusion bonding, temperatures above 1000 °C need to apply for silicon fusion bonding, while temperatures around 600 °C are necessary for glass fusion bonding. The bonding of silicon to glass (anodic bonding) is performed at a temperature ranging from 300 to 500 °C, with an applied voltage ranging from 500 to 1000 volts. These methods usually involve high temperature, high voltage, or high pressure. Several low temperature-bonding technologies (100 ~ 200°C) have been developed [Sayah et al., 2000]. However, they usually need assistance from hydrofluoric acid (HF), plasma, or high pressure (up to 50 MPa). Therefore, among these bonding techniques for silicon/glass materials, only adhesive bonding may be applied to polymer-based microfluidic devices.

Among the above-mentioned techniques, only the adhesive bonding can be applied for polymer-based MEMS devices. Lamination by adhesive tapes or thermal adhesive films is probably the simplest and fastest one, but at the risk of creating channels with different top and bottom surfaces. When using the adhesive bonding, care needs to be taken in order to prevent the adhesive from flowing and filling up the micro channels during bonding. Although several studies have been conducted to investigate and explore a better bonding technique for polymer-based MEMS devices, it still remains as a challenging task.

Various bonding techniques for polymer-based MEMS devices were briefly summarized in a patent filed by Caliper Technologies Corporation [1999]. They include thermal bonding, ultrasonic bonding or welding, adhesive bonding, and solvent bonding. For thermal bonding, basically the substrate is heated up above its glass transition temperature (T_g), followed by applying compression force and cooling down below its T_g.
Although this method is simple and straightforward, deformation of polymer substrates will occur during bonding, leading to undesired part quality. For example, the channel dimension can change due to the compression pressure, variable chamber volume because of the extrusion of upper substrate into the cavity, shrinkage or warping. For ultrasonic welding or bonding, several protrusions are fabricated as energy directors. At elevated pressure and high frequency vibration, these energy directors melt and bond with the corresponding surface on the other substrate. Some problems may occur, for instance, the edges of the bonded regions tend to be relatively irregular compared to the edge of the channel. This will lead to either material encroaching into the channel or openings between the edges of the channel and bonded region. For adhesive bonding, in addition to the filling-up-channels problem mentioned previously, one also needs ensure the channels and chambers are sealed completely after bonding and avoid introducing unwanted chemicals into the devices as well. Solvent bonding is based on the softening and re-hardening of the polymeric materials to obtain the bonding effect. A solvent can be applied to partially dissolve the bonding surfaces, and evaporating the solvent will bring two halves together. This can solve the problem with dissimilar materials at the interface. In addition to the same contamination problem encountered in adhesive bonding, possible channel distortion and stress cracking on the plastic substrate may occur. In this patent, authors applied the thermal bonding technique but with additional raised ridges on the lower substrate, which has micro channels. The upper substrate has a higher $T_g$ than that of the lower one. All the ridges collapse or melt under elevated temperature and pressure, providing bonding points for both substrates. They also
claimed that these ridges could serve as energy directors for ultrasonic welding or bonding.

2.6.1 Solvent bonding

Lum and Greenstein [1999] prepared microdepressions on one substrate and microprojections on the other so that the substrates can be mated together to secure the relative position. A layer of monomer or pre-polymer was deposited on the microprojections before being mated and further polymerized to provide a bonding effect. Dreuth and Heiden [1998] applied a thin adhesive film on a substrate and the adhesive was then transferred to the elevated microstructures by stamp printing. In order to obtain a successful bonding, selection of adhesive and processing parameters need to be well controlled such as rheological behavior and surface energy of the adhesive, stamp printing pressure, geometry of the microstructures, etc. Photoresist itself can also be used (e.g., SU-8 and Polyimide [Metz et al., 2001]) as a bonding agent to fabricate photoresist-based microfluidic devices. Glasgow et al. [1999] introduced a solvent bonding technique in which a layer of polyimide precursor and solvent with dissolved precursor was placed in contact with patterned structures made of uncured polyimide precursor. The two halves were then cured with weights on top of the upper plate. They found that the bond quality was affected by the vent spacing for solvent evaporation, soft-bake duration, spin-coat speed during solvent application, and the concentration of the dissolved polyimide precursor in the solvent. However, as the solvent dissolves the bonding surface, it also partially dissolves the microchannel itself. Dimension control becomes quite difficult for very tiny channels.
McCormick et al. [1997] successfully applied a thermal activated adhesive (Top flight MonoKote, Great Plane Model Distributors, Champaign, IL) to bond a Mylar sheet with an injection molded acrylic microchip for DNA separations. Rossier et al. [1999] used a 5 µm thick polyethylene adhesive layer to seal UV laser photo-ablated polyethylene terephthalate (PET) microchannels with a PET film for electrophoretic separations. According to Klank et al [2003], the PMMA microchip can be easily bonded after the chip was soaked in a solvent such as ethonal for 10 minutes. The chip assembly was placed between a press and the temperature was set to 85°C for 90 minutes. Although there was some deformation of the microchannel, it is usable for large sized microchannels.

A gas-assisted bonding technique was developed by Lai et al [2004]. An UV curable resin was introduced into the microchannel and the resin will fill the gap between the lid and microfluidic substrate. Then, the resin was removed by vacuum or compressed gas and the assembly was illuminated by the UV radiation. The advantages of this method include surface smoothness of the channel surface and local modification of the channel surface with a photomask. For an example, the hydrophobicity/hydrophilicity of the channel surface can be modified locally.

A novel micro/nano bonding technique was developed by Yang et al [2004]. They used a sub-critical CO₂ gas as the solvent to increase the chain diffusion at the joining interface of polymeric chips. CO₂ gas has a good solubility in polymers, therefore can increase the free volume of the polymer chains and decrease the surface Tg of the polymer substrate. Nano-sized feature bonding was demonstrated by this technique.
Thermosetting plastics or dissimilar materials cannot be effectively jointed by solvent bonding. Stress cracking of components occurs more likely with this method than other bonding methods. Furthermore, the use of solvents in a production line becomes more problematic, as environmental regulations and operator safety become prime issues for manufacturers throughout the world.

2.6.2 Ultrasonic welding

While adhesive bonding does not need thermal control, especially for room temperature curing, it requires long cure times and it introduces a consumable that may over time affect the performance of the device. Welding methods are very fast and they do not introduce foreign materials in the assembly and packaging. Ultrasonic welding is a widely used welding method for joining plastic materials together due to its simplicity of use and rapid joining potential. In ultrasonic welding, high frequent ultrasonic energy is applied to produce mechanical vibration. Usually horns and traps need to be fabricated around the designed joint area. The material at the joint surface (horn) melts resulting from the heat generated due to the mechanical vibration. Traps are used to prevent molten plastic material flowing into the designed joint area. This can pose problem for bonding of microfluidic device with very tiny channels (e.g., less than a hundred microns) because it would be difficult to fabricate even smaller horns and traps around the microchannels. This process can be done in a very fast manner (e.g., several seconds) depending on substrate materials, joint design, and the ultrasonic energy applied. However, the ultrasonic welding limits to thermoplastic materials with similar melting point. In addition, significant investment of capital equipment is necessary.
In general, these bonding techniques alter the surface of the microdevices by using external forces (e.g. solvent, adhesive, ultrasonic, laser) and applying pressure to bring two halves together. However, the same driving force that allows the bonding also tends to deform the channel shape. Therefore, these methods have problems with either blocking the microchannels or changing their dimensions and are mainly applicable for relatively large microchannels (several hundreds of microns to millimeters).

2.6.3 Laser welding

Of the wide range of welding methods that are available, laser technology offers the greatest thermal control. One application of laser welding that offers great promise of success is Through Transmission Infrared Welding (TTIr). It works by passing IR radiation through one of the parts while the radiation is absorbed by the other part at their interface. This causes the melting of the base part, which in turn, due to direct contact results in melting of the transmitting part and the formation of a weld bead at the part interface [Becker, 2001; Grewell, 2002(a); Kim, 2001; Russek, 2001]. Recent work on TTIr microwelding shows that this approach has great potential but requires more work to make it faster and more robust. For examples, more work is also needed to characterize the joints and the residual stress levels and their effects on performance.

Compared with other welding methods, laser welding of plastics offers a wide range of advantages. For example it is a non-contact method and the energy is localized. The laser energy for welding of plastic parts can be applied as a spot, line or an area. If the laser is applied as a spot, it can be scanned across the faying surfaces by steering of mirrors or translation of the parts. Often this type of application is referred to as contour
welding. The main advantage of contour welding is its flexibility; virtually any welding path can be programmed. In contrast, simultaneous welding utilizes one or more laser spots optically steered by high-speed mirror or light guides.

Line scan micro laser welding studies using scan TTIr were widely performed. For example, D. Grewell [2003(a) and (b)] studied polycarbonate (PC) and polystyrene (PS) sheets with and without microchannels. A single diode laser, manufactured by Coherent, with an 828 nm wavelength (F-system) was used as a laser source. Figure 2.1 shows the experimental setup. The effect of laser power and travel speed on melt width was evaluated. In addition, the effect of laser power, travel speed, and pressure on weld quality was determined from burst pressure testing. Welds lines that are as narrow as 11 µm were produced.

![Experimental setup for TTIr scan microwelding of PC and PS](image)

Figure 2.1 Experimental setup for TTIr scan microwelding of PC and PS
The main advantages of simultaneous welding are its relatively short cycle time and ability to allow melt down of the parts. Mask welding uses a laser line to produce a weld, but unlike simultaneous welding as shown in Figure 2.2, it uses a mask to block the transmission of the laser line in order to define the weld geometry. The main advantage of this process is that it allows for precise and relatively small weld lines. Weld lines as narrow as 100µm have been successfully made with the mask welding process [Ouellette, 2003]. In addition, this process allows the possibility of producing welds with elaborate structures or contours.

Figure 2.2 Schematic of mask welding

[http://www.nanosciences.us/pdfs/mask.pdf]

However, in sealing of microfluidic devices, standard joining methods are typically not applicable because they produce flash that can block the microchannels. In some
experiments, this problem has been solved by placing the weld some distance away from
the weld [Lee and Shen, 2001]. This gap may trap small amounts of analyte such as DNA
or gas bubbles during sample loading and testing. Another less practical solution is to
very accurately control the laser and positioning.

2.7 De-molding issue

High aspect ratio metal structures could be molded and de-molded with polymers
quite easily, as long as a polymer with a small adhesive power and rubber-elastic
properties, such as silicone rubber, is used. However, rubber-like plastics have low shape
stability and would not be adequate for high aspect ratios. Shape-preserving polymers on
the other hand, require a mold with extremely smooth inner surfaces to prevent form-
locking between the mold and the hardened polymer after hardening. De-molding has a
geometry aspect to it i.e. smooth and slightly inclined mold walls and a chemical aspect
i.e. mold release agents. A mold release agent may be required for de-molding. Using
external mold release agents is difficult because of the small dimensions of the
microstructures to be molded, as typically they are sprayed onto the mold. Consequently,
internal mold release compounds need to be mixed with the polymer, without
significantly changing the polymer characteristics.

Poor de-embossing may damage the molded parts and/or the mold. Typically, an anti-
adhesion layer can be coated on the mold surface in order to facilitate demolding. In
compact disks (CDs) manufacturing, aluminum layer has served for this purpose. For hot-
embossing, ion sputtering or plasma polymerization of thin (~ 5nm) Teflon-like film has
provided clean de-embossing. However, some materials tend to transfer from the Teflon-
like film to the embossed polymer during embossing [Jaszewski, 1997 and 1999]. Using alkanthiolate self-assembled monolayer as anti-adherent molecular coating on the copper-made mold has showed good de-embossing performance in nanoscale embossing [Azzaroni, 2001], Internal mold release agent must be used, which tends to reduce the thermomechanical properties and increase the autofluorescence of molded polymer [Becker and Gartner, 2000]. External mold release agents are often unsuitable, since they are difficult to introduce into the micro-features of the mold insert and may contaminate surfaces of molded parts. Using fluorine plasma to treat the mold surface was also reported [Yan, 2005]. It was found that CF$_4$ plasma treatment will form a thin layer macromolecular fluorocarbon on the PDMS mold surface, which reduced the surface energy and therefore, reduced the demolding force. However, when the treating time increases, the etching effect of the plasma will dominate and therefore, the surface roughness will increase.

Esch et al [2003] investigated the influence of the mold fabrication methods on the quality of embossed microfeatures. They suggested that positive draft angle will facilitate the de-molding process. If the mold has a close thermal expansion coefficient such as a polymer mold, the embossed sample will have a better surface quality and there is no difficulty for de-molding. But the mold can only survive low embossing cycles. A silicon mold is more robust. However, the mold feature tends to be broken because of its brittleness.

The de-molding step in LIGA is carried out by means of a clamping unit at preset temperatures and rates. De-molding is facilitated by slightly inclined mold walls and an internal mold release agent such as PAT 665 (Wurtz GmbH, Germany), normally
employed with polyester resins [Hagmann, 1988]. The optimum yield with this agent and Plexit M60 PMMA occurs at 3 to 6 wt%. The yield drops very fast below 3 wt% as adhesion between the molded piece and the mold insert becomes stronger. These adhesive forces are estimated by qualitatively determining the forces necessary to remove the plastic structures from the mold insert. An upper limit is 10 wt% where the MMA does not polymerize anymore. Above 5 wt%, the Young’s modulus and, hence, the mechanical stability decrease, and above 6 wt%, pores start forming in the microstructures. The internal mold release agent also has a marked influence on the optimum de-molding temperature. The de-molding yield decreases quickly above 60°C for a 4 wt% PAT internal mold release agent. With 6 wt%, one can only obtain good yields at 20°C. The molding process initially led to a production cycle time of 120 min. For a commercial process, much faster cycle times are needed, for instance by optimizing the mold release agent. With that in mind, the KfK group started to work with a special salt of an organic acid leading to a 100% yield at a release agent content of 0.2 wt% only and a temperature of 40°C (at 0.05 wt% a 95% yield is still achieved). At 80°C, a 100% demolding yield was obtained and, significantly, a cycle time of 11.5 min was reached. During these experiments the mold was filled at 80°C and heated to 110°C within 7.5 min. As the curing occurs at 110°C, the material needs to be cooled down to 80°C for demolding. Moreover, the 0.2 wt% of the ‘magic release agent’ did not impact the Young’s modulus and the glass transition temperature of Plexit M60 [Hagmann, 1988].

Simulation of de-molding has also been conducted. Schomburg et al [2005] worked on both experimental and simulations of large-area hot embossing using commercial software of MOLDFLOW and ANSYS. They focused on the de-molding forces and
shrinkage of the molded part and reported the importance of additional structures around the micro features on the reduction of contact force during de-molding.

Another research group, Worqull et al [2004] suggested a two step simulation, i.e. the simulation of mold filling and de-molding using two kinds of software because of the available models of different software. For mold filling, MOLDFLOW was used and for de-molding, ANSYS was used.

From the above, we can see that the relationship between the material properties, processing conditions and parts quality has been widely investigated. There are still many issues remaining to be solved such as de-molding, long cycle time, automation, etc. On the other hand, there may be well-established information available for the continuous hot embossing process with commercialized equipment in market, the structure made is with low aspect ratio and the features are relatively simple and repetitive. The information may not be suitable for the situation where the devices are either complicated, or with dimension in micron range or higher aspect ratio. It is the object of this thesis to investigate the hot embossing process through new process development. For instance, the effects of different embossing methods, i.e. isothermal vs. non-isothermal embossing on the capacity of hot embossing and part quality will be studied. Furthermore, the potential applications of these new embossing methods in larger size microdevice such as CD-ELISA chip will be tested and discussed.

2.8 Microfluidic devices

Microfluidic devices are utilized in a variety of applications to control the flow of microliters of fluid or gases. Perhaps the best known commercial applications of
Microfluidic devices are inkjet print cartridges and lab-on-a-chip cards as shown in Figure 2.3. However, these devices represent only the early stages of this new and exciting technology. A variety of new innovations for manipulation of the fluid and its molecules including pumps, valves, and molecular separators bring about new applications, especially in the biomedical field [Ouellete, 2003]. These include detectors for biological agents, detection of specific proteins and cells, separation and replication of RNA and DNA and drug delivery devices. For example with microcapillary electrophoresis chips (μ-CE) [Lee, 2001], the time for analysis is reduced from hours to seconds due to miniaturization effects [Becker, 2001].

Microfluidic devices are also beginning to be used in the rapidly growing field of genomics. Prototypes for detecting substitution of an amino acid in a protein or for selectively amplifying specific gene segments are currently being developed. Other potential growth areas include devices for chemical separation and detection, and metering and control of miniature reactors for unstable or expensive chemicals. As assemblies of microfluidic devices together with microelectronics and Micro-Electro-Mechanical-Systems (MEMS) devices are developed an even wider range of applications becomes possible.

Figure 2.3  Photograph of "lab-on-a-chip" product
Until recently fabrication of microfluidic devices was done through conventional lithography techniques with silicon and glass substrates [Becker, 2000]. The appeal and applications for microfluidic devices have increased and will continue to increase with the development of faster and less expensive fabrication methods and the use of polymer materials. The use of polymers for these applications offers significant advantages [Becker, 2002]:

- Low-cost manufacturing
- Chemical resistance
- Electrical properties
- Optical properties

But possibly the biggest advantage of polymer based microfluidic devices is that they may allow many devices to be inexpensive and disposable. In the biomedical industry, the use of disposable devices eliminates the sterilization risks associated with reuse. This can be instrumental in cost savings, but most importantly it helps to save human lives.

With the latest advancements in lithography, microinjection molding, and related technologies, it has become possible to manufacture thermoplastic components with features in the range of microns. For example, it is possible to produce features on the order 1-10 µm with embossing techniques. In addition, studies in microinjection molding have shown that it is possible to mold complex components (gears) with similar dimensions [Kukla, 1998]. New developments for consistently producing plastic
components with precise micro-features more rapidly and economically will further increase the applications of microfluidic devices as well as other devices.

2.8.1 Microfluidic functions

The major technical challenges in making a miniaturized biomedical instrument include: design and implementation of necessary microfluidic functions; integration of these functions with complete automation; and development of cost-effective manufacturing technology [Madou 2001]. Microfluidics is the manipulation of fluids in channels, with at least two dimensions in the micron scale. Microfluidics is a core technology in a number of miniaturized systems developed for chemical, biological, and medical applications [Freemantle, 1999].

Major microfluidic components include sample introduction or loading (and in some cases, sample preparation); propulsion of fluids (such as samples to be analyzed, reagents, and wash and calibration fluids) through micron-sized channels; valving; fluid mixing and isolation as desired; small volume sample metering; sample splitting and washing; and temperature control of the fluids. A wide range of microfluidic components such as pumps, valves, mixers, and flow sensors has been demonstrated [Gravesen et al., 1993]. The main challenge in making miniaturized systems is the integration of different microfluidic components to perform certain functions at high speed and high throughput.

2.8.1.1 Pumping

Various microfluidic propulsion technologies have been reviewed and compared by Madou et al. [1998, 2000] with regard to the choice of materials, the maturity of the
technology, and the achievable volumetric flow rates. In general, the fluid propulsion can be generated mechanically, electrically, or thermally. In the pressure-based approach, a mechanical pump is often used to provide the driving pressure. The pump can be as simple as a roller in the blister pouch design [Madou and Kellogg, 1998; Findlay et al. 1993] or as complicated as a miniaturized syringe or acoustic pump [Madou and Kellogg, 1998; Moroney et al., 1990]. The former is simple, low cost, and readily available, however, there is little opportunity of it for further miniaturization or for high throughput tests. The latter is costly and the choice of materials is limited to piezoelectrics for acoustic pumping. Pressure-based propulsion does have the attractive feature of being generic for the kinds of fluids that can be pumped. Syringe pumps and silicon or plastic diaphragm pumps with piezoelectric activators can offer suitable low flow rates.

