Department of Materials Science and Engineering

# Fabrication of inorganic-organic hybrid polymer micro and nanostructures for fluidic applications

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**Susanna Aura**

Aalto University

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# Fabrication of inorganic-organic hybrid polymer micro and nanostructures for fluidic applications

**Susanna Aura**

Doctoral dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the School of Chemical Technology for public examination and debate in Large Seminar Hall of Micronova at Aalto University (Espoo, Finland) on the 16th of December 2011 at 12 noon.

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# Abstract

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## Abstract

Microfluidics is a rapidly developing branch of microtechnology with applications in chemistry, biology, medicine and other sciences. One major trend in fluidics has been the miniaturization of analytical devices. Miniaturization improves performance, mainly speed and sensitivity, and also reduces the required sample volumes. Initially microfluidic devices were fabricated from silicon and glass using microfabrication methods borrowed from the integrated circuits industry. But polymers are gaining more and more attention due to their simple processing and potential of being so inexpensive as to be disposable. Many different polymer materials have been used, including polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA) and epoxy-based SU-8. Although these polymers have proven to be useful in some applications, there is still need for easily processable materials that would have the chemical tolerance and favorable surface properties for analytical applications. One such major issue is avoidance of analyte adsorption on chip walls in separation devices.

In this thesis, a new materials class, ORMOCER©s, are introduced into microfludics. Ormocers are inorganic-organic hybrid polymers that combine the beneficial properties of organic materials and glass/ceramics. They can be cured by using UV-light, like negative photoresists, and their surface properties resemble that of glass. New UV-embossing techniques have been conceived and tested for Ormocer patterning in this thesis. Combined with a novel self-adhesive bonding process, devices made of 100% Ormocer have beed realized for the first time. Fabrication of Ormocer structures by embossing and bonding is fast and simple compared with many other polymers, and significantly easier than silicon or glass processing. Due to its unique chemical composition, nanopores can be formed in Ormocer by etching the organic sidegroups away in oxygen plasma.

The microchips produced in Ormocer have been used in applications like capillary electrophoresis (CE), integrated capillary electrophoresis and electrospray ionization (ESI), and as substrates for surface assisted laser desorption ionization (SALDI). Ormocer has proven to be highly successful in these analytical applications. Especially intriguing is the excellent CE separation performance for peptides and proteins which is explained to be due to inherent resistance of Ormocer against analyte adsorption.

**Keywords** Microfabrication, Ormocer, embossing, bonding, microfluidics, polymers

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**Tiivistelmä**

Mikrofluidistiikka on nopeasti kasvava mikrovalmistustekniikan osa-alue, jonka sovellusmahdollisuudet ovat laajat keskittyen erityisesti kemiaan, biologiaan ja lääketieteeseen. Yksi merkittävistä mikrofluidistiikan kiinnostuksen kohteista on analyyttisessä kemiassa käytettävien laitteiden koon pienentäminen. Koon pienentäminen parantaa niiden suorituskykyä, pääasiassa nopeutta ja herkkyyttä, ja lisäksi pienentää vaadittavia näytemääriä. Ensimmäiset mikrofluidistiset laitteet valmistettiin piistä ja lasista hyödyntäen erilaisia mikrovalmistustekniikoita. Polymeerit ovat kuitenkin herättäneet kiinnostusta lähinnä niiden mahdollisen edullisuuden, ja sitä myötä kertakäyttöisyyden, takia. Useita erilaisia polymeereja, kuten polydimetyylisiloksaania (PDMS), polymetyylimetakrylaattia (PMMA) ja epoksipohjaista SU-8a on käytetty analyyttisten mikrosirujen valmistukseen. Vaikka nämä polymeerit ovat osoittautuneet toimiviksi joissakin sovelluksissa, ei vieläkään ole löytynyt materiaalia, jota olisi helppo työstää ja jolla olisi tarvittava kemiallinen kestävyys ja analyyttisiin sovelluksiin sopivat pintaominaisuudet. Yksi merkittävä pintaominaisuus on mahdollisimman vähäinen näytteen adsorptio mikrosirun kanavien seinämiin erotussovelluksissa.

Tässä väitöskirjassa esitellään uusi materiaali, ORMOCER©, mikrofluidististen sirujen valmistukseen. Ormocer on epäorgaanis-orgaaninen hybridipolymeeri, jossa yhdistyvät orgaanisten materiaalien ja epäorgaanisen lasin hyvät ominaisuudet. Se voidaan kovettaa UV valon avulla, kuten negatiiviset fotoresistit ja sen pintaominaisuudet ovat lasinkaltaiset. Tässä työssä on kehitetty uusi painamistekniikka kaupallisen Ormocerin kuvioimiseksi. Tämä yhdistettynä uuteen Ormocer-Ormocer liittämistekniikkaan mahdollistaa kokonaan Ormocerista valmistettujen sirujen tuottamisen. Suljettujen Ormocer-rakenteiden valmistus painamis- ja liittämistekniikoin on nopeaa ja helppoa verrattuna moniin muihin polymeereihin, puhumattakaan piin tai lasin vastaavista menetelmistä. Ormocerin erikoisesta kemiallisesta rakenteesta johtuen nanokokoluokan huokoisia voidaan valmistaa yksinkertaisella happiplasmakäsittelyllä.

Valmistettuja Ormocer mikrosiruja on käytetty sovelluksiin, kuten kapillaarielektroforeesi (CE) ja integroitu kapillaarielektroforeesi sähkösumutusionisaatio–siru (CE-ESI) sekä alustana pinta-avusteisessa laser desorptio ionisaatiossa (SALDI). Näillä mikrosiruilla tehdyt mittaukset ovat osoittaneet että Ormocer soveltuu käytettäväksi analyyttisiin sovelluksiin.

**Avainsanat** Mikrovalmistustekniikka, Ormocer, Mikrosiru, Mikrofluidistiikka, Polymeerit

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# Preface

I worked at the Microfabrication group for over six years during which I became familiar with many different microfabrication techniques and processes. I am forever grateful to Prof. Sami Franssila who was brave enough to hire a student without any prior knowledge on the subject. We have had many fruitful discussion and he has helped me tremendously throughout the thesis project.