On the other hand, electrokinetic techniques such as electro-osmosis or electrophoresis [Effenhauser et al., 1997; Deshpande et al., 2000], electrodynamics [Jacobson et al., 1994], and electrowetting [Colgate and Matsumoto, 1990] have the advantages that they scale favorably for miniaturization. Electrokinetic pumping has been established as the method of choice for transporting and separating liquid samples in microchannels. In electro-osmosis or electrophoresis, the driving forces for flow are generated by the interaction of applied electric fields with ionic species in the fluids. In electrodynamics, the flow is generated by the interaction of electric fields with induced electric charges in the fluids. Electrowetting is based on the principle that the contact angle between a liquid and a solid surface can be changed through the application of an electrical potential. This change may result in capillary forces that provide a driving pressure in a small flow channel. An advantage of electrokinetic techniques over
mechanical pumping is that electroosmotic flow is plug flow, and it will cause less dispersion than the parabolic flow in the traditional pressure-driven system [Paul et al., 1998]. Electroosmotic flow can be quite significant, reaching velocities of around 5 cm/min. However, they need high electric fields and depend strongly on the properties of fluids to be pumped (such as pH or charges). Many organic compounds and solvents may not be able to meet the charge and pH requirements [Zheng and Dasgupta, 1994]. Moreover, the on-chip electrophoresis systems must be made in an insulating substrate in order to avoid electrical breakdown [Bousse et al., 2000].

Thermal methods can also be used for fluid propulsion. Sammarco and Burns [1999] manipulated the contact angle between a liquid and a solid surface by changing the local fluid temperature. The resulting capillary force is used to drive the fluid as in electrowetting. In the case of phase-change pumping [Jun and Kim, 1998], the driving pressure arises from the volume change due to the phase change from liquid to gas, as the liquid is heated. Considering the high heat exchange rate in small channels, this mechanism scales well down to the micro-domain. Thermal methods are still in the early research stage and they require careful control of the local temperature. By contrast, in centrifugal pumping, fluid propulsion is achieved through rotationally induced hydrostatic pressure. It is simple, uses a single low-cost motor, and is capable of fine flow control through proper design of the location, dimensions and geometry of channels and reservoirs based on fluid properties. It can also be easily integrated with the information-carrying capacity of the CD.
2.8.1.2 Valving

Another essential component in the microfluidic system is the ability to stop and start the fluid flow. Different designs use different methods of actuation, such as magnetic, pneumatic, hydraulic or thermal-electric. The majority of micro-valves consists of a diaphragm [Sammarco and Burns, 1999; Kaetsu et al., 1999; Liu et al., 2000; Cao et al., 2001] that is actuated externally to open or close a flow port. Conventional diaphragm valves can fulfill the valving task, but they usually require moving parts and an external actuation mechanism such as a change in temperature, pH or charge. Controlling the liquid flow electro-kinetically has been recently demonstrated [Jacobson et al., 1999]. This method, however, requires a high electric field, is sensitive to the properties of the fluids, and may lead to the occurrence of Joule heating. In order to improve the performance of a single valve, an array of micro-valves has been developed for a linear and more flexible control of the flow [Bousse L., et al, 1996; Wroblewski et al., 1998]. An alternative approach is to use a passive capillary-valve that relies on the capillary force to stop the flow in micro-channels. The principle of operation is based on a pressure barrier that develops when the cross-section of the capillary expands abruptly as shown in Figure 2.4. Capillary valving has the advantage of not requiring any moving parts and external actuation. Recently, this type of valve has attracted a great deal of attention and has a strong appeal for applications in various microfluidic systems [Zeng et al., 2000; Duffy et al., 1999; Madou et al., 1998, 2000, Lai, 2004].
2.8.1.3 Micromixing

Mixing of liquids inside microchannels has received increasing attention in recent years. It is a process normally necessary during sample preparation in microfluidic devices for biological analysis and separations. Because of the dimension of micron-sized flow channels, the Reynolds number of fluid flow in the microfluidic systems is extremely small (usually less than 1). The lack of turbulent flow makes the mixing in microdevices a very challenging issue. Molecular diffusion is the main driving force in micro-mixing due to the nature of laminar flow. For example, a moderately sized DNA molecule ($D \approx 10^{-6} \text{ cm}^2/\text{s}$) would require a few hours to diffuse in a 1 mm wide channel. If the width of the channel reduced to 50 µm, the required diffusion time is about several seconds.

Design of micromixers is generally based on increasing the contacting time, enlarging the contact area, and creating more chaotic flows. Several miniaturized mixers have been developed. The most common method is based on increasing the contact
surface between two fluids. The enlargement of the contact surfaces can be achieved in many different ways. Static-type micromixers (i.e., no moving parts) based on the concept of lamination or separation-reunification have been developed and studied [Bertsch et al., 2001; Branebjerg et al., 1996; Koch et al., 1998; Hinsmann et al., 2001; Schwesinger et al., 1996, He et al., 2001]. A similar approach is to divide each flow into several partial flows in order to increase the contact surface. Injecting one liquid into another liquid with microplumes can also achieve the same goal [Koch et al., 1998, Elwenspoek et al., 1994]. The basic principle of these micromixers is to divide and rearrange the fluid streams to decrease the diffusion distance required for mixing. Some of the static micromixer designs can be complicated, e.g. the lamination type where very precise alignment is required [Woias et al., 2000]. Numerical simulations of these micromixers by using the computational fluid dynamics program have also been applied to evaluate the mixing efficiency of micromixers [Bertsch et al., 2001; Hinsmann et al., 2001; Ehler et al., 2000].

A chaotic flow field can be generated with two pumps connected via source and sink to a mixing chamber [Evans et al., 1997]. This design employs chaotic advection for mixing. It is more efficient than static mixers, but requires expensive instrumentation. In electro-kinetic based microfluidic systems, convective mixing can be achieved by inducing surface charges at the interface of liquid samples that have different conductivities [Choi and Ahn, 2000]. The surface charges react with the applied electric fields to generate electric shear forces. The separate flow streams mix when passing the electrodes. Successful mixing results have been demonstrated. Lee et al. [2001] have applied unsteady pressure perturbations superposed to a mean stream to achieve chaotic-
like mixing in microchannels and use time-dependent dielectrophoretic forces to induce folding and stretching for micromixing. One can also place a solid post in the middle of the micro-flow channel. By applying one electric field to the surface of the channel wall and an opposite electric field to the surface of the post, convective mixing can be achieved when the fluids pass the post [Brahmasandra, 2001]. In addition, the composite drop may be moved to create recirculating streamline to facilitate convective transport of species through the drop [Anderson et al., 1998]. However, this kind of mixer depends on the physiochemical properties of fluids and therefore limits its general applications.

2.8.1.4 Sampling/Metering

Delivery of precisely metered fluids from one reservoir to another in a well-controlled sequence is important in many microfluidic applications. Several methods have been developed for this purpose. An on-chip technique to meter discrete nanoliter-sized liquid drops inside microchannels was developed, using a combination of a hydrophobic surface treatment and air pressure [Handique et al., 2000]. This technique involves spontaneously filling the microchannel up to a hydrophobic stop and splitting a liquid drop by injecting air through a hydrophobic side channel as shown in Figure 2.8. Accurate liquid volumes, ranging from 0.5 to 125 nl, were metered using this technique. Another method is to draw a liquid sample from a larger reservoir to a number of smaller capillaries and let the excess liquid flow into an overflow chamber. The capillaries containing metered liquid samples can then be released in sequential order by capillary valving [Madou and Kellogg, 1998]. Two-phase flow in microchannels with a constriction has also been applied to sample metering in microfluidic system [Madou et
al., 2001] because a fixed amount of liquid is trapped between two bubbles that are snapped-off.

2.8.2 Immunoassays

2.8.2.1 Immunoassay

Immunoassay (IA) is an indirect method, which measures the effect of varying concentrations of a compound/analyte in the test fluid on the reaction of the specific antibody (Ab) and the antigen (Ag). According to the design of the experiment, they can be used to detect either antibody or antigen. A wide variety of compounds can be quantified by immunoassays. These range from large polymeric proteins, nucleic acids, receptors, and structural proteins, to small molecular weight haptens of drugs or their metabolites. Immunoassays can be classified as competitive and noncompetitive IAs [Wild, 1994; Lee and Colbrun, 2002].

2.8.2.2 Enzyme-linked immnosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA), in which antigen and antibodies are immobilized on a solid surface, has also been incorporated into microchips. Several microchip-based ELISA have also been developed, based on the immunoreaction on surfaces of a single microchannel [Rossier et al., 2001] or on micro-beads, which was trapped in the microchannel [Sato et al., 2000 and 2001]. A sandwich immunoassay of D-Dimer in plasma was carried out in a disposable plastic microchip. The time required for assay is only 5 to 15 minutes, whereas a few hours are usually required for a quantitative ELISA. Sato and coworkers have integrated an immunosorbent assay system into a glass
microchip for immunoassays of human secretory immunoglobulin A (s-IgA) and determination of carcinoembryonic antigen for cancer diagnosis. The integration reduced the time necessary for the antigen-antibody reaction and shortened the overall analysis time from 45 hours to 35 minutes. These microchip-based immunoassays took the advantages of the high surface to volume ratio of the microchannel for fast immunoreaction and carried out the whole process of immunoassays on the microchip. However, each step of the ELISA was still carried out manually.

2.8.2.3 Compact-Disk based ELISA (CD-ELISA)

Centrifugal fluidic platform technology was first developed in 1969 (Anderson, 1969), and the concept since then has been extensively studied (Scott and Burtis, 1973; Klumpp, 1977; Bonte, et. al., 1977; Henry, et. al., 1978). It is advantageous in many analytical situations because of its versatility in handling a wide variety of sample types, ability to gate the flow of liquids (valving), simple rotational motor requirement, ease and economic fabrication methods, large range of flow rates attainable, and easy adaptation to existing optical detection methods. Most analytical functions required for a lab-on-a-disc, including metering, dilution, mixing, calibration, and separation have all been demonstrated in the laboratory. Moreover, the possibility of maintaining simultaneous and identical flow rates, to perform identical volume additions, to establish identical incubation times, mixing dynamics, and detection in a multitude of parallel assay elements makes the CD an attractive platform for multiple parallel assays. A recent review of CD-based microfluidic platform technology can be found in the literature [Madou, 2001]. The CD platform has been commercialized for high throughput
screening (HTS) by Tecan, sample preparation techniques for MALDI and bead-based IAs by Gyros AB, human and veterinary diagnostic blood analysis by Abaxis system, and similar applications by Gamera and Burstein Technologies.

The CD-ELISA is to perform ELISA process on the CD microfluidic platform. The concept is to utilize its unique microfluidic function, i.e. flow sequencing, to replace the stepwise procedures carried out in the conventional ELISA process. The CD-ELISA can be a self-contained microdevice that incorporates low-power microfluidic components and high-sensitivity immunomolecules capable of performing parallel and multiple tests with high precision. The CD platform integrates a number of microfluidic functions including pumping, capillary valving, washing, and mixing with required antibodies, reagents, and buffer solution in various reservoirs. By spinning the disc, the centrifugal force overcomes the capillary force and the fluid in each reservoir is pumped sequentially with increasing rotational speed from the center towards the edge of the disc. Control of fluid transfer from one reservoir to another is achieved by manipulating the spin velocity of the disc [Lai, 2004]. The flow sequencing control has been demonstrated on a CD, but has never been applied to ELISA because of the protein blocking problem, which turned the channel surface into a hydrophilic one. By coupling the CD drive with a detection system, samples on the CD can be readily analyzed (e.g. based on absorption or fluorescence). The microfluidic device requires only a minimal sample size, in the sub-microliter range, and its automation can be achieved by modifying existing CD readers. Compared to conventional ELISA (usually carried out in multiwell plates) and other immunoassays, the new CD-ELISA platform have many advantages, including improved reliability and speed, lower reagent uses, and abilities for automation, multiple detections,
and high throughput screening.

A conceptual prototype design of a CD-ELISA with 24 sets of ELISA microdevices on a 12 cm disk is shown in Figure 2.5(a). The schematic of a single assay is explained in Figure 2.5(b).

Figure 2.5 Schematic of (a) a CD-ELISA design with 24 sets of assays, (b) a single assay (1. waste; 2. detection; 3. first antibody; 4,6,8,10. washing; 5. blocking protein; 7. antigen sample; 9. second antibody; and 11. substrate), and (c) photo of a single assay [Lai, 2004]

The substrate, conjugate, washing, primary antibody, blocking protein, and antigen solution can be preloaded into corresponding reservoirs before the test. The centrifugal and the capillary forces are used to control the flow sequence of different solutions involved in the ELISA process. In brief, the capillary force will hold the liquid from a small channel to an expanded area, while the centrifugal force may release the fluid from
its reservoir when it is larger than the capillary force. The angular frequency at this moment is called the burst frequency, which can be calculated by comparing the centrifugal force and the capillary force. A computer controls the rotation speed of the disk to achieve proper flow sequencing and incubation.

The flow sequence is designed in such a way that the antigen solution is released into the measurement site first at a low rotation speed. This action allows the first antibody to bind onto the microchannel surface. The solid surface at the measurement site needs to be modified so that it has a high protein affinity. After incubation, the washing solution is released to wash out the unbounded antibodies into the waste reservoir. Then the blocking protein, the washing solution, the antigen (sample or standard), the washing solution, the conjugate solution, the washing solution, and finally the enzyme substrate are delivered to the measurement site, one by one sequentially at increasing rotation speeds.

The reagent consumption and assay times of each step were compared in Table 2-2. It can be seen that the consumption of reagents (the most expensive ones are antigen and antibodies) on the microchip was 30 µl, which is one tenth that of the 96-well.
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Reagent volume (ml)</th>
<th>Incubation time(min)</th>
<th>Microchip (Goal)</th>
<th>Volume (ml)</th>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96-Well</td>
<td>Microchip *</td>
<td>96-Well</td>
<td>Microchip *</td>
<td></td>
</tr>
<tr>
<td>1st antibody</td>
<td>100</td>
<td>10</td>
<td>480</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Blocking</td>
<td>300</td>
<td>10</td>
<td>60</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>IgG/sample</td>
<td>100</td>
<td>10</td>
<td>120</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>2nd antibody</td>
<td>100</td>
<td>10</td>
<td>120</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>HPPA</td>
<td>100</td>
<td>100</td>
<td>40</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>700</td>
<td>140</td>
<td>820</td>
<td>76</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.2 comparison of performance between 96-well plate and microchannel
CHAPTER 3

LASER/IR ASSISTED MICRO-EMBOSSING

In order to shorten the cycle time in conventional hot embossing, a laser/IR-assisted micro-embossing process was investigated in this study. Since infrared laser (laser/IR) heats the substrate rapidly and locally, the heating and cooling time can be substantially reduced. Two different modes of IR embossing were tested. In one case, the polymer substrate was the IR-transparent poly(methyl methacrylate) (PMMA) and a carbon black filled epoxy mold was used. In the second case, the polymer substrate was an IR-absorbent PMMA and an IR transparent epoxy mold was used. A commercially available finite element (FEM) code DEFORM™ was used for process simulation. The relationship between the penetration of radiation energy flux from the laser/IR heating source and temperature distribution inside the polymer substrate was considered in the simulation. The embossing resolution was evaluated in terms of embossing depth replication.
3.1 Background

As mentioned in Chapter 2, hot embossing, a relatively simple and low-cost process, is a widely used technique for fabricating planar polymeric devices with micro-/nano- features [Gale, 1997; Becker 2000; Heyderman 2000, Scheer 2001; Schulz 2003; Lin 2002; Juang 2002; Hirai 2001]. However, in conventional hot embossing, both the mold and the substrate are heated above the glass transition temperature ($T_g$) or melting temperature ($T_m$) during embossing and cooled down below $T_g$ or $T_m$ during de-embossing, resulting a larger cycle time than that in micro-injection molding. Because embossing is an open mold process, the polymer substrate can not be heated to a very high temperature in order to maintain its dimensional integrity during embossing. This often leads to high embossing forces and correspondingly high molded-in stresses [Juang 2002]. Laser heating technology has been applied in the microfabrication of polymers due to its fast heating characteristics and small heat affected zone (HAZ). Examples include the micro-welding of polymeric micro-parts [Klein 1999] and laser ablation for micro-features [Alonso-Amigo, 2000]. Recently, an embossing/imprinting process has been developed for silicon materials using the laser heating technique. [Chou, 2002]. A high energy pulsed Excimer laser was used to heat the silicon substrate through a transparent quartz mold, followed by subsequent imprinting to form nano-sized features on the silicon surface. The cycle time was very short (less than 250 nano-seconds) for feature size around 100nm. In our group, to produce micron-sized features on a larger polymer surface, a similar Laser/IR-assisted micro-embossing method was developed [D. Grewell and C. Lu, 2003 and 2004]. An infrared assembly device (IRAM) from Branson Ultrasonics was applied to illuminate a local area of the top surface of the polymer
substrate instead of heating the whole substrate in conventional hot embossing. Because of the high power density and good beam focusing of the laser source, fast local surface heating was demonstrated. In these studies, parts and mold have to be transparent or opaque just at the wavelength of the laser that is used during embossing. Therefore, special additives are needed to make the thermoplastic, although visually transparent, opaque to a laser diode or Nd:YAG laser. Similarly, it is possible to make a thermoplastic that a visually black but is transparent to a laser diode or Nd:YAG laser.

Three rapid surface heating methods (infrared radiation (IR) heating using laser diode bars, hot gas heating, and ultrasonic heating) had been compared for embossing microfeatures on polymers [Grewell and Lu, 2003]. While all the methods worked well, IR heating followed by pressing with an aluminum mold produced the best quality with sharp edges and excellent transfer of even the machining features from the mold to the part as shown in Figure 3.1 (a), (b), and (c). Figure 3.1 (a) is an epoxy replicate from the aluminum mold.
Figure 3.1 Photograph of channel produced with IR embossing of high density polyethylene (a) cross section the mold replicate (b) and (c) top and cross section views of the embossed HDPE microchannel

It was found that with a power density of 26 W/cm$^2$, sufficient melting was produced within 8 sec, which allowed embossing with polyethylene (PE). Higher power densities or longer time resulted in over heating and evidence of degradation (smoke). From Figures 3.1 (b) and (c), it is seen that the edges are well defined and that the machine markings from the embossing tool were transferred to the plastic. It is believed that the surface temperatures were relatively high since nearly all the absorption is at the surface with IR heating because of carbon black added to the substrate. This caused the
surface viscosity of the plastic to be relatively low and allowed the image to transfer to
the plastic relatively well. Thus it is believed that that technique proves a feasible
process for micro embossing.

Figure 3.2 shows a micro-feature that was fabricated with 20 kHz ultrasonic
embossing. The cycle time for this sample was 10 sec and the amplitude was
approximately 40 µm. During these experiments it was found that fixture to sample
alignment was critical. In addition it was found that preheating the sample with a hot gun
for several seconds resulted in better transfer of the image. In general, ultrasonic
embossing produce relatively sharp corners, but because the heating was limited to only
the area of the feature, mass displacement of the material resulted in rough regions
adjacent to the micro feature. In more detail, ultrasonic heating of thermoplastics is the
result of cyclic strain/stress, thus, only the region defined by the micro feature is heated.
The region outside this region is not exposed to the cyclic stress/strain therefore does not
heat.

Figure 3.2 Micro channel sample with ultrasonic embossing
In addition, it was found that PE did not heat significantly and the resulting micro-feature did not have well defined corners. Because it was found that better results (more heating and melting) occurred with thinner samples, it is believed that materials with relatively low modulus dissipated the ultrasonic energy through the bulk of the sample and preventing localized heating.

Similar results were seen with polycarbonate (PC), but in this case the heating time required to produce a sufficient melt area was between 16 and 18 sec.

Figure 3.3 shows a photograph of PE embossed with hot gun. It is seen that the edges of the image are not well defined and the machining marks from the tool were not transferred to the plastic.

![Figure 3.3 Micro channel sample with hot air embossing](image)

So, these experimental results showed us the feasibility and advantage of laser fast heating method applied into the hot embossing process.

In this study, we analyzed two laser/IR micro-embossing methods: Transparent Mold Embossing (TME) and Transparent Substrate Embossing (TSE). TME is designed for IR-absorbent substrate materials such as semi-crystalline or pigmented polymers
while TSE is for optical clear polymers that are often used for micro-optic components or bio-chips.

Possible transparent mold materials include glass, epoxy and polyimide. Opaque mold materials can include the above mentioned transparent mold material with laser absorbing paint or coating in addition to steel and aluminum molds. Prototypes for this embossing method can be done by photolithography or CNC machining.

Epoxy was selected as the mold material in this study. Using thermoset polymers as the mold material in hot embossing has been evaluated by several researchers and good replication was achieved [Xing, 2003, G. S. Fiorini, 2003]. One advantage of polymeric molds is the small difference of thermal expansion coefficient between the mold and the substrate. The epoxy mold is advantageous because it is highly transparent to the infrared radiation (transmittance is greater than 85% in our measurement) and can be easily fabricated through the resin-casting process. Also, epoxy has a low thermal conductivity that can prevent heat loss during embossing. Once the infrared energy is converted into heat at the solid surface, the heat travels and dissipates into the solid by conduction. Materials with high thermal conductivity such as metals will quickly distribute the heat away from the mold surface by conduction. Conversely, polymers, like epoxy, may maintain high mold surface temperature during embossing. This is essential to the TSE process.

With this technique, a variety of polymers including PC, polystyrene (PS), Poly(methyl methacrylate) PMMA, polypropylene (PP) and polyethylene (PE) can be used for embossing. PMMA was chosen for the embossing trial. PMMA is the most widely used material for polymeric microfluidics because of its optical clarity, low
fluorescence background, easy manufacturability, and again, low cost compared with glass and silicon.

In laser embossing, heating is done by radiation, in some cases with either a stationary or moving heat source. Therefore, measurement of the temperature rise and distribution in the parts can be done using thermocouples and IR thermography, which is possible because the IR detector is sensitive to much longer wavelength. Models for heating and cooling of the parts during welding can be developed using the finite element method with temperature dependent properties. Predicted melt areas and HAZ can be compared with measured melt areas and HAZ for embossing.

To simulate the deformation and flow that occurs during embossing it is necessary to characterize the rheological behavior of the polymer near \( T_g \) or \( T_m \) (for embossing) and much above \( T_g \) and significantly above \( T_m \). Conventional methods using a Rheomterics mechanical spectroscopy (RMS-800, TA Instruments) and RME (Rheometrics Melt Elongation Spectroscopy) were used to characterize the rheological behavior of the polymer at temperatures much above \( T_g \) and \( T_m \).

The heat flow results together with the rheological properties of the materials were used in an uncoupled viscoelastic finite element analysis using DEFORM (Scientific Forming Technologies Corp.) to model the deformation and flow that will result during embossing. Methods for coupling the heat flow and deformation analysis will then be studied also because of the non-isothermal process.

The quality of the embossed parts were determined from dimensional analysis in relation to the mold used, visual evaluation of the edges and features to insure high quality replication and microscopic examination of the morphology within the HAZ.
Samples with multicolored layers were used for embossing to measure and compare with the models the deformation that occurs in the parts in Juang’s work. Unfortunately, for laser embossing, it is not easy to find a tracer which only help to visualize but does not absorb the laser radiation. However, it will be especially helpful to perform these experiments using time stepped samples that show the progression of the deformation as heating time increases, i.e. short shot.

3.2 Experimental

3.2.1 Equipment and Materials

A Branson IRAM laser welding system as shown in Figure 3.4 was used to carry out the experimental work. The system consists of a Branson fiber bundle (beam shaping), a diode laser, a cooling system, a pneumatic cylinder, and a programmable controller. The Branson fiber bundle is used to improve the radiation uniformity. Selected local heating was achieved with a fiber optical bundle that can generate a heating area of 50 mm x 4 mm. The output of the power system can be adjusted by increasing the current and is reported as the “power level”. A pneumatic air cylinder exerts a constant pressure on top of the substrate or the mold during heating, embossing, and holding/cooling. Both the mold and the substrate are cooled down naturally without additional cooling facilities.
The substrate material used in our experiment, PMMA from Plaskolite Inc., has a molecular weight near 150,000 g/mol and a $T_g$ of 105°C. PMMA is visibly transparent and the transmittance to infrared radiation is greater than 95%. In TME, PMMA was filled with 0.5wt.% carbon black to absorb the laser radiation energy.

Two different mold features were used in this study. Mold #1 has a dog-bone shape feature, which was manufactured by curing an epoxy monomer (Epon 862, Shell Chemical) in a poly(dimethyl siloxane) (PDMS) (Sylgard® 184, Dow Corning) female mold. The PDMS mold was fabricated from a SU-8 photoresist structure on a silicon wafer by photolithography. It has two 1.5 mm wide reservoirs connected with a channel that was 220 µm in width and 290 µm in depth (Figure 3.5a). Mold #2 has only a single channel, which was manufactured by curing the same epoxy monomer in a PDMS female
mold fabricated from a CNC machined aluminum mold. The channel is 20 mm long, 1 mm wide and 250\(\mu\)m deep (Figure 3.5b).

Figure 3.5 Dog-bone shaped mold and (b) single-channel mold
3.2.2 Methodology

This technique can be divided into three different embossing methods;

1) *No preheating-transparent mold with black substrate.* In this technique a transparent mold and black substrate are clamped together under a constant force while the laser energy is applied for a preset length of time. This method is called transparent mold embossing or TME.

2) *Preheating-transparent mold with black substrate.* In this technique a clear mold and black substrate are held together at a very little force while the laser energy is applied for a preset length of time. After the laser energy is discontinued, the mold and substrate are clamped together with a pre-selected force. So this is another type of TME.

3) *Black mold with transparent substrate.* In this technique a black mold and transparent substrate are clamped together under a constant force while the laser energy is applied for a preset length of time. This method is called transparent substrate embossing or TSE.

In the laser/IR-assisted micro-embossing process, the embossing force can be applied before, during or after heating. In our study, the embossing force was applied after heating in TME, while it was applied before heating in TSE.

Figure 3.6 (a) is a schematic of the TME process. The transparent epoxy mold was attached to the plunger of the IRAM, while a 0.5wt% carbon black filled PMMA plate served as the IR-absorbing substrate. Infrared laser radiation can pass through the mold to heat and soften the substrate. After the heating stopped, the mold was pressed down to
transfer the pattern onto the substrate. The mold was separated from the substrate after holding and cooling period.

Figure 3.6 Schematics of the Laser/IR-Assisted micro-embossing (a) Transparent Mold Embossing (TME) (b) Transparent Substrate Embossing (TSE)

Figure 3.6 (b) shows a schematic of the TSE process. A transparent PMMA substrate was placed between the plunger and the mold under force. The IR laser radiation went through PMMA and heated the mold made of epoxy blended with 2.0wt% of carbon
black. The heat can be transferred to the substrate via intimate contact and soften the substrate. When the desired pattern was transferred from the mold to the substrate, the radiation was discontinued. After holding and cooling, the mold was separated from the substrate.

In this study, a laser source with a unique wavelength was used. However, if an IR lamp is used, we can also take advantage of the wide range of wavelength of the radiation, it is possible not to pigment the substrate or mold to become absorbent, but with a longer heating time. However, the embossing process must be modified because the mold and the substrate can not be heated by the radiation at the same time. One possible solution is to use reciprocating embossing mention in the literature review, which means we need to modify the first kind of laser embossing, i.e. the mold cannot be clamped together with the substrate before laser radiation in order to avoid the mold to be heated up.

The variables that were evaluated were heating time and laser intensity. A constant clamp force of 100 N was used. The materials that were used included carbon black filled PE and PMMA as well as transparent PMMA. These materials were selected based on their relatively low processing temperatures in order to reduce the probability of thermal damage to epoxy molds.

The resulting embossed samples were examined using optical microscopy to determine quality of the embossing. Depth of embossing feature was measured using Starret® depth gauge.

In this study, #1 mold was used to do the depth measurement on embossed black PE samples and transparent PMMA samples. To compare with simulation, experiments
with both the #1 and the #2 molds were carried out on both transparent and filled PMMA. Especially, a series of short-shot experiments were carried out for TSE in order to observe the flow pattern and to make comparison with the simulation results. Three heating times were studied: 6, 10, and 14 seconds.

### 3.2.3 Simulation

Numerical simulation of polymer flow in hot embossing has been carried out by several researchers using various finite element methods [Heyderman, 2000; Scheer, 2001; Schulz, 2003; Lin, 2002; Juang, 2002; Hirai, 2001]. In this study, two commercial FEM codes, DEFORM™-3D V5.0.3 and DEFORM™-2D V7.2, were used to simulate the flow pattern of the polymer substrate during embossing. Tensile properties of PMMA below $T_g$ was obtained from literature [C G’Sell, 1997]. Extensional viscosity of PMMA slightly above $T_g$ was obtained by Rheometrics Melt Elongational Rheometer (RME, TA Instruments) with the stretching rate varied from 0.001 to $1 \text{1/s}$. The shear viscosity of PMMA much above $T_g$ was measured by Rheometrics Mechanical Spectrometer (RMS-800, TA Instruments) where the strain was set at 1% and two 25 mm parallel plates were used as the sample holder. The gap between the two plates was around 1.5 mm. The data thus obtained were converted into effective flow stress in DEFORM™ using the following equations:

$$\bar{\sigma} = \sqrt{3} \tau_{ij} \quad \text{and} \quad \dot{\varepsilon} = \frac{\sqrt{3}}{3} \dot{\gamma}_{ij} \quad (1)$$

where $\bar{\sigma}$ and $\dot{\varepsilon}$ are effective flow stress and effective strain rate in DEFORM™ respectively while $\tau_{ij}$ and $\dot{\gamma}_{ij}$ are shear stress and shear rate respectively. In DEFORM™, a tabular data input format was used and the flow stress vs. strain, strain rate and
temperature can be directly incorporated into a KEY file. More detailed rheological measurements and data analysis were available elsewhere [Juang, 2002].

DEFORM™-3D V5.0.3 was used for a qualitative comparison with results obtained using Mold #1 because it cannot be simplified to a 2D problem. Through the 3D simulation, the polymer flow at different locations can be calculated. DEFORM™-2D V7.2 simulation was carried out to quantitatively compare the experimental and calculated flow profiles, and Mold #2 was used in this case.

Heat capacity and thermal conductivity of epoxy and PMMA are 1.05 (J/g.°C) and 0.19 (N/sec), and 1.46 (J/g.°C) and 0.20 (N/sec.°C) respectively [Charles A. Harper, 2000]. Because the carbon black content is only 0.5wt% for PMMA and 2.0wt% for epoxy, we assume that the pure and filled polymers have the same thermal properties. The conductivity of the filled PMMA was verified using modulated differential scanning calorimetry (MDSC 2920, TA Instruments) and the measured value is 0.178 (N/sec.°C).

Another important heat transfer parameter is the heat transfer coefficient that controls the rate at which heat flows from the substrate to the surroundings and from the substrate to the mold. The heat transfer coefficient between the epoxy mold and the polymer substrate was varied from 0.5 to 2 (N/mm.sec.°K) in this study. The polymer was cooled by free convection without any additional cooling device. The heat transfer coefficient at the polymer free surface is assumed to be 0.0057 (N/mm.sec.°K) [T. Kanai, 1984, G.N.Nagarajan, 1995, A. Yousefi, 2001].

Unlike the conventional isothermal hot embossing, there is a temperature distribution inside the substrate in the laser/IR-assisted micro-embossing process because the substrate is only locally heated and the embossing occurs before the substrate reaches
thermal equilibrium. Since laser radiation can penetrate into the opaque sample and heat the materials below the surface, it cannot be assumed that all of the laser power is absorbed at the surface even for a substrate filled with strong IR absorber like carbon black [H. Klein, 1999].

Figure 3.7 (a) Schematic and (b) experimental setup of laser transmission measurement

To determine the power level that reaches the surface during embossing and welding it is necessary to measure the power transmission through the material. Figures 3.7 (a) and (b) showed the schematic and experimental setup respectively for laser power
transmission measurement. The IR transmission measurement system includes a fiber
coupled diode laser system (Coherent Inc.) with a wavelength of 808 nm and a total
power of 1 watt, a Coherent’s Thermal SmartSensors™, and a Coherent’s FieldMaster™
laser power meter. In this study, the input power used was 0.5 watt. The sample with
known thickness was placed between the laser and the meter. First, the power will be
measured without the sample and then the power will be measured with the sample. Since
the sample will reflect and absorb some of the laser energy, the power measured with the
sample will be lower than that measured without the sample. The output power at
different locations was measured and averaged. The ratio of power measured with the
sample divided by the power measured without the sample gives the fraction of power (%)
(FOP) transmitted through the material.

In order to calculate the temperature distribution inside the substrate, the following
equations are used:

\[ \rho C_p \frac{\partial T}{\partial t} = k \left[ \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right] + \dot{Q} \]  \hspace{1cm} (2)

\[ \dot{Q} = \left| \frac{\partial I}{\partial z} \right| = I_0 \beta_L \exp[-\beta_L z] \]  \hspace{1cm} (3)

\[ I = I_0 \exp[-\beta_L z] \]  \hspace{1cm} (4)

where \( \dot{Q} \) is the rate of radiation absorption, \( I \) is the incident laser power density at
different \( z \) locations (the direction of laser radiation), \( I_0 \) is the power density of laser
radiation reaching the substrate surface, which usually has a 2D distribution but is
assumed to be uniform in this study. \( \beta_L \) is the absorption constant of the substrate.
material that can be calculated by fitting the transmittance data into Lambert-bougler law, in which the incident power density of the laser attenuates exponentially.

3.3 Results and Discussion

3.3.1 Experimental

In visual inspection of the channel, both approaches produced channels of good quality with sharp edges. To quantify the experiments, the depth of the channel was measured and compared to the depth of the channel of the embossed black PE samples with #1 mold. It is interesting to notice that in both cases longer heating times resulted in poorer replication of the channel depth. This is probably due to creation of a larger heat-affected-zone (HAZ) for the longer heating times resulting in greater shrinkage.

![Graph showing channel depth as a function of time at various laser powers (clear mold-no preheating)](image)

Figure 3.8 Channel depth as a function of time at various laser powers (clear mold-no preheating)
Figure 3.8 shows feature depth as a function of heating time with no preheating using a transparent mold at various laser intensity levels. As expected, it is seen that the feature depth is proportional to heating time. In addition, the time required to fully replicate the feature (~290 µm) increases with decreasing laser intensity. For example, it only takes approximately 2 sec to fully replicate the feature at 100% laser intensity (heating time dominates); while at 85% intensity the required time is approximately 3.5 sec. The possible reason is that once the maximum feature depth is achieved, additional heating cause the feature depth to decrease. This is not expected and very difficult to explain at this time. While additional studies are required to fully explain this observation, it is theorized that it caused by localized thermal expansion of the substrate material. In more detail, because a mask was not used in these experiments and the laser spot was slightly larger than the embossing feature, there was a region adjacent to the embossing zone that was heated. This region (HAZ – heat affected zone) is softened as the mold is pushed into the plastic and experiences a volume increase due to mass flow from the embossing region as well as thermal expansion. Once the mold is fully projected into the sample, the face of the mold engages the sample forming a closed volume. Thus, with additional heating, the mold is pushed out of the sample due to hydraulic pressure caused by thermal expansion. However, from Figure 3.8, the depth difference is huge compared with the mold, so another possible reason is the mold deformation. With a 100% power level, the heat input from a longer radiating time is too much for the epoxy mold because the mold contacts the substrate all the time in this process. With a lower power level such as the 70%, the heat generated will not be enough to decrease the strength of the epoxy mold as well as the depth of the embossed samples.
Figure 3.9 shows the results with a transparent mold and preheating. As expected, feature depth is proportional to heating time. However, unlike the previous results, the feature depth never started to decrease with longer heating times. This is probably due to the fact that longer times were not evaluated. It is important to note that only relatively low laser intensity was evaluated in order to reduce thermal degradation of the based sample. That is to say, smoke was evident when higher power levels were used. This maximum intensity is limited with preheating because the mold is only lightly pressed against the substrate material, so the contact between the mold and the substrate is not so good that heat buildup occurred only in the substrate. At the same time, the convection will promote the heat loss before embossing, so it is possible for the mold not to be deformed with a much longer heating time.

Figure 3.9 Channel depth as a function of preheating time
Figure 3.10 shows the results of a transparent mold with an absorbing substrate. Again as expected, feature depth is proportional to heating time. In addition, it is again seen that once the maximum feature depth is achieved, additional heating promotes a reduction of feature depth. This could be explained by the same theory for the transparent mold embossing with pre-heating. It is important to note that only very low laser intensity was studied. High intensity caused mold damage.

Figure 3.10 Channel depth as a function of heating time with black mold

While the balance of experiments conducted in this study used a mold fabricated using photolithography, some initial experiments were conducted using a tool fabricated with CNC micromachining. In these experiments with mold by photolithography, tool damage was observed after the mold and base materials were separated as shown in Figure 3.11. Cross sectional examination of tool showed evidence of a negative profile
(“dovetail”). This is a typical result of UV diffraction. Surprisingly, many samples were successfully embossed, as shown in Figures 12(a) and 12(b), without tool damage. This is possible due to the elasticity of the mold and substrate. In contrast, tools fabricated with micromachining were relatively square and the resulting features were also relatively square.

Figure 3.11 Tool damage after de-molding
3.3.2 Simulation

Figure 3.13 shows the measured transmittance of 0.5-wt% carbon black filled PMMA and 2.0-wt% carbon black filled epoxy measured at various thicknesses. The thin film samples of PMMA were prepared with a microtome and the thin film samples of epoxy were prepared with a slow speed cutter. It can be seen that nearly all laser radiation is
absorbed within the first 60 µm of the sample depth for PMMA. Although laser penetration is relatively small, it could be significant when the mold feature is of a similar dimension in micro-embossing in this study. However, for 2.0-wt% carbon black filled epoxy, the laser radiation is absorbed within a 20 µm surface layer, so, it is reasonable to assume the total absorption of the laser radiation at the surface. In this study, the calculated $\beta_L$ for PMMA with 0.5wt% carbon black equals 18 (1/mm). For 2.0wt% carbon black filled epoxy mold, the calculated $\beta_L$ is about 1200 (1/mm).

![Figure 3.13 Transmittance of carbon black filled polymers: Epoxy (2.0wt% carbon black) and PMMA (0.5wt% carbon black)](image)

Figure 3.13 Transmittance of carbon black filled polymers: Epoxy (2.0wt% carbon black) and PMMA (0.5wt% carbon black)

Figure 3.14 shows the calculated temperature distribution within a PMMA sample filled with 0.5-wt% carbon black with the penetration of laser radiation was considered. For comparison, the temperature distribution assuming the laser radiation to be totally
absorbed by the top surface of the substrate is also plotted. One can see that a higher temperature is predicted in the top 200 µm with surface absorption only.