I am very grateful to Dr. Tiina Sikanen and Professors Risto Kostiainen and Tapio Kotiaho from the department of Farmacy, University of Helsinki for collaboration. Without the collaboration, this thesis would not have been finished. I am especially grateful to Tiina who has performed the microchip measurements and helped me understand the analytical part of my work.

My thanks goes also to all my colleagues and co-authors of the articles. Without a great atmosphere at the office, coming to work every morning would not have been as easy as it was. My special thanks go to Ville Jokinen and Lauri Sainiemi who both have helped me a lot with my research work and beyond.

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My final thanks go to my family and friends, with whom I have spent many great moments outside the office. And special thanks to Janne how has lived with me throughout the thesis project.

Helsinki, November 17, 2011,

Susanna Aura

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# List of Publications

This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.

**I** S. Aura, T. Sikanen, T. Kotiaho and S. Franssila. Novel hybrid material for microfluidic devices. *Sensors and actuators, B*, 132, 397-403, 2008.

**II** S. Aura, V. Jokinen, L. Sainiemi, M. Baumann and S. Franssila. UV-embossed inorganic-organic hybrid nanopillars for bioapplications. *Journal of Nanoscience and Nanotechnology*, 9, ,6710-6715, 2009.

**III** T. Sikanen, S. Aura, L. Heikkilä, T. Kotiaho, S. Franssila and R. Kostiainen. Hybrid ceramic polymers- New nonbiofouling and optically transparent materials for microfluidics. *Analytical Chemistry*, 82, ,3874-3882, 2010.

**IV** T. Sikanen, S. Aura, T. Kotiaho, S. Franssila and R. Kostiainen. Microchip capillary electrophoresis-electrospray ionization mass spectrometry of intact proteins using uncoated Ormocomp microchip. *Accepted to Analytica chimica acta*, .

**V** S. Aura, V. Jokinen, M. Laitinen, T. Sajavaara, S. Franssila. Porous inorganic-organic hybrid material by oxygen plasma treatment. *Journal of Micromechanics and Microengineering*, 21, 125003, 2011.

# Author's Contribution

### Publication I: "Novel hybrid material for microfluidic devices"

Novel bonding method was invented by the author. Part of the planning of the experiments and all the microfabrication work except the ICP-RIE ething was done by the author. The article was written by the author with the contribution of Dr. Tiina Sikanen

### Publication II: "UV-embossed inorganic-organic hybrid nanopillars for bioapplications"

Part of the planning of the experiments and the microfabrication work except the fabrication of black silicon. The article was written by the author with the contribution of Ville Jokinen

### Publication III: "Hybrid ceramic polymers- New nonbiofouling and optically transparent materials for microfluidics"

All the microfabrication work and related text. The analytical part was written by Dr. Tiina Sikanen.

### Publication IV: "Microchip capillary electrophoresis-electrospray ionization mass spectrometry of intact proteins using uncoated Ormocomp microchip"

All the microfabrication work and related text. The analytical part was written by Dr. Tiina Sikanen.

### Publication V: “Porous inorganic-organic hybrid material by oxygen plasma treatment”

The initial concept was invented by the author. Planning of the experiments and the microfabrication work except the ALD deposition. The article was written by the author with the contribution from co-authors.

# List of Abbreviations

| | |
|---|---|
| 2PP | Two photon polymerization |
| ALD | Atomic layer deposition |
| BHF | Buffered hydrofluoric acid |
| BSA | Bovine serum albumin |
| CE | Capillary electrophoresis |
| CNP | Combined nanoimprint and photolithography |
| COC | Cyclic olefin copolymer |
| CVD | Chemical vapor deposition |
| CZE | Capillary zone electrophoresis |
| DLW | Direct laser writing |
| DXRL | Deep x-ray lithography |
| EBL | Electron beam lithography |
| ESI | Electrospray ionization |
| EUV | Extreme ultraviolet |
| FITC | Fluorescein isothiocyanate |
| GC | Gas chromatograph |
| HF | Hydrofluoric acid |
| HMDS | Hexamethyldisilazane |
| IC | Integrated circuit |
| ICP-RIE | Inductively coupled plasma reactive ion etching |
| IR | Infra red |
| LC | Liquid chromatography |
| LDI | Laser desorption ionization |
| LIGA | Lithographie galvanoformung und abformung |
| MCE | Microchip capillary electrophoresis |
| MEMS | Micro electro mechanical system |
| MIMIC | Micro molding in capillaries |

| | |
|---|---|
| MS | Mass spectrometry |
| $\mu$TAS | Micro total analysis system |
| NIL | Nano imprint lithography |
| ORMOCER | Organically modified ceramic |
| ORMOSIL | Organically modified silicate |
| PAC | Photoactive compound |
| PBS | Phosphate buffered saline |
| PC | Polycarbonate |
| PDMS | Poly(dimethyl siloxane) |
| PLA | Poly lactic acid |
| PLGA | Poly lactic-co-glycolic acid |
| PMMA | Polymethylmetacrylate |
| PU | Polyurethane |
| PVD | Physical vapor deposition |
| RBS | Rutherford backscattering spectrometry |
| REM | Replica molding |
| RIE | Reactive ion etching |
| rpm | Rotations per minute |
| SALDI | Surface assisted laser desorption ionization |
| SFIL | Step and flash imprint lithography |
| TCB | Thermo-compression bonding |
| $T_g$ | Glass transition temperature |
| TOF-ERDA | Time-of-flight elastic recoil detection analysis |
| UV | Ultraviolet |
| XPS | X-ray photoelectron spectroscopy |

# 1. Introduction

The first microfluidic components were introduced more than 30 years ago. These components were ink jet printing nozzle array that was etched in silicon [1] and gas chromatograph (GC) fabricated on a silicon wafer [2]. This early microfabricated gas chromatograph fitted into 2 inch silicon wafer making it many times smaller than the standard gas chromatograph. Furthermore, even though the separation of closely related chemicals was not as good as with standard GC, it was already shown that separations could be done faster when using this hand-held device.

After the introduction of first miniaturized analytical device there was over a decade when no significant progress in the field was made. A new boost to the research was given by the article of Manz published in 1990 [3]. Manz introduced the concept called "miniaturized total chemical analysis system", later shorten to micro total analysis system ($\mu$TAS). In this article the main goal was not the reduction of size but rather the enhancement of the analytical performance. The theoretical examination of hydrodynamics and diffusion indicated that the separations could be done faster and more efficiently by using miniaturized total analysis systems. The consumption of sample and reagents is also significantly reduced. Furthermore, by utilizing microfabrication techniques it is possible to integrate several functions on the same chip, such as sample introduction, pre-treatment and separation.

The above mentioned benefits initiated active research in miniaturized analytical systems which started to grow rapidly in the beginning of 90's. Capillary electrophoresis (CE) [4–8], along with liquid chromatograph (LC) [9, 10] were the most studied system in the early stage of research. More complex systems, where different functions were integrated on the same microchip quickly followed [11, 12]. Since then all kinds of analytical devices have been miniaturized and many different functions have been integrated on a single microchip, such as ionization sources for mass spectrometry [13].

At the beginning silicon and glass where used as substrates but in the late 1990's polymers started to draw more attentions since most applications demanded devices which can be disposable in order to eliminate risk of sample contamination. Furthermore, polymers had already been used in laboratories since many standard laboratory items like Eppendorf tubes, microtiter plates, pipette tips and cell culture plates are made out of polymers. Capillary electrophoresis [14, 15] was the first microfluidic chip made out of plastics due to its simplicity. Simple fabrication is the reason why polydimethylsiloxane (PDMS) fast became the most popular material for microfluidic polymer chips. Other widely used polymers for microchip fabrication are SU-8 [16] and polymethylmethacrylate (PMMA) [17].

This thesis gives an overview of microfabrication techniques for relatively new hybrid material, ORMOCER®(trademark of the Fraunhofer institute, Germany). The chips produced have been used for analytical applications, such as capillary electrophoresis, electrospray ionization (ESI) and laser desorption ionization (LDI). The thesis is divided into six chapters. The first chapter gives the overview of the traditional microfabrication techniques, the second presents the most used polymers for microfluidic structures and the third, the microfabrication methods used for polymer. The next two chapters concentrate on microfabrication of ORMOCER and the applications produced by using the presented techniques. The last chapter will conclude this thesis.

## 1.1 Materials for microchips

Silicon and glass were the first materials used in fabrication of microfluidic devices [2, 18]. Silicon was used since its microfabrication methods were known from the IC-industry. Due to this there is a large toolbox of techniques available for silicon microprocessing and almost any geometry is possible with high precision. Silicon is also clean room compatible and highly pure material is readily available. Silicon has a well characterized oxide which can be used when electrical insulation is needed. Mechanically silicon is strong which makes it especially suitable for devices where moving parts are required. However, in many analytical applications electro osmotic flow is used to drive the analytes which makes the use of silicon difficult due to its semiconductivity. Also the opacity of silicon makes the optical detection inside the microchannels impractical.

Glass [19], on the other hand, is popular material in analytical applications since its surface properties are well known and many times ideal for devices in contact with biological agents. It is an insulating material which makes its use in electrically driven application such as capillary electrophoresis possible. Also the transparency of glass makes it suitable for simple optical detection.

The fabrication techniques for glass are however often too time consuming which has led to study of new materials, like polymers. Most common polymers used in microfluidics will be discussed in chapter 2. Some of the old methods like optical lithography can be used also with polymers, such as SU-8, that are structural part of the device. Many polymers however require the development of completely new fabrication techniques. The fabrication techniques for polymers are reviewed in chapter 3.

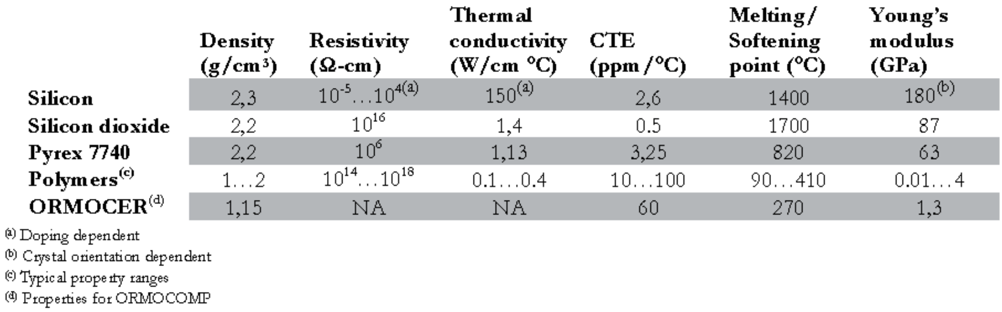
The selection of material for certain application depends on the required material properties. As can be seen from the table 1.1, the material properties of different material classes differ significantly from each other. The resistivity of the material is important if electrically driven flow is used. Thermal properties are also important, especially when high temperatures are used. Thermal expansion coefficient of most polymers is significantly higher than silicon and glass which can be an issue when silicon or glass is used as a substrate or as stamp material. When using polymers, the usable temperature range can also be limited because polymers do not tolerate as high temperatures as silicon and glass. Optical clarity of glass is superior compared to silicon and most of the polymers but there are some polymers that are transparent over a wide wavelength range. Autofluorescence of material may also be an issue when UV detection is used since many polymers exhibit autofluorenscence in the UV region. The chemical tolerance of material is also important since many different solvents, acids or bases are used as buffers. In some applications the oxygen and carbon dioxide permeabilities are important which is of course only possible with certain polymers.