Figure 3.14 Comparison between temperature distributions with and without considering IR radiation penetration (PMMA, 100% power level, 2.5 seconds preheating, 0.5-wt% carbon black)

Figures 3.15 and 3.16 show simulated and experimental flow patterns in TSE and TME respectively. In TSE, only the substrate material near the mold feature reaches a high temperature because of its contact with the “hot mold” as shown in Figure 3.15(a). Once the mold embossed into the substrate, the molten polymer was pushed out showing characteristics similar to a “squeezed” flow as shown in Figures 3.15(b) and (c). In TME, the substrate was preheated to form a molten pool at the surface, i.e., HAZ. The molten polymer near the mold feature and at the edge of the HAZ would have lower
temperatures (i.e. higher flow stress/viscosity) compared to that in other locations. The initial temperature of the substrate after preheating is shown in Figure 3.16(a). Once the mold embossed deeper into the substrate, the molten polymer with higher flow stress/viscosity near the mold feature tended to push away the molten polymer with a lower flow stress/viscosity, resulting in a “wavy” flow pattern as shown in Figure 3.16(b). An embossed sample with TME is shown in Figure 3.16(c).

Figure 3.15 Polymer flow pattern in TSE (PMMA; force: 100 N; power level: 50%) (a) Simulated temperature distribution in the substrate at 8 seconds, (b) simulated flow pattern and (c) an embossed sample.
Figure 3.16 Polymer flow pattern in TME (0.5wt% carbon black filled PMMA; force: 100 N; power level: 100%) (a) Initial temperature distribution in the substrate at 2 seconds, (b) simulated flow pattern and (c) an embossed sample

The advantage of localized heating is obvious when the laser embossed flow pattern is compared with that of conventional isothermal embossing as shown in Figure 3.17. One can see that the whole polymer piece is distorted during embossing because all the substrate was heated above the softening temperature. By contrast, only a portion of the substrate around the features would deform in the laser embossed part.
Figure 3.17 Simulated flow pattern in isothermal embossing (PMMA, 170 °C, 100 N)

Although 3D simulation can predict flow patterns of complicated mold geometry, it is difficult to be used for quantitative analysis. Therefore, some experiments were conducted using Mold #2. The micro-feature is a simple straight channel with the length to width ratio of 20, so it can be simplified into a 2D flow problem.

The heat transfer coefficient between the mold and the substrate is evaluated. According to the literatures, the heat transfer coefficient is often taken as 0.1~5 (N/mm.s.K) in injection molding [K. Ainoya, 2001, F. Ilinca, 2000], but the actual value is difficult to determine under the embossing conditions. A heat transfer coefficient of 1 or less and 2 (N/mm.s.K) or more were compared in this study. From the simulation, it was found that when a heat transfer coefficient of 2 or higher (N/mm.s.K) is used, the flow front tends to fold. On the other hand, with a heat transfer coefficient of 1 or lower
(N/mm.s.K), the flow front doesn’t show any folding, which agrees with the experimental observations. Therefore, a heat transfer coefficient of 1 (N/mm.s.K) is used in the remaining simulation.

Figures 3.18(a) and 3.18(b) show the 2D simulation of the initial temperature distributions in the PMMA substrate containing 0.5-wt% carbon black at different preheating times in TME. The temperature is high enough to cause melting (above $T_g$ or $T_m$) and HAZ increases as the preheating time increases. Because of local heating by laser illumination, a radial temperature distribution is observed.

Figure 3.18 The calculated temperature distribution inside 0.5wt% carbon black filled PMMA substrate with (a) 1 second and (b) 2 seconds preheating time at 100% power (Substrate is 2 mm in thickness and 10 mm in width)
Figure 3.19 Simulated and experimental flow pattern in TSE at different heating times (Viewed in x-y plane) (a) 6 seconds, (b) 10 seconds, and (c) 14 seconds (PMMA; Power level: 50%; Force: 240N)

Figure 3.20 Simulated and experimental mold displacement curve in TSE (Filled symbol: Simulation; Empty symbol: Experimental)
Figures 3.19 and 3.20 compare the flow pattern and displacement vs. time between experimental and simulation in TSE. There is a good agreement of the flow pattern, but a slight over-prediction of displacement curve by simulation.

Figures 3.21 and 3.22 compare the flow pattern and displacement curve between experimental and simulation results in TME respectively. The substrate was PMMA with 0.5wt% carbon black, the power level was 100%, the embossing force was 240N and the preheating time was 1 and 2 seconds respectively. It can be seen that the simulated flow pattern agrees fairly well with experimental results. However, the calculated HAZ seems to be larger than the experimental one. A possible explanation is our assumption of uniform power density. The radiation density of the laser array is not totally uniform. Intensity is a Gaussian distribution with a higher power density in the center of the radiation, which would result in a narrower HAZ. The simulation again over-predicts the experimental displacement curve. The simulated curve levels off quickly, but the experimental curve shows a more gradual change. The Gaussian radiation density distribution could be the reason because it would affect the viscosity and viscosity distribution in the polymer substrate.
Figure 3.21 Simulated and experimental flow pattern in TME (Viewed in x-y plane) (PMMA with 0.5wt% carbon black; Power level: 100%; Force: 240N) (a) Preheating time: 1 second (b) Preheating time: 2 seconds

Figure 3.22 Simulated and experimental mold displacement curve (Preheating time: 2 seconds)
Comparison between the depth measurement and the simulation results, one can see that thermal expansion was not considered during heating and cooling. More advanced software might be needed to get more accurate results.

3.4 Conclusion

In this study, a novel fast heating embossing method, laser assisted embossing, were evaluated. We showed that the material flow patterns of an semi-crystalline polymer, PE and an amorphous polymer, PMMA, in both TME and TSE of the laser/IR-assisted micro-embossing technique can be well simulated by FEM simulation. The displacement curve, however, cannot be simulated well, particularly in TME. The reason that TSE could be simulated well is because the heat is transferred to the substrate via the contacted area between the mold and the substrate, which results in less influence on the overall temperature distribution in the substrate.
CHAPTER 4

SACRIFICIAL TEMPLATE MICRO-EMBOSSING

4.1 Background

High-aspect-ratio microstructures (HARMs) can be found in many applications, such as microsensors, microactuators, and high-throughput microfluidic systems [Becker, 2000]. LIGA (a German acronym that stands for lithography, electroplating, and molding) and LIGA-like processes have been used to directly manufacture HARMs. However, those methods usually involve expensive equipment and a cleanroom environment. For polymeric microstructures, there have been many efforts to develop cost-effective fabrication methods. Micro-embossing, micro-reactive-injection molding and reactive casting are enabling manufacturing techniques.

Rigid materials, such as silicon, quartz, tool steel, nickel, or even polymers, are usually used to make molds in micro-embossing. Adhesion between the mold surface and the molded polymeric structure, however, tends to damage either the mold or the substrate, especially for microstructures with high aspect ratios. Although this problem
can be reduced by making molds with very smooth surfaces and positive draft angles or by applying internal or external mold release agents, these are insufficient for fabricating HARMs, especially those with high feature densities [Gourgon, 2003; Heydermann, 2000]. Another approach is soft lithography, where a rubbery polymer, poly(dimethyl siloxane) (PDMS), is used as the mold material for easy de-molding because of its flexibility and low surface energy [Xia, 1998]. A series of molding methods related to soft lithography have been developed. Microstructures with high aspect ratios can be produced at the cost of long cycle time because high temperatures and low embossing force must be used to avoid the deformation of the soft PDMS molds during embossing [Narasimhan, 2004]. The low rigidity of PDMS, however, makes it difficult for HARMS with high feature density.

Sacrificial polymer materials are widely used in fabricating MEMS (Micro-ElectroMechanical-System) devices. For example, photoresists can be considered a sacrificial material in photolithography and the electroplating process [Dellmann, 1998]. Photo- or thermal-degradable polymers have been used to form sealed microchannels [Jayachandran, 2003; Li, 2003; Park, 2003]. Water-soluble polymers were used to release free-standing microstructures [Horvath, 2003]. However, sacrificial polymer molds have not been reported in the micro-embossing process.

In this study, we describe a sacrificial template method and its applications for fabricating HARMs using a biodegradable polymer, poly(DL-lactide-co-glycolide) (PLGA), and an optic clear polymer, PMMA, as examples.
A water-soluble polymer, PVP, was chosen as the mold material because it has a $T_g$ higher than most water soluble materials (about 185 °C for commercially available PVP), and it is highly hydrophilic because of the formation of hydrogen bonds in water [Szaraz, 2000]. After embossing, the mold can be dissolved in water quickly to release the micro-structures, and the PVP sacrificial material may be reused.

For PMMA, IR laser surface heating [Lu, 2005] is combined with the sacrificial template embossing process. Because heating is localized on the polymer surface, the material there can be heated to a high temperature and, correspondingly, a low viscosity, while the temperature of the remaining substrate and the sacrificial template can be maintained to avoid deformation of the PVP mold. This temperature distribution can provide very low stresses on the mold and can result in fast cooling.

This phenomenon is verified experimentally and by a FEM analysis.

4.2 EXPERIMENTAL

4.2.1 Materials

1-vinyl-2-pyrrolidone (VP) was purchased from Aldrich and used as received. Polypyrrolidone (PVP, K60) was purchased from Aldrich as a 45% aqueous solution. PMMA plates were purchased from McMaster Carr with dimensions of 25.4cm x 25.4cm x 1.51cm. They were placed in a vacuum oven at 70°C for at least 48 hours before use. The Poly(dimethyl siloxane) (PDMS) precursor (Sylgard 184 silicone kit) was purchased from Dow Corning. The photo-initiator, Irgacure 819, was purchased from Ciba Specialty Chemicals. Poly(DL-lactide-co-glycolide) (PLGA, 4A) was obtained from Alkermes. A
perfluoroalkyl silane, 1H,1H,2H,2H-Perfluorooctyl-trichlorosilane, was purchased from Aldrich.

Two different microstructures were tested in this study. One is a microchannel array with dimensions of 10µm in width, 20µm in depth and a 30µm distance between two channels, i.e. an aspect ratio of 2. The other is a microwell array with a diameter of 20µm and a well height of 100µm, i.e. an aspect ratio of 5.

The polymer substrates were either PMMA or PLGA. PMMA has a $T_g$ of 105°C, and PLGA, a biodegradable polymer, has a $T_g$ of 43°C.

4.2.2 Mold Preparation

4.2.2.1 SU-8 master fabrication

A SU-8 mold was prepared using standard photolithography. For the high-aspect-ratio well array, a negative photoresist, SU-8 100 (MicroChem, MA), was spun at 3000 rpm with a conventional spin coater. The film was pre-baked on a flat hot plate at 65°C for 10 min and then at 95°C for 30 min. The exposure was performed at the dose of 400mJ/cm² using a standard contact aligner (EVG 620, EV Group). After post-exposure baking at 95°C for 10min, the SU-8 was developed using PGMEA (propylene glycol monomethylether acetate, from MicroChem, MA) for 1 hour. For the multi-channel array, the SU-8 50 photoresist was used. The parameters used can be found at www.microchem.com.
4.2.2.2 PDMS replicates (daughter mold)

The surface of the SU-8 mold was treated using fluorine plasma, CF$_4$ with a power of 100W and a pressure of 300mTorr using a bench-top reactive ion etching system (Technics 800 II Series).

After surface treatment, the premixed PDMS precursor and curing agent (10:1.05) was poured over the SU-8 mold. The mixture was degassed under vacuum for 30 min to remove trapped air. Then the system was heated up to 65°C for 2-3 hours. Finally, the PDMS daughter mold was peeled off of the SU-8 mold. The pre-cured PDMS was further heated to 150°C for 1 hour to complete the cure.

For the HARMs, the PDMS mold was further treated before fabricating the sacrificial templates in order to reduce surface adhesion. The PDMS mold was treated with oxygen plasma for 30 sec and soaked in the Perfluoroalkyl Silane isopropanol solution for 1 hour. Then, the mold was rinsed in isopropanol for 10 min and placed in an oven at 70°C overnight.

4.2.2.3 Sacrificial templates

Sacrificial templates with a high softening temperature, superior mechanical strength, and good surface quality are essential for the micro-embossing process.

Among the water soluble polymers listed in Table 4.1, PMAA has the highest T$_g$, but its solubility is much lower than that of PVP. When curing MAA in a PDMS mold, the MAA monomer would readily diffuse into the PDMS mold, and the cured PMAA could not be separated after polymerization. Both table salt and table sugar are readily soluble in water. However, the template usually has a very rough surface because of strong
crystallization. PVP has a high $T_g$ and is highly soluble in water. Furthermore, the VP monomer has very good UV curability and PDMS compatibility, which is important when preparing a PVP template by direct polymerization in a PDMS mold.

<table>
<thead>
<tr>
<th>Materials</th>
<th>$T_g$ ($^\circ$ C)</th>
<th>Water Solubility</th>
<th>UV curability</th>
<th>PDMS compatibility</th>
<th>Surface quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP(K60)</td>
<td>175</td>
<td>highly</td>
<td>good</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>PMAA</td>
<td>220</td>
<td>medium</td>
<td>poor</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>PVA</td>
<td>50/85 (&lt;100)</td>
<td>medium/low</td>
<td>N/A</td>
<td>N/A</td>
<td>good</td>
</tr>
<tr>
<td>Chitosan</td>
<td>70</td>
<td>acid</td>
<td>N/A</td>
<td>N/A</td>
<td>good</td>
</tr>
<tr>
<td>Table salt</td>
<td>800.8 ($T_m$)</td>
<td>good</td>
<td>N/A</td>
<td>N/A</td>
<td>poor</td>
</tr>
<tr>
<td>Table sugar</td>
<td>186 ($T_m$)</td>
<td>good</td>
<td>N/A</td>
<td>N/A</td>
<td>poor</td>
</tr>
</tbody>
</table>

Table 4.1 Comparison between different water-soluble materials

Using the microchannel array as an example, two mold preparation approaches were studied: direct polymerization of a monomer in a PDMS daughter mold, i.e. reactive molding [Apoga, 2002; Choi, 2004], and casting of a spin-coated polymer layer in a PDMS daughter mold, i.e. solvent molding [Kim, 2003; Kim, 2001]. The schematic of the reactive molding process is shown in Figure 4.1a, where the VP monomer and 1.0wt% Irgcure 819 were mixed and partially cured with a UV lamp (0.2Wcm$^{-2}$, 1 min) to prepare the VP prepolymer. The VP prepolymer then was poured into the PDMS mold. A prepolymer, instead of a monomer, was used because a monomer may diffuse into the
PDMS mold and cause it to swell and deform, although VP is much better than other monomers, such as MAA or 2-hydroxylethyl methacrylate (HEMA). A piece of glass then was placed on top of the mold so that oxygen in the air could be isolated to increase the conversion of the bulk polymerization. A UV intensity of 15 mw/cm$^2$ was used for 10 min to complete the curing process.

Figure 4.1 (a) Schematic of reactive mold to prepare a PVP sacrificial template. (b) A SU-8 mold with microchannel array. (c)Cross section and (d) top views of a PVP microchannel array prepared by reactive molding.

UV curing at elevated temperatures and the influence of micro-/nanoparticles on the $T_g$ of PVP were investigated. UV curing was conducted on a hot plate (Fisher Scientific) at 175°C for 5 min, and micro-/nanoparticles used included fumed silica (14nm, from
Aldrich) and MIN-U-SIL® Ground silica (10µm, from U.S. Silica). The $T_g$ of the template was measured using TMA (Thermal Mechanical Analyzer, TA Instruments).

The schematic of the solvent molding method is shown in Figure 4.2. Again, the PDMS daughter mold was replicated from the SU-8 mother mold using standard photolithography. PVP was diluted to 25wt% with DI water, and a thin layer of PVP was spin-coated onto a ceramic wafer at 1000 rpm for 30 sec using a spin coater (G3p-12, Cookson Electronics Equipment). The PDMS daughter mold was then pressed gently onto the wet PVP layer. The setup was left to dry for overnight before the PDMS daughter mold was peeled off the PVP template. The PVP template was then placed in a vacuum oven for 24 hours at 70ºC to dry completely.

Figure 4.2 Schematic of solvent molding to prepare a PVP sacrificial template
For the high-aspect-ratio array, only the solvent molding method was used. A thin layer of PVP solution was spin-coated onto the PDMS mold, and the setup was degassed for 10 min to ensure that the mold cavity was completely filled. The extra PVP solution was removed using a razor blade. A ceramic wafer was also spin coated with a thin layer of the same polymer solution. Finally, the pre-filled PDMS mold was placed directly onto the ceramic wafer. The entire setup was then dried through the same procedure mentioned previously.

For the solvent molding method, a porous ceramic wafer, rather than a glass or silicon wafer, was used as the support because the ceramic wafer is water permeable and therefore capable of achieving uniform shrinkage across the whole wafer during drying. Directly casting the PVP solution onto the PDMS mold without the porous ceramic wafer resulted in a warped mold because of the non-uniform shrinkage. In addition, the dissolution of the polymer mold after embossing was much faster than using non-porous materials.

### 4.2.3 Hot Embossing

An Instron 5848 micro-tester was used as the embossing equipment in this study. Both constant and programmed force and speed can be achieved easily.

The substrate and the mold were first heated above the $T_g$ of the substrate material. Then, the top press was held against the mold for a specific holding time (5 min in this study). The mold was then cooled down until it reached below $T_g - 20^\circ C$, or room temperature, under the embossing force. Finally, the embossing force was released, and the sample was placed into a stirred beaker with water for 60 min to dissolve the
sacrificial template. The solution thus obtained could be reused and the samples separated from the ceramic wafer need to be rinsed further by fresh water for a couple of times.

Figure 4.3 (a) Schematic of the setup for embossing of microporous membrane with a sacrificial bi-layer. (b) Embossed microporous membrane with PLGA.

In addition to microwell structures, the micropillar array was also used to emboss PLGA through-hole structures (membranes). The schematic of this process is shown in Figure 4.3a. A PLGA thin film with a thickness of 100µm was placed onto a glass plate oated with a thin layer of polyvinyl alcohol (PVA from Aldrich), and then the film was heated to 130°C. The PVP template was pressed down and held for 5 min with an embossing pressure of 0.14Mpa. Finally, the setup was cooled to room temperature, and the embossed sample was released by dissolving the PVP template in water.
4.2.4 Laser/IR Surface Heating Assisted Sacrificial Template Embossing

A laser/IR system [Lu, 2005] was used to generate a molten layer on the PMMA surface. The diode laser system with a wavelength of 808nm and a peak power density of 0.26w/mm² can generate a heating area of 25mm x 2mm. A mold of a single channel with a width of 100µm and a depth of 300µm was used in this transparent mold embossing process (TME). The sacrificial template obtained by solvent molding was placed between the laser radiation and a piece of PMMA filled with 0.5% carbon black. The laser beam passed through the transparent PVP template and illuminated the absorbent PMMA substrate for 6 sec. Then, an embossing pressure of 0.14 Mpa was applied via a pneumatic cylinder, which transferred the mold features from the template to the PMMA substrate. After 30 sec of cooling, the embossed sample was released by dissolving the PVP template in water. Using an IR thermometer, the maximal temperature at PMMA surface before embossing was around 250°C. For comparison, an isothermal embossing was also carried out at 250°C, i.e. the whole PMMA substrate was heated to 250°C.

4.2.5 FEM Simulation

A commercial FEM code, DEFORM™-2D V7.2, was used to simulate the stress distribution on the template mold and the PMMA substrate during the embossing under both isothermal and non-isothermal conditions. The methods to measure the stress-strain relationship of PMMA near and above T_g have been reported in detail elsewhere [Lu, 2005; Juang, 2002; G’Sell, 1997]. The stress-strain rate data thus obtained were converted into the effective flow stress in DEFORM™ using the following relationships:
\[
\bar{\sigma} = \sqrt{3} \tau_{ij} \quad \text{and} \quad \dot{\varepsilon} = \frac{\sqrt{3}}{3} \dot{\gamma}_{ij} \tag{1}
\]

where \(\bar{\sigma}\) and \(\dot{\varepsilon}\) are the effective flow stress and effective strain rates in DEFORM™, respectively, while \(\tau_{ij}\) and \(\dot{\gamma}_{ij}\) are the shear stress and shear rates, respectively. In DEFORM™, a tabular data input format was used, and the flow stress vs. strain, strain rate and temperature can be directly incorporated into a KEY file.