In this thesis, an inorganic-organic polymer, ORMOCER, is introduced into field of microfluidics. ORMOCERs are a new class of materials that combine the properties from different materials. ORMOCERs are composed of inorganic backbone formed by sol-gel process, where organic sidegroups are attached by crosslinking. Due to their formation chemistry, several different types of ORMOCERs can be formed with tailored material properties. In table 1.1 properties of the commercially available ORMOCER material, Ormocomp®, are presented. The main advantages of ORMOCER for microfluidics, are its easy processing, glass-like surface properties, optical clarity even near UV-region and tolerance to chemicals and relatively high temperatures.

## 1.2 Basics of microfabrication

**Lithography** Optical lithography has been used in IC-industry for decades. The patterns on the mask are transferred to the photosensitive polymer layer called photoresist.

**Table 1.1.** List of selected material properties for different materials



| | Density (g/cm³) | Resistivity (Ω-cm) | Thermal conductivity (W/cm °C) | CTE (ppm/°C) | Melting/ Softening point (°C) | Young's modulus (GPa) |
|---|---|---|---|---|---|---|
| **Silicon** | 2,3 | $10^{-5}\ldots10^{4}$[a] | 150[a] | 2,6 | 1400 | 180[b] |
| **Silicon dioxide** | 2,2 | $10^{16}$ | 1,4 | 0.5 | 1700 | 87 |
| **Pyrex 7740** | 2,2 | $10^{6}$ | 1,13 | 3,25 | 820 | 63 |
| **Polymers**[c] | 1…2 | $10^{14}\ldots10^{18}$ | 0.1…0.4 | 10…100 | 90…410 | 0.01…4 |
| **ORMOCER**[d] | 1,15 | NA | NA | 60 | 270 | 1,3 |

[a] Doping dependent
[b] Crystal orientation dependent
[c] Typical property ranges
[d] Properties for ORMOCOMP

Photoresist is composed of the base resin, solvent and light sensitive material, called photoinitiator. The base resin determines the mechanical and thermal properties and photoinitiator the sensitivity to radiation. Photoinitiator is a molecule (usually organic), which absorbs light and forms reactive initiating species like anion radicals, $H^+$, or cation radicals. Solvents are used to control the viscosity of the resist. Viscosity has direct impact on the achievable layer thickness. Depending on the type of the resist, either positive or negative, the areas that are exposed to light or the areas that are not exposed to light will be removed in development, respectively (see figure 1.1). In standard optical lithography a photomask, made of glass or quartz with light blocking metal layer, usually chromium is used.

In addition to standard UV-exposure, the radiation can also be X-rays, electrons, ions or UV-laser. Like UV-lithography, in the X-ray lithography the mask is used to transfer the pattern but since x-rays can penetrate through thicker layers the radiation blocking areas have to be made of thick metal layers (about 10 um) which makes the mask fabrication very expensive and complicated. In electron and ion beam lithography masks are not needed but the beam will scan through the area to be patterned. Due to a sequential nature of these methods, the patterning is very slow but narrow linewidths, down to nanometers, can be achieved. Electron and ion beam patterning are mostly used in applications where only small areas have to be patterned or when the same structure can be used several times eg. stamps for nanoimprint lithography. The patterns can also be produced by direct laser writing using UV-lasers.

**Etching** After photoresist patterning, usually some further processing is needed. Most often the resist is used as a mask for etching of the underlying layer. Etching processes can be divided into different classes depending on the point of view. Sometimes they are divided into wet and dry etching according to the phase of the etchant. Sometimes division to isotropic and anisotropic etching is used. Isotropic etching means that etching propagates on all directions with the same etch rate. This can be a problem when the structures to be etched are on the same size range as the etch depth. When this is the case, anisotropic etching should be used. There etching propagates faster in some direction than others. All the wet etching processes are not isotropic nor are all the dry etching methods anisotropic or vice versa. Dry etching is also many times called plasma etching since plasma is used to excite the etchants. Typical etching profiles are presented in figure 1.1.

**Replication methods** Apart from lithography and etching, there are several other methods to fabricate three dimensional microstructures. These are generally called replication techniques and are used for fabrication of polymer micro- and nanostructures. These methods include, but are not limited to, casting (also called molding), hot and UV embossing and injection molding.

Casting (molding) is a straightforward process where a liquid polymer is cast on top of the master. The master can usually be used multiple times which is an advantage since the master fabrication is most commonly done in clean room. After the master is fabricated, no special tools are needed which makes the method accessible for most research laboratories. Casting is often used for rapid prototyping of the microfluidic structures but can also be used for the fabrication of molds for subsequent processes like micromolding in capillaries (MIMIC) which will be reviewed in chapter 3.

In casting, the polymer mixture which contains the two components; base and curing agent is poured on the master and cured. The polymer will conform to the structures of the master and after curing (usually by heating) the polymer replica can be detached. The casting process for polydimethylsiloxane (PDMS) [14], the one of the most used polymer in microfluidics, is presented in the figure 1.1. The master for the casting process is most commonly made of silicon, silicon dioxide or silicon nitride or by using some photoresist such as SU-8. Especially the SU-8 is used since fabrication of high aspect ratio structures is relatively fast and simple [20]. The master is coated with some anti-adhesion layer, typically fluoropolymer, to facilitate the release of the PDMS from the master after curing. The elastic nature of PDMS also makes the release of the PDMS easy even from complex and fragile structures [21].

Casting can also be done on an elastic mold, usually PDMS. Casting of polyurethane (PU), PMMA and epoxy has been demonstrated [22]. If elastomeric mold is used, the structures can also be fabricated on non-planar surfaces [23].

In hot and UV embossing, similar types of masters (sometimes called stamps) are used

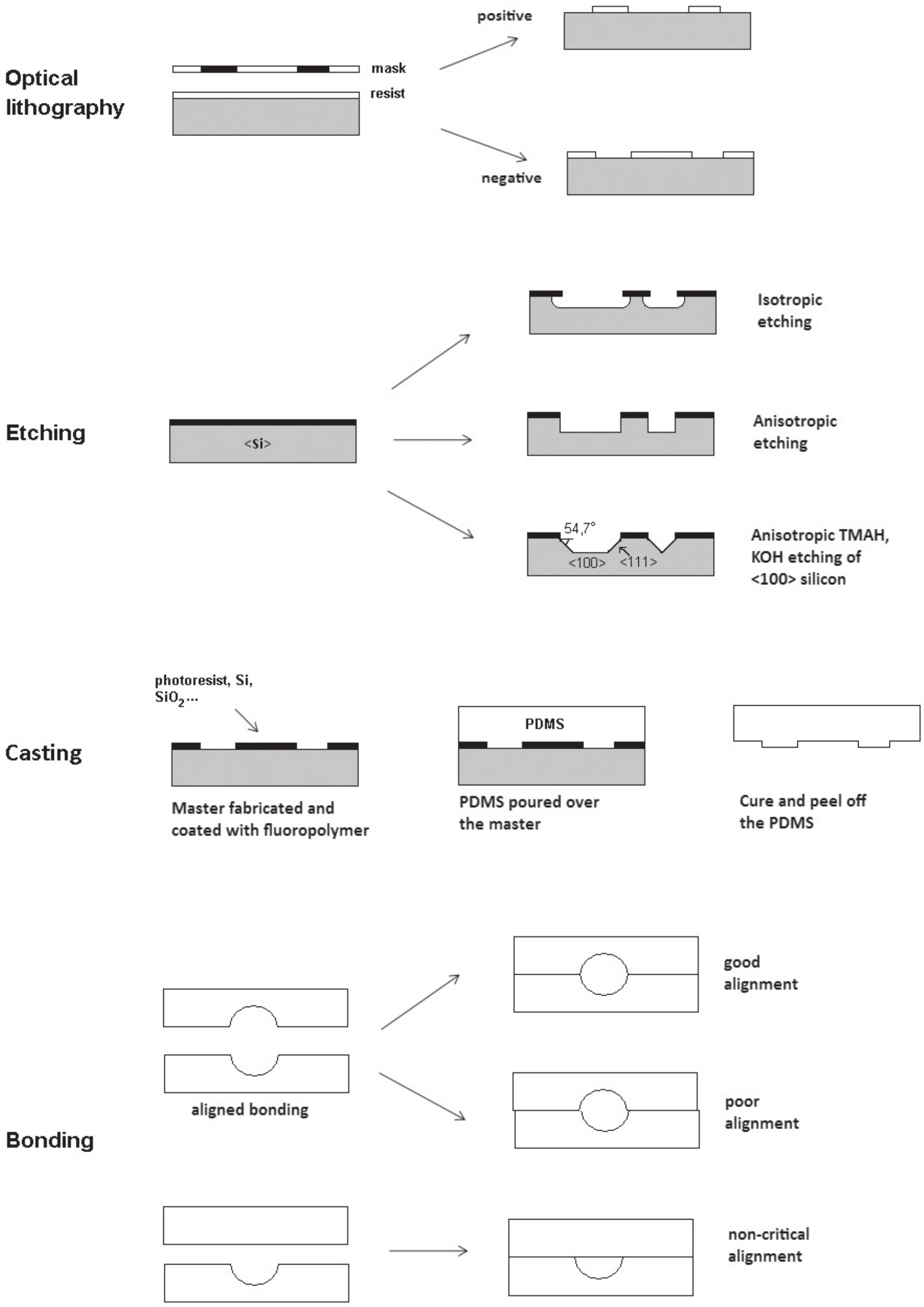


**Figure 1.1.** Common microfabrication techniques

as in casting process. In hot embossing, the structures of the stamp are transferred to thermoplastic polymer, such as PMMA or polycarbonate (PC), by applying heat and pressure. In UV-embossing, UV-light is used to cure the structures on the desired material. In injection molding the polymer melt is injected into a preheated closed, evacuated, mold. All of these and many other microfabrication techniques for polymers are reviewed in chapter 3.

**Thin film deposition** An important process step in most microfabrication schemes is thin film deposition. Thin films can be deposited with variety of techniques, such as sputtering, evaporation, thermal chemical vapor deposition (thermal CVD), plasma enhanced chemical vapor deposition (PECVD) and atomic layer deposition (ALD) [24]. The first two are called physical vapor deposition techniques (PVD) whereas the others are called CVD techniques. This is due to a difference in the deposition method. CVD involves a chemical reaction at the surface to be coated, whereas PVD process is purely physical condensation.

Depending on the application, there can be many requirements for the thin films (such as thickness uniformity, good step coverage and fast deposition) and not any of the above mentioned methods fulfill all of them. For example the step coverage and thickness uniformity is excellent in atomic layer deposition (ALD) but the method is very slow and the thickness of the thin films is limited to few hundred nanometers and in some cases only tens of nanometers. ALD is mostly used to deposit oxides, nitrides and sulphides, although some metal depositions have also been presented [25]. The evaporation on the other hand is relatively simple method and almost all metals can be deposited, but it does not work well for compound deposition. It also suffers from poor adhesion and poor step coverage. Typical thin film deposition processes are summarized in table 1.2. Thermal oxidation is a special type of thin film deposition method which can only be done on top of silicon. In thermal oxidation, a high temperature, together with either water vapor (wet oxidation) or molecular oxygen (dry oxidation) is used to oxidize the silicon. Silicon is consumed in the process so that 46 % of oxide thickness will lie below the original surface and 54 % above it.