The single microchannel feature was used in the simulation because of its symmetry. For isothermal processes, temperatures of 130 and 150°C were used to evaluate the influence of temperature on the stress distribution. The initial thickness of the PMMA substrate was 300µm, and the embossing pressure was 1.6Mpa. For laser surface heating, a constant heat flux boundary condition of 0.18 w/mm² was applied to generate the surface molten polymer layer, assuming the laser radiation was totally absorbed by the polymer surface. The pre-heating time was 10 sec, and the embossing pressure was 1.6Mpa.

4.3 RESULTS AND DISCUSSION

4.3.1 SU-8 master fabrication

The SU-8 masters of the microchannel and micropillar arrays are shown in Figure 1b and Figure 4.4a, respectively. According to the measurement, the microchannel array has a dimension of 10µm in width, 18µm in depth, and a 30µm distance between two channels. So the actual aspect ratio equals 1.8. The micropillar array has a diameter of 20µm and a height of 120µm. So the actual aspect ratio equals 6.
For preparing the high-aspect-ratio micropillar array, the post-bake time must be longer than the time recommended by MicroChem. After post-bake, the wafer should be cooled down and kept at room temperature for 4-6 hours before exposure. A hard contact mode should be used during exposure. These steps could reduce the residual stresses and improve the replication resolution.

Figure 4.4 (a) SU-8 master mold with micropillar array. (b) Replicated PDMS daughter mold. (c) Replicated PVP sacrificial template.

4.3.2 PDMS replicates (daughter mold)

The PDMS mold could be separated easily from the SU-8 mother mold in the case of multichannel structure because of its low aspect ratio. However, a surface modification was needed for the high-aspect-ratio micropillar structures. Otherwise, micropillars would break during de-molding from the SU-8 master. Surface treating the PDMS daughter mold solved this problem, as shown in Figure 4b.
4.3.3 Sacrificial Template

A microchannel PVP template by reactive molding is shown in Figures 1c and 1d. This method is fast, low-cost, and has high fidelity. The dimensions of the replicated microstructures were very close to those of the SU-8 master mold, according to our measurements (less than 1% variation). However, the template thus obtained had a lower \( T_g \) than the commercial PVP. The \( T_g \) and the coefficient of thermal expansion (CTE) measured by TMA are listed in Table 4.2. When cured at elevated temperatures and mixed with micro-/nanoparticles, the \( T_g \) of the cured PVP template increased. A UV cure conducted at room temperature resulted in a low \( T_g \). When the cure temperature was increased, the reaction reached a higher conversion and, correspondingly, a higher \( T_g \). Adding silica micro-/nanoparticles increased the \( T_g \) further. According to Table 2, the \( T_g \) of UV-cured, pure PVP at a temperature of 175\(^\circ\)C reached 120\(^\circ\)C, which is much higher than the PVP cured at room temperature. Adding a 6wt% of fumed silica further increased the \( T_g \) to 140\(^\circ\)C. A combination of fumed silica nanoparticles and grounded silica microparticles increased the \( T_g \) to 150\(^\circ\)C.

Similarly, the thermal expansion coefficient of PVP decreased when cured at elevated temperatures and when the micro-/nanoparticles were added. This improved dimensional stability is important for embossing precision micro-/nanofeatures.

The hydrogen bond formation between the hydroxyl groups on the silica surface and the carbonyl groups in the PVP molecules increased the \( T_g \). However, when the micro-/nano-sized silica particles were added to the PVP solution, the \( T_g \) decreased instead. This is because the added micro-/nanoparticles damaged the ordered hydrogen bonding formed between the PVP molecules. Therefore, the influence of micro-/nanoparticles on
the $T_g$ deserves further investigation. For this reason, all of the following embossing experiments in this study were conducted using the PVP sacrificial template prepared by the solvent molding method because of its high $T_g$.

<table>
<thead>
<tr>
<th>Formula</th>
<th>$T_g$ (°C)</th>
<th>Coefficient of Thermal Expansion ($\mu$m/m.°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure VP cured at room temperature</td>
<td>89</td>
<td>321</td>
</tr>
<tr>
<td>Pure VP cured at 175 °C</td>
<td>115</td>
<td>196</td>
</tr>
<tr>
<td>VP mixed with 4% fumed silica cured at 175°C</td>
<td>124</td>
<td>223</td>
</tr>
<tr>
<td>VP mixed with 6% fumed silica cured at 175°C</td>
<td>143</td>
<td>194</td>
</tr>
<tr>
<td>VP mixed with 6% fumed silica and 8% ground silica cured at 175°C</td>
<td>152</td>
<td>166</td>
</tr>
</tbody>
</table>

Table 4.2 Thermal properties of UV cured PVP

4.3.4 Hot Embossing

A PVP sacrificial template with the microchannel structure prepared by solvent molding is shown in Figures 4.5a and 4.5b. According to the measurement, both the width and the depth of the female features decreased by around 15%. This is due to the shrinkage of the template during the drying process. Both the isothermal and non-isothermal embossing processes were tested for the PMMA substrate, and the resulting microchannel arrays are shown in Figure 4.6. In the isothermal process, both the substrate
and the mold were heated above the $T_g$ of the polymer substrate, while only the substrate or the mold were heated up in the non-isothermal process[Lu, 2005; Juang, 2002]. In Figure 4.6a, a substrate temperature of 25 °C above $T_g$ (i.e. 130°C), was used and the mold remained unheated. At an embossing force of 1.6Mpa, the sample was not fully embossed. When both the mold and the substrate were heated to 130 °C and the same embossing pressure was used, the feature was distorted, as shown in Figure 4.6b. This implies that while the temperature and pressure were high enough to achieve good material flow, the high stress damaged the mold feature. When the embossing force was decreased to 0.34Mpa, fully embossed samples without any defects were achieved, as shown in Figure 4.6c.

Figure 4.5. (a) Cross section and (b) top view of the sacrificial template prepared by solvent molding, (c) the photo of a ceramic wafer with PVP feature via solvent molding.
Figure 4.6 (a) Embossed multi-channel array on PMMA non-isothermally. Embossed micro-pillar array on PMMA isothermally at pressures of (b) 1.6Mpa and (c) 0.34Mpa

A PVP template of a micropillar array prepared by solvent molding is shown in Figure 4.4c. When this high-aspect-ratio pillar array was used to emboss PMMA using the same embossing conditions as in Figure 4.6c, it was impossible to achieve good replication. A typical embossed PMMA substrate is shown in Figure 4.7a. Apparently, the stresses on the mold during embossing were too much to form features with the high aspect ratios.

For the low $T_g$ PLGA embossed at a temperature of 120°C and a pressure of 0.14Mpa, a well-defined microwell array was achieved, as shown in Figure 4.7b. The top surface was not as smooth as the bottom surface of the SU-8 mother mold because of air entrapped during the embossing. Embossing in a vacuum facility may help to solve this problem.
With a PVP and PVA sacrificial bi-layer, a 120μm thick PLGA micro-porous array can be formed, as shown in Fig. 3b. When a SU-8 pillar array was used to directly emboss PLGA, broken pillars became trapped in the PLGA, as shown in Figure 4.7c.

![Image](image1.png)  
(a) \hspace{1cm} (b) \hspace{1cm} (c)

Figure 4.7 Embossed (a) PMMA and (b) PLGA microwell array with PVP sacrificial templates. (c) Embossed PLGA with a SU-8 mold.

### 4.3.5 Laser/IR Surface Heating Assisted Sacrificial Template Embossing

An embossed PMMA sample using the laser/IR surface heating method in conjunction with the PVP sacrificial template micro-embossing process is shown in Figure 4.8a. One can see that the feature was fully transferred to the PMMA substrate with excellent replication accuracy. However, the isothermal embossing at a temperature of 250°C deformed the template, as shown in Figure 4.8b.
Figure 4.8 Embossed PMMA single channel (a) isothermally and (b) non-isothermally using laser/IR surface heating.

The FEM simulation results at the isothermal condition using the same embossing pressure and initial substrate thickness as used in Figure 4.6c are shown in Figures 4.9a and 4.9b for embossing temperatures of 130°C and 150°C, respectively. The maximum stresses of the mold feature, i.e. the nodal tensile stress at the root of the female mold feature, for these two cases were 12.0Mpa and 24.6Mpa, respectively.

With the surface heating, the simulated initial temperature distribution in carbon-blacked PMMA is shown in Figure 4.10. A maximal surface temperature of 209° was achieved. As shown in Figures 4.11a and 4.11b, a very short embossing time, 0.5 sec in this case, was enough for the micro-features to be nearly filled. The stress of the mold feature was less than 1Mpa. Compared with isothermal micro-embossing, surface-heated embossing can greatly reduce the stresses on the mold. Furthermore, the highest temperature in the mold was only 66.2°C at 6.5 sec, which is lower than the Tg of PVP.
Figure 4.9 Simulation results of isothermal micro-embossing at temperatures of (a) 130°C (12.0Mpa) and (b) 150°C (24.6Mpa).

Figure 4.10 Simulated initial temperature distribution of PMMA substrate with surface heating.
Figure 4.11 Simulated (a) temperature distribution and (b) material flow of laser/IR surface heating assisted sacrificial template embossing
4.3 CONCLUSION

Sacrificial–template-based micro-embossing provides a way to form high-aspect-ratio microstructures. However, the isothermal embossing of high T\text{g} polymers, such as PMMA, is difficult because of the limitations of the template materials. Laser/IR heating can generate a molten layer on the surface of the polymer substrate. By combining the surface heating method and the sacrificial template micro-embossing process, it is possible to mold microstructures with high aspect ratios for high T\text{g} polymers.
5.1 Background

Polymer based microfluidic biochips have great potential to serve as low-cost and easy-to-fabricate analytical tools in clinical applications. A critical component of microfluidic biochips is valving for fluid manipulation. There are both active and passive valve designs. Examples of active valves include thermally actuated gels [Luo, 2003; Yu, 2003], paraffin [Liu, 2004] or liquid itself by rapid cooling and thawing [Gui, 2004], magnetically actuated ferrofluid [Hartshorne, 2004] or monolith [Kirby, 2002], pneumatically controlled membrane [Schomburg, 1993], electro or electrochemically controlled bubble [Suzuki, 2003], and controlled surface wetting [Cheng, 2004]. Most active valves require a mobile part or external stimuli, which restricts their applications in microfluidics when the devices become smaller and need to be low-cost. Passive valves provide a more affordable method to control the liquid flow because they do not need any moving part. They include capillary valve [Madou, 1998], polymer check valve [Nguyen, 2004], elastomer valve [Jeon, 2002], and hydrophobic valve [Feng, 2003].
Among them, the capillary valve is the simplest and can be easily implemented into the microfluidic design and fabrication. However, this type of valve tends to lose its function when holding protein solutions or after the blocking treatment on the microchannel/reservoir surfaces because protein binding on the solid surface would change the surface hydrophobicity and contact angle. Protein blocking is a necessary step to prevent non-specific binding in many biological assays.

One way to overcome this problem is to develop valves with a super-hydrophobic surface. Super-hydrophobicity is widely observed in nature (e.g. lotus leaves), caused by the hierarchical roughness of micro-sized papillae having nano-sized protrusions covered with hydrophobic wax. The contact angle of such a “composite” surface, i.e. a surface consisted of fractions of air and solid, can be described by Cassies’ equation [Cassie, 1944]:

\[
\cos \theta^* = f \cos \theta - (1 - f)
\]  

where \( \theta^* \) is the contact angle on the composite surface, \( \theta \) is the contact angle on the flat surface and \( f \) is the fraction of the solid on the composite surface. The contact angle can be larger than 150° (super-hydrophobic) when a water droplet sits on a super-hydrophobic surface. There are various methods to create super-hydrophobic surfaces [Xie, 2004; Shiu, 2004; Erbil, 2003; Lu, 2004; Feng, 2003; Zhang, 2004; Lau, 2003; Woodward, 2003; Sun, 2004; Favia, 2003]. Among them, the fluorine plasma treatment on a rough surface is the most efficient and affordable surface modification method. However, super-hydrophobic valves capable of functioning in microfluidic biochips requiring surface protein blocking have not been reported in the literature.

The development of a biologically permissive packaging method for bonding microfluidic and microarray devices with preloaded proteins is also critical for the design
and fabrication of biochips. Although there are many methods available for bonding polymeric devices, such as adhesive bonding, plasma bonding, lamination, thermal bonding, and solvent assisted bonding [Rossier, 1999; Dreuth, 1998; McCromick, 1997; Metz, 2001; Duffy, 1998; Lai, 2004], these approaches involve either high temperatures or an organic solvent. They may also introduce foreign materials into the microchannels, which will denature the preloaded proteins or contaminate the assay reagents. Recently, a CO$_2$-assisted polymer fusion approach has been developed in our lab [Yang, 2004]. This method is able to bond polymeric micro- and nanoscale structures at low temperature and low pressure without using any organic solvent.

In this work, we present a new “fishbone” micro-valve design based on the concept of super-hydrophobicity and demonstrate the feasibility of using a CO$_2$-assisted polymer fusion approach for the packaging of microfluidic biochips containing proteins for enzyme-linked immunosorbent assay (ELISA) applications.

5.2 EXPERIMENTAL

5.2.1 Valving

5.2.1.1 Materials and Reagents

Poly(methyl methacrylate) (PMMA) plates were purchased from McMaster Carr (Cleveland, OH) with dimensions of 25.4cm x 25.4cm x 1.6cm. They are stored in a vacuum oven at 70°C for at least 48 hours before use. Poly(dimethyl siloxane) (PDMS) precursor (Sylgard 184 silicone kit) was purchased from Dow Corning (Midland, MI). Bovine serum albumin (BSA; Fraction V) was purchased from Fisher Scientific (Pittsburgh, PA). BSA solutions (0.2wt% and 1.0wt%) in DI water was prepared with a
concentration of 1.0wt% as the blocking solution and 0.2wt% for the use in ELISA. Food dye was purchased from McCormick & Co. Inc. (Hunt Valley, MD).

5.2.1.2 Fabrication of Super-hydrophobic Surface

Parallel microchannels were machined using a CNC machine (Sherline Products Inc., Vista, CA) on the flat PMMA surface. The channel width and depth were 180 and 50 µm, respectively, with a spacing of 200 µm. The solid content of the composite surface was 10%. The fluorine plasma treatment was carried out at 13.56 MHz using a bench-top reactive ion etching system (Technics 800II RIE system). Prior to plasma treatment, the plasma chamber was cleaned with 2-propanol, dried and further cleaned using a 20 sccm oxygen plasma at 200 W for 30 min. The sample was then placed in the chamber, followed by evacuation to a pressure of 12 mTorr. CF₄ gas was then introduced at a flow rate of 50 sccm and the glow discharge was ignited at 300 W. After 2 minutes, the power and CF₄ were turned off and the chamber was evacuated to 12 mTorr again. The chamber was then purged and the samples were taken out.

5.2.1.3 Contact Angle Measurement

To examine the surface hydrophobicity, contact angles of droplets of 10µl DI water and 0.2wt% protein solution on various surfaces (pristine flat, fluorine-plasma treated flat, and fluorine-plasma treated surface with microstructures) were measured under a temperature (air conditioning at 20°C) and humidity (a sealed container with water at the bottom with a relative humidity of more than 95%) controlled environment. Surface blocking with the protein solution was performed by soaking the plate surfaces in 1.0wt%
BSA solution for 10 minutes, and then rinsing it in DI water before blowing it dry. The contact angle was obtained by measuring the sessile drop profile taken by a COHU high performance CCD camera (1.27 cm view area) and was analyzed with a MATLAB code.

5.2.1.4 “Fishbone” Valve Design

The design of a fishbone micro-valve is shown in Figure 1a. To test the function of the valve, two reservoirs were connected by a microchannel with the valve in between. The microchannel had a width of 200µm and a depth of 200µm. Unlike the conventional capillary valve as shown in Figure 5.1b, the valve shown in Figure 5.1a is designed to have several parallel microscale channels, or “fishbone”, mimicking a microfeatured surface. In this study, all fishbone channels were 200 µm in depth, length(l) and width(w). The distance(d) between two fishbone channels was the same as the channel width. The fishbone were treated with fluorine plasma using the same parameters described earlier.

![Diagram of fishbone valve](image)

Figure 5.1. Schematics of (a) fish-bone valve and (c) conventional capillary valve design.
5.1.2.5 Chip Fabrication

A photoresist mother mold (SU-8 100, Microchem) with the fishbone design was fabricated through a standard photolithographic process. The PDMS daughter mold was obtained through reactive casting in which a 10:1 (w/w) base/curing agent of PDMS was thoroughly mixed and degassed under vacuum for 30 minutes, followed by pouring over the mother mold and curing on a hot plate at 70°C for 2 hours. The PDMS daughter mold was used to produce fishbone valves on the PMMA surface through a hot embossing process using a digitized hot press (Carver, Wabash, IN). If not otherwise mentioned, all the microfeatures were fabricated using this procedure. Microchips with a conventional capillary valve as shown in Figure 5.1b were also fabricated. Again, the microchannel had a width of 200 µm and a depth of 200 µm. The circular junction was 1 mm in diameter. One of these chips was plasma treated using the parameters mentioned earlier.

5.1.2.6 Protein Blocking

The chips were sealed with a scotch tape (Clearview, Premium grade, Staples). For testing, 1.0wt% BSA blocking solution was introduced into the microchannels for 10 min. Then the solution was removed by vacuum and the channel was rinsed by DI water three times followed by vacuum drying. Finally, the 0.2wt% BSA solution in DI water colored by food dye was introduced into the sample reservoir. Channels without BSA blocking were used as control.
5.1.2.7 Valve Testing

Figure 5.2. Photos and schematics of (a) the cross section and (b) the top view of the capillary forces working on the liquid front at the edge of the valve.

Photos of the liquid fronts at the valve junction were viewed from the side and top surfaces are shown in Figures 5.2a and 5.2b. The schematics in the figures show the force balance at the liquid front. At the side surfaces, the liquid is held back due to the surface tension of the liquid. At the top and bottom surfaces, the liquid is dragged forward by the wetting force on the surface. If the applied external force is \( F_0 \), the force balance over a unit area can be written as follows:
where W and H are the channel width and depth respectively, θ is the contact angle from the top view, and \( \phi_{\text{top}} \) and \( \phi_{\text{bottom}} \) are the contact angles of the lid and the bottom of the channel from the side view, respectively. If P is negative, the valve would fail and the liquid would burst through. From equation (2), it can be seen that the holding capacity is very sensitive to the contact angles.

Figure 5.3. (a) Schematic of conventional capillary valving. (b) CAD design of a 5-well CD-chip for flow sequencing.

The CD-based microfluidic platform technology has been proposed for various biomedical applications [Scott, 1973; Klumpp, 1977; Bonte, 1977; Henry, 1978]. CD platform have been commercialized for high throughput screening (HTS) by Tecan, for
sample preparation in MALDI by Gyros AB, for human and veterinary blood analysis by Abaxis system, and similar applications by Burstein Technologies [Madou, 2006]. The core of this technology lies on the balance between the capillary force and the centrifugal force such that it is capable of holding the solutions in the reservoirs by capillary valving and of dispensing the solutions in sequence by varying the centrifugal force. In the CD microfluidic platform as shown in Figure 5.3(a), the centrifugal force \( F_c \) provides the pumping pressure. The pumping force per unit area \( P_c \) is:

\[
\frac{dP_c}{dr} = \rho \omega^2 r
\]  

(3)

where \( \rho \) is the density of the liquid, \( \omega \) is the angular velocity of the CD platform, and \( r \) the distance of a liquid element from the center of the CD. The integration of the above equation from \( r = R_1 \) to \( r = R_2 \) results in:

\[
\Delta P_c = \rho \omega^2 (R_2 - R_1)(\frac{R_1 + R_2}{2}) = \rho \omega^2 \cdot \Delta R \cdot \bar{R}
\]  

(4)

where \( \Delta R \) equals to \( R_2 - R_1 \), \( \bar{R} \) equals to \( (R_1 + R_2)/2 \), \( R_1 \) and \( R_2 \) are the two distances of the liquid element from the center of the CD.