**Bonding** Bonding is used to fabricate closed structures. Common to all bonding techniques is that two substrates have to be brought to sufficiently close contact to adhere each other. There are four basic bonds that can form between the substrates; covalent,

**Table 1.2.** Summary of the thin film deposition techniques

| | **Method** | **Deposition rate** | **Deposition temperature** | **Step coverage** | **Typical materials** |
|---|---|---|---|---|---|
| **Physical** | Sputtering | Up to 100 nm/min | RT | Fair | Most metals, metallic compounds |
| | Evaporation | Typically 1 - 10 nm/min | RT | Poor | Low melting point metals |
| **Chemical** | ALD | ~1 nm/min | 80 °C to 500 °C | Excellent | Oxides, Nitrides |
| | Thermal oxidation | 1nm/min (dry)<br>6 nm/min (wet) | 850 °C to 1050 °C | Good | Silicon dioxide |
| | Thermal CVD | 10-100 nm/min<br>5 µm/ min (epi) | 300 °C to 900 °C | Good | Oxides, Nitrides, W, Poly |
| | PECVD | ~few hundred nm/min | 100 °C to 400 °C | Fair | Oxides, Nitrides amorphous Si |

Van der Waals, metallic and ionic bonds. Covalent and Van der Waals bonds are the most dominant bonding mechanisms. In order to accomplish any type of bonding, the atoms of two opposing surfaces must be less than 0,5 nm apart. The resulting bond strength then depends on the type of bonds formed [26].

Most common bonding techniques for microfluidic devices are direct bonding (sometimes called fusion bonding in case of silicon and thermal bonding in case of polymers), anodic bonding and adhesive bonding. There are also several types of polymer-polymer bonding methods, such as solvent bonding that will be discussed in section 3.6. In fusion bonding the wafers are brought in contact at room temperature and high temperature anneal is used to form strong bond between the wafers. Fusion bonding between silicon-silicon [27], silicon-glass [28] and glass-glass [29] are possible. Fusion bonding typically includes cleaning of the wafers since very clean surfaces are needed for good contact. This cleaning step will be beneficial in two different ways. First it will remove all the particles that are on the surface. And second, if a standard RCA-1 cleaning is used, it will leave the surface of the wafers hydrophilic, which makes the siloxane (Si-O-Si) bond formation possible when annealing is done.

The main method of silicon-glass bonding is anodic bonding. Lower temperatures can be used than in fusion bonding since electric field is used to facilitate the bond formation. Due to high attraction force that is created by the electric field, anodic bonding is more tolerant to surface roughness than fusion bonding. Anodic bonding is however possible only between silicon and glass (with few exceptions [30]) since free ions are needed to form the depletion layer at the bond interface.

In adhesive bonding, a polymer adhesive is placed on top of the one of the wafers to be bonded. The polymer adhesive has to be in liquid or semiliquid form in order to achieve sufficiently close contact between the wafers. Furthermore, the adhesive has to transform into a solid to achieve lasting bond. There are three basic types of polymer adhesives:

- Drying
- Hot melts
- Chemically curable

In drying adhesives water or some other solvent will evaporate from the adhesive, hardening the polymer. Hot melts are thermoplastic polymers that can be heated to their melting temperature and will solidify when cooled back below their melting temperature. In chemical curing, the polymer precursors polymerize by chemical reactions that form larger molecules or polymer chains. The curing can be triggered for example by mixing two (or more components) together, by heating, by UV exposure or by combination of UV exposure and heat [31].

By using adhesive bonding different type of wafers can be bonded together. Other main benefit of the adhesive bonding is the insensitivity to the surface topography which means that structures, defects or particles will not affect the bonding. The roughness of the surfaces is less critical since the intermediate polymer layer will deform to fit the surfaces to be bonded. The wetting of the surface is, however, important since the more complete the polymer adhesive fills the cavities of the surfaces, the better is the resulting bond quality. In addition to adhesive bonding by using intermediate polymer adhesive, there are some special adhesive bonding strategies like bonding with the material itself (see paragraph 3.6.3) or self-adhesive bonding of PDMS (see paragraph 3.6.1).

Apart from the above mentioned bonding methods there are several others, although much less common bonding methods for microfluidics. For example in thermo-compression bonding (TCB), pressure and heat are simultaneously applied on the sample. This technique is mostly used to bond metal to metal. Oxide on the surfaces prevent the formation of a strong bond and for that reason the use of oxidation resistant material such as gold is preferred [32]. Glass frit bonding is an other example of TCB [33].

In all the bonding methods, alignment is often needed since usually both of the wafers contain structures that need to be aligned relative to each other. In figure 1.1 the problem is presented. The alignment has to be done by using a bonding tool that contains microscopes for visual inspection. For glass and other transparent substrates visible light can be used but with silicon infrared transmission or backside microscopes are necessary.

## 1.3 ORMOCERs

In mid 1980's two independent groups reported on the preparation of new sol-gel based materials, inorganic/organic hybrid polymers. Wilkes termed the materials, CERAMERs [34] and Schmidt named them first as ORMOSILs [35, 36], organically modified silicates, but later changed the name to ORMOCERs, organically modified ceramics, to emphasize their ceramic nature. In many articles the difference between inorganic-organic and organic-inorganic polymers is made [37]. Inorganic-organic polymers have inorganic elements in their main chain and organic side groups. If the inorganic backbone is formed through traditional sol-gel process followed by organic crosslinking then the hybrid material is called ORMOCER [38]. Organic-inorganic polymers on the other hand are formed of organic main chain and some inorganic side groups. Organic-inorganic polymers are, however, out of the scope of this thesis.

The main motivation for the hybrid material development is the formation of the material that combines the properties of different material classes (see figure 1.2). In classical composites different macroscopic components are mixed together to form a composite material [39]. For example in the case of glass-fiber reinforced polymer, the polymer is the matrix where glass-fibers are mixed to make stronger material. When the component size decreases below microns nanocomposites are formed, for example carbon nanotubes mixed with polymers. When component size decreases even further, the so-called molecular composites can be formed where phases no longer exist and the material is homogenous. ORMOCER®s are members of these molecular composites.

ORMOCERs combine the easy processing of polymers with the hardness and chemical and thermal stability of glass and ceramics. The formation process of ORMOCER makes the modification of material properties easy and for that reason different types of ORMOCERs are used in several different applications. ORMOCERs have been used in optical device [40, 41], as functional coatings [42–44], as filling material in dentistry [45], for fabrication of three dimensional microneedle arrays [46] and as cell growth templates [47]. ORMOCERs have been especially used in optical applications since the refractive index of ORMOCERs can be tuned over a wide range by mixing various resins. There are even two commercial ORMOCER materials, Ormocore and Ormoclad, availble for micro-optical applications. Ormocore is used as core and Ormoclad as cladding material in waveguides.

Fabrication of ORMOCER micro- and nanostructures have been done with several different methods, such as UV-lithography [I], UV-embossing [48], [II], two-photon polymerization (2PP) [49], three-photon polymerization [50] and direct laser writing (DLW)

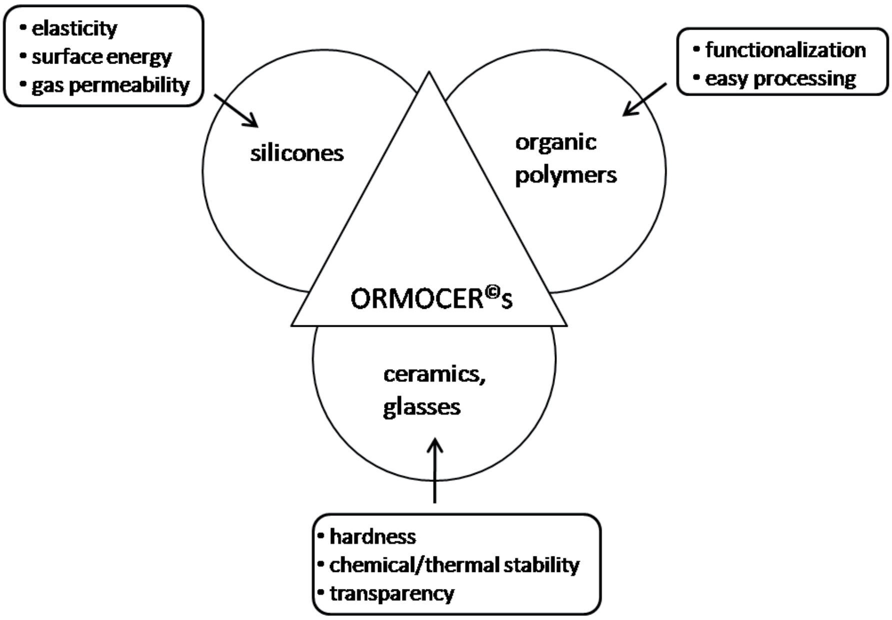


**Figure 1.2.** ORMOCER materials combine the properties of different material classes

[51]. While the first two methods readily available in most clean room facilities, the other three require much more complicated systems with one or more high power lasers. They are also sequential processes, which means that typically structures are fabricated one at the time. In our own studies, commercially available Ormocomp®(a trademark of the Fraunhofer-Institute, Germany) [52] has been used to fabricate microchips for analytical applications. The suitability of Ormocomp for separation devices has also been evaluated. The main results are presented in chapters 4 and 5.

# 2. Polymers for microfluidics

Polymers are gaining more and more attention as materials for microfluidic devices due to relatively simple fabrication methods and their potential of being inexpensive. Both of these aspects make the fabrication of disposable devices possible which is crucial in most medical applications. Polymers have been used as photoresists for several decades but their use as structural material has been limited until the 1990's. Polymers include a large number of materials with completely different properties which can make the selection of material for certain application difficult. Fabrication methods used will depend on the chosen polymer and for these reasons only a small group of polymers have been extensively used in microfabrication [53].

Polymers are commonly divided into three major groups; thermoplastics, thermosets and elastomers [54]. The classification is based on the nature of the interactions between the polymer chains. In thermoplastic polymers, the interactions are mainly Van der Waals interactions and for that reason the thermoplastics can be heated to relatively high temperatures and reformed into new stable structures. In thermosets and elastomers the bonds between the chains are covalent and can not be reform by heating. The difference between thermosets and elastomers is the degree of crosslinking. The crosslinking of elastomers is low making them soft and flexible.

For microfluidic applications there are many material properties that need to be considered, such as processability, surface charge, molecular adsorption, electro-osmotic flow mobility, optical properties and many others. This chapter will introduce some of the most used polymers in microfluidic applications and discuss their strength and weaknesses. The most important material properties for selected polymers are listed in tables 2.1 and 2.2.

## 2.1 Polydimethylsiloxane (PDMS)

The most used polymer for microfluidics is polydimethylsiloxane (PDMS). The chemical structure of PDMS is presented in figure 2.1. PDMS is a silicone elastomer and it has several desirable properties such as optical transparency in UV-VIS regions and low cost. It is also non-fluorescent, biocompatible and non-toxic. Young's modulus of PDMS can be tailored by changing the base curing agent ratio [55]. The simple fabrication of PDMS structures by casting method (see section 1.2) is one of the main reasons why PDMS has become the most used material in microfluidic applications [14, 56, 57]. The bonding of PDMS to PDMS is also easy which makes the fabrication of closed structures possible [58]. Bonding of the PDMS will be explained in more detail in section 3.6.1.

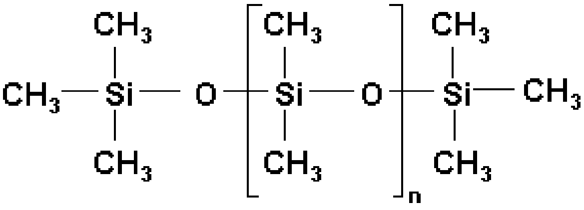


**Figure 2.1.** Structure of PDMS

One of the main disadvantages of PDMS is its poor non-polar solvent compatibility [59]. PDMS swells when in contact with the solvents like hydrocarbons, toluene and dichloromethane. There has been some attempts to overcome this problem by coating the surface of the PDMS microchannel. PDMS microchannels has been coated with poly(urethane acrylate) by using an intermediate oxygen plasma treatment [60]. The coating somewhat efficiently inhibited the swelling of the PDMS by nonpolar solvents, but the poly(uret-hane acrylate) is not optimum for biological applications. The other approach presented modified the PDMS microchannels with a borosilicate glass coating [61]. The coating was shown to prevent swelling of the PDMS when exposed to toluene. The high gas permeability of PDMS can also be an issue although it has also been used as an advantage in applications, like cell culture devices [57], where oxygen can be supplied through the material.