Combining equations (2) and (4), the rotating speed at which the liquid would burst through can be calculated using equation (5).

\[
\omega = \sqrt{\frac{2H \sin(\theta) - W (\cos(\phi_{top}) + \cos(\phi_{bottom}))}{\rho \cdot W \cdot H \cdot \Delta R \cdot \bar{R}}}
\]  

(5)
Although the CD-based platform technology works well for the aqueous solutions, it encounters difficulty when dealing with protein solutions or in an enzyme-linked immuno-sorbent assay (ELISA) [Lai, 2004] that requires protein blocking on the chip surface because the capillary valve fails.

ELISA is the most widely used method among various immunoassays for the detection and quantification of biological agents (mainly proteins and polypeptides). It requires a series of reaction/incubation and washing steps. Typically, ELISA carried out in a 96-well microtiter plate would take many hours to several days. A CD-ELISA combining microchannel ELISA and microfluidic flow sequencing can lead to a much shorter cycle time, decreased consumption of reagents and an easy automation [Lai, 2004]. In the CD-ELISA process, the reagents (antibody, antigen, substrate and washing solution) were placed in reservoirs at different locations on the CD and held by microscale valves. The delivery sequence of the reagents to the reaction/diction channel was controlled by the rotation speed.

A CD-ELISA chip with fishbone valves was designed as shown in Figure 5.3b. This design contains 5 sample reservoirs and each reservoir is located between two fishbone valves. The dimension of all microchannels and reservoirs are given in Table 5.1. The blocking solution used is the Tris assay buffer (TAB, 50 mM Tris, 0.5M NaCl, 1% BSA, and 0.1% Tween 20, pH 7.5) and the testing protein solution is 0.2wt.% BSA solution in DI water colored by 5.0wt.% food dye. After fluorine plasma treatment, the CD chip was bonded by Scotch tape. The blocking solution was then added into reservoirs through the loading holes and the CD was spun at 4000 rpm to make sure all the blocking solution would burst through. The blocking solution flowed downward to the waste reservoir and
a thin layer of protein remained on the channel surface. After protein blocking, the CD was dried in a vacuum chamber for 2 hours. Then the testing protein solution was loaded for the flow sequencing test.

<table>
<thead>
<tr>
<th>No.</th>
<th>Channel width (µm)</th>
<th>Channel depth (µm)</th>
<th>(R_1)(mm)</th>
<th>(R_2)(mm)</th>
<th>Calculated Burst frequency (rpm)</th>
<th>Experimental burst frequency (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>230</td>
<td>22</td>
<td>24.33</td>
<td>1480</td>
<td>1420±60</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>230</td>
<td>25</td>
<td>28.5</td>
<td>1124</td>
<td>1040±55</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>230</td>
<td>30</td>
<td>32.33</td>
<td>870</td>
<td>820±40</td>
</tr>
<tr>
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<td>225</td>
<td>230</td>
<td>35</td>
<td>38.5</td>
<td>610</td>
<td>660±35</td>
</tr>
<tr>
<td>5</td>
<td>375</td>
<td>230</td>
<td>40</td>
<td>43.5</td>
<td>417</td>
<td>470±30</td>
</tr>
</tbody>
</table>

Table 5.1 Dimensional parameters and burst frequency of the 5-reservoir CD-chip design

5.2.2 Packaging

5.2.2.1 Materials and Reagents

Poly(DL-lactide-co-glycolide) (PLGA, 4A) was obtained from Alkermes. A bone-dry grade carbon dioxide was supplied by Matheson at a purity of 99.8%. Lysozyme was purchased from Sigma (St Louis, MO). Alexa Fluor® 488 goat anti-rabbit IgG (GAR-488) was purchased from Invitrogen (Carlsbad, CA). The BioRad protein assay dye reagent was purchased from Bio-Rad Laboratories (Hercules, CA).
5.2.2.2 CO$_2$-assisted Bonding

![Figure 5.4 Schematic of CO$_2$ assisted bonding device](image)

A pneumatic cylinder (2A series heavy duty, Parker) with an inside diameter of 20.32 cm was modified to act as the CO$_2$ bonding device as shown in Figure 5.4. A flexible silicon rubber heater (OMEGALUX™ heating tape) was wrapped around the cylinder to control the bonding temperature. Two Praxair gas regulators were used to control the pressure of the compressed nitrogen and CO$_2$ gas. The CO$_2$ is connected to the cylinder chamber as the bonding medium. Nitrogen gas drives the piston to provide the contact pressure. A stopper is used to control the position of the piston.

A PLGA solution was prepared by dissolving 2g of PLGA in 10g of acetonitrile. A thin layer of PLGA film was spin-coated onto a blank PMMA cover at 1500 rpm for 1 minute using a spin coater (G3p-12, Cookson Electronics Equipment). The spin coated
cover was then placed in a vacuum oven overnight for drying. Before bonding, the PMMA cover was aligned with the microfluidic chip and the whole assembly was placed inside the pneumatic cylinder at 37°C. The CO₂ pressure was introduced and increased to 1.38 MPa for half an hour while the stopper held the piston as shown in Figure 5.4. Then a contact pressure of 0.28 MPa was applied by the piston on the assembly by adjusting the nitrogen pressure. The bonding took another half an hour, followed by a slow pressure release of CO₂ gas at 0.3 MPa per min to avoid foaming. The low pressure was chosen to allow low-cost pneumatic cylinders, instead of expensive autoclaves, to be used in mass production.

5.2.2.3 Protein Activity Test

BSA, lysozyme and GAR-488 were chosen to represent the blocking reagent, primary antibody and secondary antibody used in ELISA, respectively. BSA (5 mg in powder), lysozyme (5 mg in powder), and GAR-488 (40 µg/ml) in triplicate were subject to CO₂-assisted bonding, and thermal lamination bonding conditions. For CO₂-assisted bonding, the samples were placed in a pressure chamber (PAAR 5660) at 37°C. An ISCO 500C high-pressure syringe pump was used to deliver and control the CO₂ pressure in the chamber. The CO₂ pressure was increased to 1.38 MPa at an approximate rate of 0.28 MPa per min, maintained at 1.38 MPa for 30 min and followed by a slow pressure release at 0.3 MPa per min. The thermal lamination bonding condition was mimicked using a hot plate heated at 140°C for 10 s. Samples without any treatment were used as control. After treatment, BSA and lysozyme were collected and dissolved in 20 ml of
phosphate-buffered saline (PBS). The protein content of BSA, the lysozyme activity, and the fluorescence intensity of GAR-488 were measured.

The protein content of BSA was determined with the BioRad protein assay dye reagent. Briefly, 10 µl of BSA samples in duplicate were placed into each well of the 96-well plate and 200 µl of diluted BioRad protein assay reagent (1:4 in water) was added to each well. After incubating the plate for 5 min, the absorbance of the wells was detected using a Tecan GENios Pro™ microplate reader (Durham, NC) set at 595 nm. BSA in series concentration (0 - 0.5 mg/ml) was used as standard. The protein content of BSA after treatment was normalized with control and expressed as percentage (%).

The lysozyme activity was measured using an EnzChek Lysozyme Assay Kit (Invitrogen) according to the manufacturer’s specifications. Briefly, the lysozyme in solution was further diluted 1:30 with PBS and 50 µl of samples in duplicate were placed into each well of a 96-well plate. Lysozyme in series concentration (0 - 500 U/ml) was used as standard. Fifty µl of the substrate working solution was added to each well and incubated at 37°C for 30 min. The lysozyme activity was detected using Tecan GENios Pro™ microplate reader set at a filter set of 488/535 nm. The data were normalized with control and expressed as a percentage (%).

GAR-488 was diluted 1:100 in water and 50 µl of samples in duplicate were placed into each well of a 96-well plate. The fluorescence intensity of GAR-488 was detected using a Tecan GENios Pro™ microplate reader set at a filter set of 488/535 nm. The data were normalized with control and expressed as a percentage (%).
5.3 RESULTS AND DISCUSSION

5.3.1 Valving

5.3.1.1 Super-hydrophobic Surface

The shape of water droplets on PMMA, fluorine-plasma treated PMMA, and fluorine-plasma treated microfeatured PMMA surfaces is shown in Figures 5.5(a), (b) and (c), respectively. The corresponding contact angles are 73°, 108°, and >160°, respectively.

![Figure 5.5](image)

Figure 5.5. Images of water droplets on various surfaces: (a) PMMA, (b) fluorine plasma treated PMMA, and (c) Microfeatured PMMA with fluorine plasma treatment.

After protein blocking, the contact angle with 0.2wt.% BSA solution would change with time, but eventually leveled off after several minutes. So the contact angles at 5 minutes were used to calculate the valve holding capacity. The initial contact angles and the contact angle after 5 minutes on various solid surfaces are listed in Table 2.

Compared with pristine PMMA, the contact angle of protein blocked PMMA without fluorine plasma treatment decreased the most (32°), showing why the conventional capillary valve would fail after protein blocking. The protein blocked
PMMA with plasma treatment showed less contact angle decrease (23°), but the contact angle was still too low for the conventional capillary valve to function in microfluidic applications. It should be mentioned that the contact angle of pure DI water on all solid surfaces did not show any obvious change as a function of time.

The fluorine-plasma treated surface with microstructures, i.e. the super-hydrophobic surface maintained its super-hydrophobic property after protein blocking with a small contact angle decrease from 161° to 150°. Because of air trapped in the microfeatures, the protein solution would not flow into the microchannel when the chip was soaked in the blocking solution. In this case, the solid fraction is 0.1. Using the contact angle value given in Table 5.2 and the Cassie’s theory in equation (1), the predicted value for the super-hydrophobic surface is 158° and for the super-hydrophobic surface after protein blocking is 147.7°. Both agreed well with the experimental measured values.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>plasma treatment</th>
<th>protein blocking</th>
<th>Contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>PMMA</td>
<td>No</td>
<td>No</td>
<td>73</td>
</tr>
<tr>
<td>PMMA</td>
<td>No</td>
<td>Yes</td>
<td>74</td>
</tr>
<tr>
<td>PMMA</td>
<td>Yes</td>
<td>No</td>
<td>108</td>
</tr>
<tr>
<td>PMMA</td>
<td>Yes</td>
<td>Yes</td>
<td>80</td>
</tr>
<tr>
<td>PMMA with microfeatures</td>
<td>Yes</td>
<td>Yes</td>
<td>161</td>
</tr>
<tr>
<td>Scotch tape</td>
<td>No</td>
<td>Yes</td>
<td>106</td>
</tr>
</tbody>
</table>

Table 5.2. Contact angle of 0.2wt% BSA solution on various surfaces
5.3.1.2 Performance of Conventional Capillary Valve

The conventional capillary valve worked well for both pure water and protein solution in the absence of protein blocking (Figure 5.6(a)). However, the capillary valve function was lost after protein blocking as shown in Figure 5.6(b). The protein solution wicked through the entire valve surface even without any external driving force. Even with the fluorine plasma treatment, the valving function was still totally lost after protein blocking as shown in Figure 5.6(c), but the wicking occurred slower.

Figure 5.6. (a) Capillary valve can stop the flow of pure water and protein solution. (b) Capillary valve fails to stop the 0.2wt.% BSA solution with food dye after protein blocking. (c) With the fluorine plasma treatment, capillary valve still loses its function.

5.3.1.3 Performance of Fishbone Valve

When the protein solution containing food dye was loaded through the sample loading hole, the solution filled the microchannel and the reservoir easily by means of the capillary force, indicating that the solid surface has become more hydrophilic after protein blocking. The fishbone valve was able to stop the liquid flow as shown in Figure
5.7(a), while the conventional capillary valve failed when it was subjected to the same fluorine plasma surface modification. Because the multiple-gate design of the fishbone valve provides the necessary redundancy, a long holding time of the reagents/washing buffer in the reservoirs can be achieved during the ELISA process. In this case, each microchannel of the fishbone provided a holding time of several hours.

Figure 5.7. (a) Fishbone valve is able to stop the flow of 0.2wt.% BSA solution with food dye after protein blocking. Flow profile of protein blocking solution in microchannel (c) with and (d) without fluorine plasma surface modification. 

Protein buffer+food dye
Figures 5.7(b) and 5.7(c) were taken during the protein blocking process for chips with and without fluorine plasma treatment. With the treatment, the blocking solution only wetted (or blocked) a portion of the fishbone surface as shown in Figure 7(b). Most of the channel surface in the fishbone remained hydrophobic because of the trapped air. While without the fluorine plasma treatment, the solution would eventually fill the entire surface of the fishbone valve because the blocking solution in the fishbone (Figure 7(c)) tended to stay in the fishbone microchannels during removal of the blocking solution by vacuum or centrifugal force.

The calculated and experimental burst frequencies in rpm are given in Table 5.1. The burst frequency can be predicted well by equation (5) and the flow sequence of the protein solution can be successfully achieved using the fishbone valve design in the ELISA process.

5.3.2 CO$_2$-assisted Bonding

Since CO$_2$ is a good solvent for certain polymers to increase the free volume of the polymer molecules and the chain mobility at the bonding interface, both micro-/nanobonding can be achieved via molecular diffusion [Yang, 2004]. CO$_2$ can be released after bonding without causing any deformation of the substrate. A CD-sized microfluidic chip was sealed with a lid using the CO$_2$-assisted bonding methods as shown in Figure 5.8. Figures 5.9(a) and 5.9(b) show the cross section and top view of the CO$_2$ bonded chip. A hermetic bonding was achieved without any leakage as tested by the food dye solution.
The effect of the CO₂-assisted bonding method on the activity of biomolecules was also investigated and compared with the widely used thermal lamination bonding method. Biomolecules inside microchannels without going through any bonding process were used as control. From Figure 5.10(a) the protein content of BSA inside microchannels was reduced by almost 50% after going through thermal lamination bonding. However, the protein content of BSA after going through CO₂-assisted bonding was only reduced by about 10%. From Figure 5.10(b), the lysozyme activity after CO₂-assisted bonding was maintained by more than 95% while that after thermal lamination bonding was less than 90%.

![Figure 5.8 CO₂ bonded 5-inch CD-ELISA chip](image)
Figure 5.9. (a) Cross section and (b) top view of a CO$_2$ bonded chip tested with food dye solution.

More significantly, the fluorescence intensity was reduced almost 80% after thermal lamination bonding as shown in Figure 5.10(c). This poses a major detection challenge in the ELISA process since the fluorescence intensity is substantially reduced compared to that using the 96-well micro titer plate. The CO$_2$-assisted bonding, on the other hand, suffered little fluorescence intensity loss, which greatly benefits the packaging of microfluidic devices involved with preloaded proteins.
Figure 5.10. Effect of bonding conditions on (a) the protein content of BSA, (b) the bioactivity of lysozyme, and (c) the fluorescence signal of Alexa Fluor® 488 goat anti-rabbit IgG.
5.4 CONCLUSION

When the solid surface is subjected to protein adsorption, the contact angle may change greatly. This is why the conventional capillary valve fails to hold/stop the protein solution. Based on the concept of super-hydrophobicity, a fishbone valve was developed, which provided a robust valving function when dealing with protein blocking and protein solutions. The valving capacity was demonstrated using a 5-reservoir CD chip design. Low pressure CO$_2$ bonding was successfully used to package CD-sized chips. Test results showed that this method had little influence on the protein loading amount, bioactivity and fluorescent signal intensity. It is feasible to use this low-cost packaging approach for assembling biochips containing proteins.
CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The overall goal of this study is to design and fabricate polymer-based microfluidic devices for BioMEMS applications. The emphasis is on the development of new hot embossing methods, new packaging techniques, and design of microfluidic functions. These low-cost microfabrication techniques can be used to produce polymer-based microfluidic devices for research and commercial applications.

Two new hot embossing techniques were developed in this study. The laser/IR assisted embossing process could achieve a cycle time of less than 1 minute, which is comparable with that in micro-injection molding. At the same time, high precision localized micro-patterning could be achieved on the polymer surface due to localized heating, which decreased the bulk deformation of the substrate. With a sacrificial template, microstructures with an aspect ratio of 6 were achieved successfully on a biodegradable polymer, PLGA. Furthermore, a combination of sacrificial template and
fast surface heating was able to fabricate high aspect ratio microstructures on high temperature polymers such as PMMA.

Two approaches were used to mimic the lotus effect in nature, i.e. CNC machined microstructures and chemical synthesized conductive polymer hierarchical structures on the polymer surface. To achieve super-hydrophobicity, these micro/nano structures were treated by fluorine RF plasma by coating the surface with a very thin layer of Teflon-like low free energy material. A very high contact angle of water, more than 175°, was obtained. Furthermore, the influence of protein absorption on these super-hydrophobic surfaces was tested. It was found that protein binding had little effect on the contact angle. Based on this phenomenon, super-hydrophobic and fishbone valves were designed and tested, which showed excellent valving performance.

A microfluidic platform has been designed on a compact disk (CD) for medical diagnostics in our laboratory [Lai, 2004], which includes functions such as pumping, valving, sample/reagent loading, metering, reaction and detection. The fluid propulsion is based on the centrifugal force, which is achieved through rotationally induced hydrostatic pressure. A passive capillary valve, which is based on a pressure barrier that develops when the cross-section of the capillary expands abruptly, was used to control the fluid flow. However, these capillary valves lost their function due to the change of the surface property after protein blocking. The fishbone microvalve remained its function even after protein blocking. Compared with the super-hydrophobic valves based on chemical synthesis, the fishbone valve can be easily incorporated into the microchip design and can be produced in the typical chip replication process such as micro-injection molding and hot embossing.
A polymer CD microfluidic platform integrated with these different fluidic features was designed and fabricated for enzyme-linked immunosorbent assays (ELISA). We have successfully demonstrated that flow sequencing could be achieved on this CD-like microfluidic platform by integrating the necessary microfluidic functions such as centrifuge pumping and fishbone valving.

For packaging the microfluidic platforms, a new interstitial bonding technique was developed, which could bond the polymer-based microfluidic platforms without introducing any alien materials into microchannels. A multi-channel DNA sequencing chip was demonstrated experimentally. Another bonding method, CO$_2$ assisted bonding, was also demonstrated for bonding a CD platform. By applying a thin PLGA interlayer, the CD can be bonded at low temperatures to achieve a hermetic bonding. ELISA tests showed that these two bonding methods have no or little effect on the activity of preloaded proteins, which is essential for microfluidic designs that requires preloading of regents such as proteins, antibody/antigen and cells.

### 6.2 Recommendations

#### 6.2.1 Applications of the new laser heating technique

Laser embossing and laser welding have many common features enabling some of the experimental tool and theoretical concepts to be applicable in both cases. For example, the laser heating theory has been successfully applied to analyze both TME and TSE embossing according to the simulation results [Chapter 3]. However, the energy efficacy is not high because the mask blocked most of the incident laser radiation.
One approach to increase the efficacy is to use the diffractive imaging technique. Diffractive imaging has been widely used for entertainment and illustration processes, such as laser pointers, but it has not been widely used with high powered laser (>1 W).

Diffractive optics function by diffracting light and interference of waves that have defined phase shifts. As shown in Figure 6.1, a collimated beam that passes through two relatively thin slits in a mask will produce interference patterns on a screen. So it is possible to use diffractive optics to reshape a laser beam and other light sources into complex shapes as shown in Figure 6.2. What’s more, nearly all of the light is allowed to pass through the lens. This greatly increases the efficacy of the lens, which can be as high as 70% [Grewell, 2005]

Grewell et al [2005] has developed this technique to achieve microwelds on plastics. As shown in Figures 6.3 (a) and (b), circular and cross shaped heat-affected-zones (HAZ) were generated. Micro-weld feature as small as 300 microns with a weld line as thin as 75 microns could be produced and a cycle time as short as 50ms could be achieved. This technique has a great potential to be applied for fast replication of polymer-based MEMS and micro-fluidic devices. For example, the cross shaped HAZ in Figure 6.3(b) is designed for the fabrication of a DNA separation device.
Figure 6.1 Schematic of light diffraction

[http://homepage.univie.ac.at/Franz.Embacher/KinderUni2005/waves.gif]

Figure 6.2 Diffractive optics
6.2.2 Applications of numerical analysis

Many FEM simulations were carried out in this research. Simulation can provide better understanding of the processes and decrease the cycle time for production development. For example, the software Deform has been successfully used to predict the flow front during embossing [Chapters 3 and 4].

There is also a growing interest in using simulation and modeling to guide the design of microfluidic devices. Jensen et al [1998] used FEM simulations to design microfluidic devices for chemical reactions. Two- and three-dimensional fluid flow, thermal fields, and chemical species concentrations in microfluidic devices were simulated to predict the performance of microreactor systems. The reactor simulations are in excellent agreement with experimental observations, demonstrating the ability of FEM solution to provide accurate simulations of microfluidic chemical devices. Bergkvist et al [2002] investigated...
a silicon microextraction chip (SMEC) structure both numerically and experimentally. The finite element analysis of the microfluidic flow in the microchip was analyzed numerically by the use of computational fluid dynamics module FLOTRAN in the ANSYS software package. The overall analysis performance was verified by the experimental result.

Several companies have developed commercial simulation software for modeling MEMS, microsystems, sensors and actuators, and microfluidics devices. FlumeCAD™ (Fluid-molecular CAD, now called Biochip Developer software) by Microcosm Technologies (renamed to Coventor) is the first integrated CAD system targeting the general design of integrated chemical and biochemical analysis or synthesis systems. Biochip Developer software (then FlumeCAD) uses finite element methods to solve the Navier-Stokes equations including the thermal fields in fluidic devices. In addition, it calculates the coupled electrostatic equations for applications in ionic transport in microchannels. Biochip Developer software supports 3D simulation of chemical transport in electrophoretic, electroosmotic, and pressure driven systems and includes tools for the design and analysis of DNA separation and PCR amplification systems on chip. It also includes 3D modeling of fluidic microcomponents; performing flow, chemical transport, and containment analysis; enabling the analysis of complex fluidic networks; and calibrating microchemical flows with valid simulations. This software has been applied in the analysis of micromixing [Wang et al., 2001] and optimization of sample injection components in electrokinetic microfluidic systems [Bousse et al., 1999].

CFD Research Corporation provides software (CFD-ACE+) for biochip and microfluidic development for microreactions, bioanalytical (genomics, proteomics,
cellomics), medical and pharmaceutical applications. CFD-ACE\textsuperscript{+} enables the strongly coupled simulation of fluid flow, heat and mass transfer, particle magnetics, structural dynamics, and others. It is capable of modeling microfluidic functions such as sample preparation, extraction, injection, cleaning, dispensing, filling, mixing, preconcentration, separation, fluid and particle handling, electrochemical sensing, biochemical sensing, and PCR. This software system has been applied in the optimization of DNA hybridization kinetics [Lenigk et al, 2002], thermal management (i.e., heater input power, fluid flow rate, sensor placement, and air-gap size and placement) inside microchannels for PCR [Sadler et al, 2002; Chou et al, 2002], DNA analysis (i.e., extraction, concentration of DNA from fluidic samples and on PCR amplification, hybridization, and electrophoretic separation) in microfluidic networks on a chip [Przekwas et al., 2000], and convective-diffusive mass transport as well as biochemical reaction kinetics in a coupled manner [Makhijani et al., 1999].

FLOW-3D (Flow Science, Santa Fe, NM) is a general purpose CFD software package capable of fluid flows. Besides its specialty surface flows, FLOW-3D also has excellent potential for simulations of external flows and confined flows.

Computer simulation can help design engineer to gain insight into the physics of a microdevice, as well as the interactions between physical domains that can greatly affect the device performance. For the fishbone valve design as an example, a good simulation can lead to answers to the following questions: what is the optimal channel dimension such as the width, depth and length of the fishbone? What does the flow front look like during the blocking process by centrifugal force? What is the optimal rotating speed for blocking? What is the fraction coverage and thickness of the absorbed proteins? and
many more. Compared to traditional empirical and laboratory analysis, this method provides a fundamental and detailed understanding of the microdevice and may lead to higher performance designs in less time and at lower cost.

The computer simulation can also improve the performance of CD microfluidic platforms. With the computer simulation, it should be able to model the centrifugal pumping and capillary valving precisely and predict the burst frequency in a more accurate way. For example, the contact angles varied as a function of time after the channel surface was blocked by protein, i.e. there is not a fixed burst frequency before the contact angle reaches equilibrium [Chapter 5].

An example of simulation of protein blocking for the fishbone design with Flow3D is given in Figure 6.4. Snapshots from the simulation results are captured. In this simulation, a channel size of 200µm x 200µm and a protein injection speed of 2mm/s are used.
Figure 6.4 simulation results of (a) Pristine PMMA surface and (b) plasma treated PMMA
As one can see, the simulation can predict the flow front change during the protein blocking process. Compared with the experimental results shown in Figure 5.6, the liquid will not fill the fishbone for chips with plasma treatment and the liquid will wet at least part of the fishbone surface for chips without plasma treatment.

However, for the complex CD microchips, the blocking was conducted by centrifugal force at a high rotating speed. It is not easy to capture the flow front of protein solution. A simulation will provide a way to optimize the design parameters.

Another question involved in centrifugal blocking is how to quantify the protein absorbed onto the channel surface. To answer that question, we need to know the fraction coverage. Although many literatures have presented how to simulate fraction coverage, the inner coating based on centrifugal force has never been reported. The development of software to solve this problem is critical for a better performance of ELISA process.

So far, no commercial software is available to predict the protein binding process and interaction between the binded protein and the protein contained in the protein solution. Although contact angle change has been quantified experimentally, a software that can predict the amount of absorbed protein based on the interaction between the protein and the substrate is still abstractive not only for the microfluidic design in this study, but also for protein containing devices in other bio-related areas.

6.2.3 Super-hydrophobicity

In this study, two kinds of micro-/nano features were used to generate a super-hydrophobic surface. One is micro-features by CNC machining and the other is from chemical synthesized nano-features by in-situ polymerization of conductive polymers. The latter can create localized nano-features with in a very short of time compared with
standard photolithography process. However, this study did not take the advantage of the characteristic of conductive polymers.

6.2.3.1 Electro-wetting micro-valve

Electro-wetting controlled micro-valves were widely studied. To form such a valve, a conductive material such as ITO is usually patterned in the microchannel to act as the electrode. A hydrophobic material such as Teflon is then patterned on top of the electrode material to act as the isolator and the hydrophobic barrier. When the electricity is turned on, the surface tension of the liquid decreases because of the electric induced charge changes on the free surface and the micro-valve will open. After the electricity is turned off, the micro-valve will close. Patterning of the electrode and the hydrophobic material is usually done photolithographically, which takes a longer time and needs a cleanroom environment. The contact angle of Teflon-like materials is not high (~105°) because of the absence of nano-features. With a proper micro-feature design, however, nano-feature of conductive polymers can be easily generated locally and rapidly. The polymer itself can serve as the electrode material and after hydrophobicization, the contact angle of the valve surface can be very high, i.e. a larger design window can be provided.

Another advantage of conductive polymer is that it can be doped easily by an acid and un-doped by a base. So besides the fluorine plasma, a super-hydrophobic surface could be generated by doping the nano-featured surface by a perfluoroalkyl acid. Under electricity, the polymer surface is positively charged, the acid is doped and the surface changes from hydrophilic to hydrophobic. Without electricity, the polymer surface is undoped, the surface changes to hydrophilic again.

6.2.3.2 Super-hydrophilicity
Another characteristic of the synthesized nano-features by conductive polymer is super-hydrophilicity, i.e. a contact angle of less than 5° could be achieved. This can be explained by Wenzel’s theory [Wenzel, 1944], i.e. a surface roughness can enhance both hydrophobicity and hydrophilicity. The super-hydrophilicity together with a good UV absorption property of conductive polymer such as Pani provides a potential application of conductive polymer in the field of anti-fog and UV protection products such as sunglasses.

Another potential application of such a super-hydrophilic surface is to stretch DNA and conjugate DNA with PEI for gene therapy applications. For example, Pani has a positive charged function group and DNA has negative surface charge. When DNA solution flow on a Pani super-hydrophilic surface, the DNA could be anchored onto the Pani surface and also stretched because of the high flow speed of the DNA solution on such as super-hydrophilic surface. After drying, the PEI solution can be introduced and conjugated onto DNA molecules because of the positive surface charge on the PEI molecules. The conjugated DNA could then be released from the Pani surface because of the same kind of surface charge as the Pani substrate.
APPENDIX A: INTERSTITIAL BONDING

A.1 Background

The fabrication of microfluidic devices typically includes the following steps: Manufacturing of one or more substrates with micro-channels and other micro-features, this is usually done by lithography, soft lithography, micro-injection molding, and hot embossing; Application of coatings or reagents to the channels and other features; Assembly of the substrates to create three dimensional flow paths with the incorporation of pumps and valves.

This multi-step fabrication process is complex with many requirements. For example, consider a relatively simple microfluidic device that includes an array of micro-channels. The substrate must have precisely dimensioned channels (to control the fluid volume) with specific surface properties (to accept hydrophilic or hydrophobic coatings, proteins, or chemical reagents) and acceptable physical, thermal and structural properties with low residual stresses (to minimize surface attack or micro-cracking). The assembly and packaging methods must permit precise control of heating and heat transfer and elimination of vibration that is common in many industrial plastics welding techniques, such as vibration and ultrasonic welding.

In most cases, the coating is temperature-sensitive and will not function properly if over-heated. Thus, it is important to confine any heat generated during welding or
adhesive bonding to the joint area to prevent damage to the micro-channels and to minimize residual stresses due to assembly. In addition, it will be important to prevent any flash/particulates that are produced during the welding process from entering the channels. Finally, the joining process must be robust as joining is done near the end of the manufacturing cycle.

Owing to the unique properties of polymeric materials such as biocompatibility, optical quality, high impact strength, low cost, good processibility for mass production, and being recyclable, myriads of fabrication and bonding techniques for plastic microfluidic chips have been reported in recent years. Referring to bonding techniques for plastic microfluidic chips, several approaches have been demonstrated, including thermal bonding/lamination, solvent bonding, adhesive tape or glue layer, and plasma-aided bonding. However, they are problematic associated with channel deformation, contamination of solvent residue, introduction of multiple materials to form non-homogeneous microchannel surface and altering the original surface properties. Most important is that when reagent needs to be preloaded onto channel surface before bonding, most of the bonding methods are problematic because of the denaturing of the reagent such as proteins.

Liquid flow driven by capillary force inside a microchannel and a spherical shape of liquid drop at the tip of a capillary tube due to its surface tension are widely known phenomena. We have recently found that good bonding of plastic microfluidic chips can be achieved by placing the chip and the cover plate together without clamping, dispensing a UV-curable resin in between the assembly, and applying UV exposure. It was realized that the resin filling of the interstitial space in between the assembly was
driven by the capillary force owing to the microscale gap. As the resin flow encountered the microchannels, an open area leads to the resin in contact with only one side of solid surface of the gap. The surface tension of the resin is able to overcome the wetting and hold off the resin flow. This is the reason why the UV-curable resin can bond the entire microfluidic chip without flowing into the microchannels.

The new bonding technique, dubbed as interstitial bonding, has many advantages: it is a simple process and capable of bonding plastic microfluidic chips with complicated designs at high yields. In addition, there will be no issues regarding channel deformation, and formation of substantial non-homogeneous microchannel surface. Selection of polymeric materials for both the chip and the cover plate is flexible, i.e. polymethyl methacrylate (PMMA), polystyrene (PS), polycarbonate (PC), polyethylene terephthalate (PET), polyolefin, etc. can be used. Moreover, they can be with different thickness. Although the bonding technique involves two-stage curing, the first stage can be completed within seconds if a powerful UV lamp is provided. By adjusting the resin filling time and first-stage UV curing time, bonding of microfluidic chips can be easily converted into continuous process. The second stage will then be carried out in batch-type fashion. In the following section, we present an example of packaging of plastic microfluidic chip via interstitial bonding.
A.2 Experimental

A.2.1 Interstitial bonding

A.2.1.1 Materials

1/16" PMMA plate was purchased from McMaster Carr with a dimension of one square feet. 2-hydroxylethyl methacrylate (HEMA) monomer was purchased from Sigma-Aldrich (St. Louis, MO). Igrcure 819 was purchased from Ciba Specialty Chemicals. Both HEMA and Igrcure 819 were used as received.

A.2.1.2 Interstitial bonding

Figure A.1 shows the schematic of the interstitial bonding. A single microchannel feature was studied with a cross section of 250µm in width and 100µm in depth by CNC machining (Sherline). Both the microfluidic chip and cover plate were thoroughly cleaned and blown dry. Several microliter (depends on the chip size) UV-curable resin (HEMA) was dispensed into four 1.5mm-in-diameter resin-loading wells, which were pre-drilled either on the chip or the cover plate. The whole assembly was placed under the UV light for around 5 minutes.

After the bonding, the gas assisted bonding was conducted by filling HEMA into the microchannel followed by removal via vacuum and UV curing of 2mw/cm² for 10minutes.
A.2.2. DNA separation

A.2.2.1 Materials and reagents

Thiazole orange (TO, DNA probe) was purchased from Aldrich. A DNA standard (ΦX174 Hae III Digest, 381 µg/ml) and the 5X Tris-Borate-EDTA (TBE) buffer solution were purchased from Sigma-Aldrich (St. Louis, MO). PEO 2wt%, sieving material, was prepared from PEO powder (Sigma, 4M) in our own lab.

A.2.2.2. Microchip fabrication

The microchip designs used for DNA separation is similar to Caliper’s DNA Labchip® for multiple DNA separation as shown in Figure A.2. In this design, a cross structure was adopted for sample injection, and the channel dimensions, 250 × 100 µm, are the same as in the interstitial bonding test. This design is able to separate 6 DNA samples. Each end of each microchannel was connected to a reservoir for buffer solution,
DNA sample, and wastes, respectively. They also provide the access of the electrodes. The detection can be carried out at any point between the sample injection point and the waste reservoir. Therefore, the actual separation length ranges from 0 to 1.5 cm.

The DNA sequencing microchips was fabricated by the CNC machining on a 24mm long, 18mm wide and 1.59mm thick PMMA plate and bonded with a same sized PMMA plate by using the interstitial bonding technique described in Section 2.1.

Before bonding, the microfluidic chip and a cover plate were then thoroughly cleaned and blown dry followed by being brought in contact without clamping. A total amount of 5 1 UV-curable resin (HEMA and 1% w/w photoinitiator, Irgacure 819) was dispensed into four 1.5mm-in-diameter resin-loading wells, which were pre-drilled either on the chip or the cover plate. The resin began to quickly fill the interstitial space between the chip and the cover plate without flowing into the microchannels as shown schematically in Figure A.3 (a). The whole assembly was placed under the UV light for around 10 minute for pre-curing (Figure A.3 (b)) and subsequently moved into the 60°C oven for 2-hour post-curing to remove residual monomers.

For visualization purpose, the food dye was filled into the loading hole instead of HEMA and after bonding, the food dye was filled into the microchannel to see if there is any leakage.
Figure A.2 DNA chip design

Figure A.3 Schematic of (a) microfluidic chip design and resin loading, (b) resin curing for bonding.
A.2.2.3 Detailed DNA separation process

The DNA standard was diluted in a 0.2X TBE buffer solution to 10 µg/ml from the stock solution (381 µg/ml). The TO dye was prepared in 100 µM in a 1X TBE buffer solution. It was further diluted to 5 µM in the sieving PEO solutions.

The experimental setup for DNA separation is shown in Figure A.4. Voltages was applied to the reservoirs via a high voltage power supply system, which consists of a low voltage, programmable power supply (72-6695, Tenma® Test Equipment, Springboro OH), two miniature DC to HV (high voltage) DC converters (G10 and G20, Emco High Voltage Corp., Sutter Creek CA), and a DPDT (double pole double throw) relay (0700A, MCM Electronics). The 72-6695 power supply is capable of voltage and current outputs, timer, and over voltage/current protection. It provides the input voltages for miniature DC to HV DC converters. The G10 is used for output voltage up to 1,000 V and G20 for output voltage up to 2,000V. The relay is used to switch the polarity of the electrodes.

Figure A.4 Nikon Epi-Fluorescence Microscopy
Detection was carried out on-chip using an inverted fluorescence microscope (Nikon ECLIPSE TE2000-U). A 100W mercury light source with a 490 nm filter and a dichroic mirror was used as an excitation source. The fluorescence signal was obtained through a dichroic mirror and a 510 nm filter. Images at the detection point were recorded sequentially by a 12-bit high-resolution monochrome digital camera system (CoolSnap HQ, Roper Scientific). The fluorescence intensities were extracted directly from the sequential video images by using an image analysis software (Fryer Metamorph Image Analysis System). The intensity was an average over a 100 µm × 30 µm detection area.

As described in the literature [Lai, 2002], the electrophoresis separation of the DNA fragments follows three steps: injection of DNA samples, pulling back to form the sample plug, and the separation as illustrated in Figure A.5. The first step is the injection of DNA samples. We chose reservoir 1, 2, 3 and 4 for the DNA separation. With an electric field applied between reservoirs 2 and 3 and no voltage applied between reservoir 1 and 4, the DNA migrated from reservoir 3 towards reservoir 2 and filled the injection cross. The next step (pulling back) is to move the sample in the injection cross forward to the separation channel and pull the rest back to reservoirs 2 and 3 to form a DNA sample plug for separation. The third step is the separation with an electric filed applied between reservoirs 1 and 4. The DNAs migrated towards reservoir 4 under the electric field and were separated through the sieving materials inside the separation channel. The voltages applied and times for each step are summarized in Table A.1
<table>
<thead>
<tr>
<th>Electrode (V)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>t (s)</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>400</td>
<td>Float</td>
<td>20</td>
</tr>
<tr>
<td>Pull back</td>
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<td>500</td>
<td>500</td>
<td>1100</td>
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</tr>
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<td>0</td>
<td>Float</td>
<td>Float</td>
<td>1100</td>
<td>150</td>
</tr>
</tbody>
</table>

Table A.1. Separation parameters

Figure A.5 Schematic of DNA chip and electrode numbers

A.3 Results and discussion

The pictures of the cross section of the interstitial bonded and CO2 bonded chips were shown in figure A.6 (a) and A.6 (b). From the cross-sectional view of the microchannel, we can see that the microchannel was well bonded and only a very thin resin layer was observed shown in Figure 6(a).
After the gas assisted coating of HEMA, the surface became very smooth according to Figure 6(b).

Figure A.6 cross section of (a) interstitial and (b) CO2 bonded microchannels.

Figure A.7 (a) shows that the food dye is able to fill the interstitial space between the chip and the cover plate without flowing into the microchannels. After bonding the microfluidic chip, we injected the food dye into the reservoirs and it filled the microchannels without leakage as shown in Figure A.7 (b).

Figure A.7 (a). Interstitial space filled with food dye (b). Microchannels of the bonded microfluidic chip filled with food dye.
The inherent autofluorescence background of the polymeric materials can be problematic for fluorescence detection in the electrophoresis separation of DNA. PMMA has a low background, similar that of the glass. Because of its good combination of optical clarity, mechanical strength and low cost, PMMA was selected as the dense material in the microfluidic experiments.

After its fabrication, the polymer microdevice has to be closed to perform microfluidic functions. The fluorescence background of the bonding materials may also cause problems for the detection. Because no alien material was introduced by the interstitial bonding, so the fluorescence background of two bonded microdevices was determined by the PMMA itself.

The separation channels were filled with 3% 4M PEO with 5µM TO. Loading of sieving materials is by capillary force only in the DNA sequencing chip. The 3.0% 4M PEO solution was loaded into an empty channel with dimensions of 250µm × 100 µm. It should be noted that the loading speed of each sieving material in the separation channel is critical to avoid bubble entrapment. By the capillary force only, filling a linear polymer solution into microchannel takes about 10 minutes.

The performance of interstitial bonded DNA sequencing microdevice was evaluated by separating the fragments in the DNA standard (ΦX174 Hae III Digest, 11 fragments with size ranging from 78 to 1353 base pair). Figure A.8 shows the fluorescence intensities of separated fragments in the DNA standard using 2.0% 4M PEO as the sieving materials, respectively. 10 fragments are resolved. A fluorescence intensity of separated fragments from the literature [Lai, 2002] is shown in Figure A.9.
The imperfection of the separation may result from the short separation channel because a 3.5 mm long separation channel is usually used, but ours is only 1.5mm long.

![Figure A.8 DNS separation result](image1)

**Figure A.8 DNS separation result**

![Figure A.9 Separation result from the literature](image2)

**Figure A.9 Separation result from the literature**
A.4 Conclusion

A new technique, interstitial bonding, has been developed for bonding and surface modification of polymer-based microfluidic devices. This method successfully bonded microfluidic platforms from a simple single-channel structure, to more complex patterns. By combining gas assisted coating, a well bonded and very smooth microchannel was achieved. This approach was also applied to multi-channel DNA separation chip. PEO solution was used as the sieving material for electrophoresis. This method achieved the similar DNA separation efficiency as reported literature.
APPENDIX B SUPERHYDROPHOBIC VALVE STUDY

Surface coating of poly(methyl methacrylate) (PMMA) with double conductive polymer layer (polyaniline followed by polypyrrole) formed hierarchical nano-sized roughness. A polypyrrole secondary layer can help increase the adhesion between the coating and the PMMA substrate. Further treatment with CF$_4$ RF plasma treatment can manipulate the surface morphology and change the surface to super-hydrophobicity. This super-hydrophobicity showed a very high contact angle of water, which is robust even being further treated with protein. This property was successfully applied in a microvalving design, where protein is required to coat the channel surface (blocking) to avoid the further absorption of valuable reagent. A proposed mechanism of morphology formation process was also presented.

B.1 Background

Wettability of the solid surfaces is governed by both surface energy and roughness or surface structures [Nakajima, 2001; Wenzel, 1936]. The surface energy is the intrinsic property of each solid material, which depends on the chemical composition. The contact angle on a composite surface can be described by the Cassies’ equation [Cassie, 1944]:
\[
\cos \theta^* = f \cos \theta - (1 - f)
\]  

(1)

where \( \theta^* \) is the contact angle on the composite surface; \( \theta \) the contact angle on the flat surface; \( f \) the fraction of solid. Super-hydrophobicity, where a contact angle of water exceeds 150°, was found in nature caused by the hierarchical roughness of microsized papillae having nanosized protrusions covered with hydrophobic wax [Barthlott, 1997]. Recently, the super-hydrophobic surfaces have been obtained via the bio-mimic micro- and nanofabrication, i.e., making a very rough surface followed by a thin layer coating of materials of low surface free energy (e.g. fluorinated compounds, CF₄) [Lin, 2002; Lau, 2003; He, 2003; Woodward, 2003; Youngblood, 1999; Morra, 1989] or making a very rough surface directly from the hydrophobic materials [Erbil, 2003; Feng, 2002].

Micro valve is a key component in microfluidics, which is used to control the liquid flow in microchannels. The valving mechanism can be divided into two categories: active and passive. The active valves require a mobile part and external stimuli [Luo, 2003; Yu, 2003; Liu, 2004; Gui, 2004; Hartshorne, 2004; Kirby, 2002; Schomburg, 1993; Suzuki, 2003; Cheng, 2004], which restrict their applications in microfluidics when the device becomes smaller and needs to be cost-effective. Passive valve provides a more affordable method to control the liquid flow without a moving part [Madou, 1998; Nguyen, 2004; Jeon, 2002]. Capillary valve is one of the simplest hydrophobic valves that can be incorporated into microfluidic chips very easily because there is no moving part as that in a active valve. Another very simple passive valve is hydrophobic valve. A typical hydrophobic valve is shown in Figure B.1 schematically [Feng, 2003] and the pressure the valve can hold can be calculated by equation (1).
\[ P = 2 \gamma \cos \theta \frac{H + W}{HW} \]  

(1)

where \( H \) and \( W \) are the width and depth of the channel respectively. \( P \) is negative and is the maximum pressure that the hydrophobic valve can hold. As we can see \( P \) is proportional to \( \cos \theta \), but the limit of \( \cos \theta \) is -1, or the contact angle reaches 180°. So, the larger is contact angle is, the larger the holding capacity will be.

Figure B.1 Schematic of hydrophobic valve [Feng, 2003]

However, some applications, such as the enzyme-linked immuno-sorbent assay (ELISA), involve the surface blocking of proteins to avoid the nonspecific binding of valuable reagent such as antigen/antibody or cells. After blocking, the valve will not be functional because of the surface property changes such as the contact angle [Please refer to Chapter 5].
This paper presents a super-hydrophobic valving design. The super-hydrophobic surface was generated by in-situ polymerized conductive polymer film followed by morphology manipulation and hydrophobicization using fluorine RF plasma. The film formation of polyaniline film on glass was reported by Irina Sapurina et al [Sapurina, 2001]. However, the wetting/non-wetting property of the film thus obtained and film formation on a polymer microfluidic substrate such as PMMA has never been reported. In this paper, the morphology changes with different plasma treatment time will be investigated. The influence of protein on the valve function and the valve capacity after protein blocking are also examined.

B.2 Experimental

B.2.1 Materials and reagents

Poly(methyl methacrylate) (PMMA) plate was purchased from McMaster Carr with dimensions of 25.4cm x 25.4 cm x 1.6cm. Pyrrole (Aldrich) was distilled under reduced pressure prior to use. Aniline (Aldrich), ammonium peroxydisulfate (98 %, Aldrich), methanol, and hydrochloric acid were used as received. Food color was purchased from a local Kroger grocery store (McCormick, Hunt Valley, MD). Bovine serum albumin (BSA; Fraction V) was purchased from Fisher Scientific (Pittsburgh, PA). BSA solutions in DI water was prepared with a concentration of 1.0wt% as the blocking solution and 0.2wt% for the use of valving test.
B.2.2 Nanostructured surface

B.2.2.1 Surface deposition

Ammonium peroxydisulfate (APS) was dissolved in 1M hydrochloric acid (HCl) to form a 0.13M solution. Aniline (Ani) and pyrrole (Py) were dissolved in 1M hydrochloric acid to form 0.2M solution. The PMMA plate was cut into 1cm x 2cm pieces. Two pieces of PMMA were placed in a 20ml vial containing 10ml of 0.2M aniline solution. Then 10ml of 0.13M APS solution. After twenty minutes of reaction under room temperature, the substrate was removed and rinsed copiously with distilled water followed by methanol. For double layer coatings the above procedure was repeated with one piece of PMMA that already had a single layer of Ani in ice bath (0~5°C). Following the wash, the single layer reaction was repeated on the substrate. For Py, only one layer samples were prepared. There are totally 4 cases of coating process. Each substrate will be coated by single layer of polyaniline, single layer of polypyrrole, one layer of polyaniline followed by one layer of polypyrrole, two layers of polyaniline.

B.2.2.2 Plasma treatment

Plasma treatment was carried out at 13.56MHz using a bench-top reactive ion etching system (Technics 800II RIE system). Prior to plasma treatment, the chamber was cleaned with 2-propanol, dried and further cleaned using a 20sccm oxygen plasma at 300W for 30min. The sample was then put into the chamber, followed by evacuation to a pressure of 12 mTorr. CF$_4$ gas was then introduced at a flow rate of 50sccm and the glow discharge was ignited at 300W. To simplify, the ani/ppy case were treated for 30sec, 1min, 2min, 4min and 10min. After that, the power and CF$_4$ were turned off and the
chamber was evacuated to 12mTorr again. Then the chamber was purged and the samples were taken out.

B.2.2.3 Surface characterization

The surface morphology was characterized by SEM to view the surface morphology. Surface treatment with protein solution was performed by soaking the sample surfaces in 0.1wt% BSA solution for 10 seconds, which was then blown dry. To examine the surface property subject to protein solution, the contact angle of a water droplet of 20µl on these surfaces were measured before and after the protein treatment for selected coating method. The contact angle was measured by analyzing the sessile drop profile taken by a COHU high performance CCD camera.

B.2.3 Superhydrophobic valve

B.2.3.1 Chip design

A microchip design is shown in Figure B.2, which is located on a piece of compact-disk (CD) made of PMMA. It has two larger reservoirs, one is for the sample loading to test the valve capacity and the other is the reaction chamber for the polymerization to generate the rough surface. Two small circular junctions were placed on both sides of the reaction well to keep the reagent in the reservoir during the reaction. A waste reservoir is placed downstream to collect the samples. The depth of the microfeature is 230µm and the diameters of the reservoirs are 3 mm and 4 mm respectively. A channel of 200µm in width connects all the reservoirs.
B.2.3.2 Chip fabrication

The designed microfluidic patterns were drawn using commercial AutoCAD software (AutoCAD 2002, AutoDesk, Inc.). Glass masks were made based on the CAD design. The chip is fabricated by micro injection molding on a piece of CD of PMMA. The mold preparation and injection molding were carried out by Ritek Inc. (Taiwan). The mold is made of Nickel by a typical electroplating process from a SU-8 (MicroChem) master mold via standard photolithography.

The Pani-Ppy double-layer coating was chosen for the valve test. The coating was generated following the procedure in the synthesis section by dropping the aniline solution (0.2M in 1M hydrochloric acid) and APS solution (0.13M in 1M hydrochloric acid) was loaded into the reaction chamber at a ratio of 1:1 volume ratio. After 20 min of
reaction, the chip was rinsed copiously by water and methanol. Then the process was repeated with pyrrole in a chiller (0-5°C).

The CD microchip was then plasma treated following the same recipe abovementioned. After that, the chip was sealed by scotch tape (Clear view, Premium grade, Staples). A 0.1wt% BSA solution was loaded into the sample reservoir and the CD was span at 2000rpm on a rotor test device as shown in Figure B.3. This device includes a spinner, a strobe and a power supply. The strobe was synchronized with the spinner so that it flashes in a frequency as the spinner rotation and the flow of liquid can be observed visually.

Figure B.3 Motor test setup

**B.2.3.3 Valve capacity test**

The 0.2wt% protein solution in DI water mixed with 5.0wt% food dye was loaded into the reservoir. Then the chip was mounted onto the spinner in Figure B.3. The
rotating speed was increased slowly and the rotating speed was recorded when the liquid bursts through, which is called the burst frequency.

B.3. Results and discussion

B.3.1 Hierarchical surfaces

B.3.1.1 Surface characterization

The SEMs of all the four coating processes with a 2 min plasma treatment were shown in Figure B.4. As we can see, single layer of Pani resulted in a rough surface as shown in Figure B.4 (a), but only a monolayer of Pani particles was formed on the PMMA surface because the gap between particles can be observed. On the contrary, the Ppy single layer coated on PMMA showed a much smoother surface and only a few particles were scattered on the surface as shown in Figure B.4(b). Pani/Ppy and Pani/Pani coated PMMA, however, showed a hierarchical morphology, i.e. larger particle-like primary structures (~500nm) are coated by smaller secondar features of a few tens of nanometers as shown in Figures B.4(c) and B.4(d). The size of the secondary features is around 50nm, so the real feature size should be around 30nm considering the 20nm sputtered gold for scanning electron microscopy (SEM).
Figure B.4 SEM of (a) single layer Pani, (b) single layer Ppy, (c) double layer Pani, and (d) Pani/Ppy on PMMA surface after 2 min fluorine plasma treatment.

The Pain/Ppy and Pani/Pani double layer coated on PMMA were further studied to see the influence of plasma treatment. Figure B.5(a) showed the SEM of Pani/Ppy double layer on PMMA without any plasma treatment. One can see that there is no nano features could be observed on the submicron particle-like features. However, after 2min plasma treatment, 30nm features could be observed as shown in Figures B.5(b). After 4min plasma treatment, the primary features were trimmed further and the secondary structure...
disappeared as shown in Figure B.5(c). When the plasma treatment reached 10min, nano-tip like structure appeared as shown in Figure B.5(d).

![Figure B.5 SEM of Pani/Ppy coated PMMA surface after (a) 0, (b) 2 min (c) 4min and (d) 10 min fluorine plasma treatment](image)

The Pani/Pani double layer on PMMA shows different morphology evolution. As shown in Figure B.6(a), there is hierarchical structure even without plasma treatment. After 2 min of plasma treatment, the size of the secondary structures increased while the
size of primary structures decreased as shown in Figure B.6(b). As shown in Figure B.6(c), after 4 min of plasma treatment, the structure became irregular with all the secondary structure disappearing compared with Figure B.5(c). The feature after 10 min of plasma treatment was shown in Figure B.6(d). As one can see, no nano-tips were observed.

Figure B.6 SEM of Pani/Pani coated PMMA surface after (a) 0, (b) 2 min (c) 4min and (d) 10 min fluorine plasma treatment
According to the observation, for a single layer Pani and a double layer Pani coated on the PMMA, some part of the features disappeared when washed by water and methanol, indicating a poor adhesion between the film and the substrate.

**B.3.1.2 Proposed Mechanism**

Although literature already proposed a primary and secondary nucleation mechanism for film-on-film structure of Pani on glass [Sapurina, 2001], the theory cannot explain all the phenomena presented in previous section. For example, the single layer Pani only formed a loose particle monolayer and the poor adhesion of Pani or Pani/Pani on PMMA.

The proposed mechanism here is the affinity between conductive polymer used in this paper and the PMMA substrate. For a single layer of hydrophilic Pani coated on hydrophobic PMMA, the electrostatic charge interaction between Ani and PMMA dominate over the hydrophilic/hydrophobic interactions. However, the PMMA has only a weak negative charge, so the affinity between Ani and PMMA is weak so that less nucleation occurred on the PMMA surface and only a monolayer of isolated Pani particles was formed. However, the hydrophobic interaction between a more hydrophobic pyrrole and hydrophobic PMMA, nucleation occurred everywhere and the surface was coated uniformly. This theory can also explain the weak adhesion of the Pani and Pani/Pani on PMMA.

Furthermore, the morphology change with different treatment time can be explained by the selectivity of CF₄ plasma on Pani/PpY and Ani/Ani double layer coating.
The assumption is that Ppy is more sensitive to the CF$_4$ plasma etching because the PPY is less stable than the Pani.

Based on this assumption, a schematic of Pani/Ppy coating and plasma treatment process is shown in Figure B.7. Firstly, a loose monolayer of Pani particles was formed on the PMMA surface. Then, PPY was formed on both Pani and PMMA because of the gap between Ppy particles and also acts as the bridge of Pani and PMMA resulting in a better adhesion. A short time of plasma treatment generates nano-sized feature, but longer plasma treatment will etched away the PPY along the direction of glow discharge. A longer plasma treatment time will trim the Pani/PPY double layer to nano-tip like structure by etching the PPY further into the PMMA substrate.

Figure B.7 Proposed mechanism of surface morphology evolution for Pani/Ppy double layer on PMMA
However, a Pani second layer will only add onto the first layer because they are totally the same material. So because of iso-tropical etching of the fluorine plasma, Pani/Pani double layer did not show nano-tip like structures after 10 min of fluorine plasma treatment when comparing Figure B.5(d) and Figure B.6(d).

**B.3.2 Contact angle measurement**

From contact angle measurement after 2 min CF$_4$ RF plasma treatment, the Pani, double Pani, and Pani/Ppy all showed super-hydrophobicity. However, longer treatment showed different results. For example, the contact angle of Pani and Pani/Pani on PMMA will continue to show super-hydrophobicity even after 4 min treatment. However, for Pani/Ppy double layer on PMMA, the contact angle decreased with longer plasma treatment, but after 10 min, the super-hydrophobicity came back again. This phenomenon is consistent with the SEM results, which showed the morphology change from rough to smoother because of the disappearance of the secondary structure and back to rough again because of the non-isotropical etching of Ppy and Pani.

The pictures of the water drop on the Pani/Ppy surface after 2 min plasma treatment are shown in Figure B.8. According to the analysis, the plasma treated double-layer particle surface shows a contact angle of 177° as shown in Figure B.8(a). Furthermore, after protein treatment, the contact angle changes to 174°, but still remain super-hydrophobic as shown in Figure B.8(b), which can be explained by Cassie’s and Wenzel’s theories [Cassie, 1944; Wenzel, 1936], i.e. when the protein solution flowed over the coated surface, the protein only bound to the peaks the nanofeatures. Because the
solid fraction is small, the surface still remained super-hydrophobic after protein treatment.

Figure B.8 Photo of water profile on achieved super-hydrophobic surface (a) before and (b) after protein treatment for Pani/Ppy double layer on PMMA after 2 min fluorine plasma treatment

B.3.3 Super-hydrophobic valve

Figure B.9 shows the enlarged part of the valve design. The surface coated by particle changed color and the food dye test shows that the valve can stop the food dye after the protein treatment. According to the experiment, protein treatment also change the plasma treated surface without coating from hydrophobic to hydrophilic, which can be observed by the capillary filling of the sample.

The recorded burst frequency is 980rpm, which is close to the predicted valve by the equation in chapter 5.
B.4 Conclusion

This article presented the formation of super-hydrophobic surfaces by synthesis of conductive polymer Pani and Ppy and its application on the design of a microvalve that remains functional after the protein treatment. Other potential application of this super-hydrophobic surface include electrically controlled microvalve taking the advantage of the property of conductive polymer.


G’Sell C., Boni S., and Shrivastava S., “Application of plane simple shear test for
determination of the plastic behavior of solid polymers at larger strains”, Journal of

G’Sell C. and Gopez A. J., “Plastic banding in glassy polycarbonate under plane simple

polymers and metals beyond the necking point”, Journal of Materials Science, 27,

G’Sell C., and Souahi A., “Influence of crosslinking on the plastic behavior of
amorphous polymers at larger strains”, Journal of Engineering Materials and Technology,

assembled monolayers of dendron thiols for electrodeposition of gold nanostructures:
toward fabrication of superhydrophobic/superhydrophilic surfaces and pH-responsive

Gu Z. Z., Uetsuka H., Takahashi K., Nakajima R., Onishi H., Fujishima A. and Sato O.,


Handique K., Burke D.T., Mastrangelo C.H., and Burns M.A., “Nanoliter liquid metering


He B., Patankar N. A. and Lee J., “Multiple equilibrium droplet shapes and design

Heckele M., Bacher W. and Muller K. D., “Hot embossing-the molding technique for

Henry V., Deutsch J. and Gifford, L., “Enzyme immunoassay of theophylline with a
centrifugal analyzer, and comparison with an ultraviolet method”, Clinical Chemistry,


Nakajima A., Hashimoto K., and Watanabe T., Monatshefte fur Chemie, 132, pp.31 (2001)


Ogawa N., Soga M., Takada Y. and Nakayama N., Jpn J Appl Phys, 32, 614 (1993)..