The other main issue is the hydrophobicity of PDMS. Hydrophobic nature of PDMS makes its use in contact with biomolecules such as proteins and peptides problematic due to hydrophobic interactions [62]. This problem has been attempted to overcome by using several different methods. The most simple one is the oxygen plasma treatment of the PDMS. By using oxygen plasma, the water contact angle of PDMS will decrease from about 108 ° to 30 ° which can be explained by the conversion of -OSi($CH_3$)$_2$O- groups at the surface to $O_n$Si(OH)$_{4-n}$ [63]. The decrease of the contact angle has several benefits. The oxidized PDMS channels fill much more easily with liquids with high surface tension, such as water. Also the bubble formation inside the channel is reduced. The oxidized PDMS channels also support electro-osmotic flow (EOF) [64], which can be used in biomolecule separation. The interactions between channel surface and biomolecules is also reduced which is also important in separation devices. The surface of the PDMS will however regenerate back to hydrophobic when in contact with air which is due to a diffusion of the hydrophobic residues into a surface [65]. When PDMS is stored in water, the regeneration is not as fast [66]. It has also been reported that by thermal aging of PDMS the hydrophobic recovery can be reduced [67].

Despite all the above mentioned problems associated with PDMS, it has been and probably will be the most used material in research laboratories for early stage testing of new concepts.

**Hard PDMS** Another siloxane polymer, called hard-PDMS (h-PDMS), was first developed for stamp material for micro contact printing where the elasticity of common PDMS (Sylgard 184) causes problems [68]. The Young's modulus of h-PDMS and 184-PDMS are 8.2 MPa and 1.8 MPa, respectively [69]. The stamps fabricated of h-PDMS are composite stamps where h-PDMS is used for patterns and common PDMS as support since h-PDMS would be too brittle to be used alone [70]. The use of h-PDMS/PDMS composite stamp enables the fabrication of nanosized patterns with high precision [II].

## 2.2 SU-8

SU-8 is a multifunctional epoxy derivative of a bis-phenol-A novolac [71]. The molecular structure of SU-8 is presented in figure 2.2. On average a single molecule contains eight epoxy groups, from which comes the 8 in SU-8. It was first developed by IBM as high resolution resist for IC-industry but was later used to replace the original LIGA (Lithographie, galvanoformung und abformung) technique which required thick layers and for that reason the use of synchrotron X-ray source. This type of X-ray source is extremely expensive and only limited number of equipments are available. The term poor man's LIGA refers to a method where UV-lithography is used instead of X-rays [72].

There are three aspects that make the SU-8 suitable for thick layer applications [71,

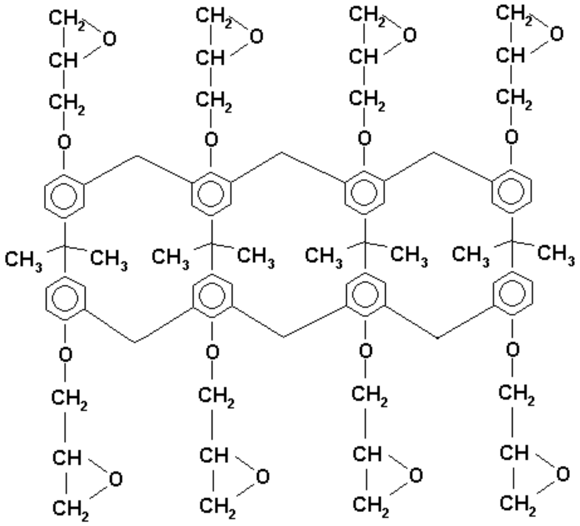


**Figure 2.2.** Molecular structure of SU-8 resin

73]. First, because of its low molecular weight, it can be dissolved in a variety of organic solvents, such as propylene glycol methyl ether acetate (PGMEA), gamma-butyrulactone (GBL) or methyl iso-butyl ketone (MIBK). The first commercially available SU-8 system was dissolved in GBL but nowadays also other SU-8 types are available (MicroChem Corporation, Newton, MA, USA). The amount of solid together with the spin speed will determine the thickness of the films and with current systems thicknesses from under 1 $\mu$m to 500 $\mu$m can be achieved with single spinning step.

The second thing important for thick resists is low optical absorption in the near-UV. Due to SU-8's high transparency, aspect ratios of 18:1 has been obtained [74]. And third, because of its aromatic functionality and highly cross linked matrix, the exposed resist is thermally and chemically stable (glass transition temperature of cured SU-8 is 240 °C [75]) , making it suitable for further processing and as a permanent part of a device.

There are, however, also some problems associated with SU-8, such as poor adhesion to materials like glass, nitrides, oxides and many metals although use of adhesion promoter can improve adhesion on certain surfaces. Also the large thermal expansion coefficient of SU-8 compared to silicon and glass causes problems especially in large area SU-8 structures. Because SU-8 is chemically stable and resistant to most acids and solvents, the removal of crosslinked SU-8 is extremely difficult, although it has been done by using laser [76] and radical plasma [77]. The relatively high water contact angle of SU-8, about 85°, can be a problem in some fluidic applications and it has been modified by using oxygen plasma treatment [78].

Despite some of the drawbacks of SU-8, it has been used for many different types of microfluidic devices. The applications of SU-8 include polymerase chain reaction (PCR) chip [79, 80], integrated microchip capillary electrophoresis and electrospray ionization [81], cantilevers for bio and chemical sensing [82] and many others [83]. The SU-8 microchannels have been characterized for electrokinetic microfluidic applications [84]. SU-8 shows similar electro osmotic mobility as a function of pH ($\geq 4$) as glass microchannels. SU-8 has also been used for fabrication of molds for PDMS casting [85], [III, IV] and as a stamp for hot embossing [86].

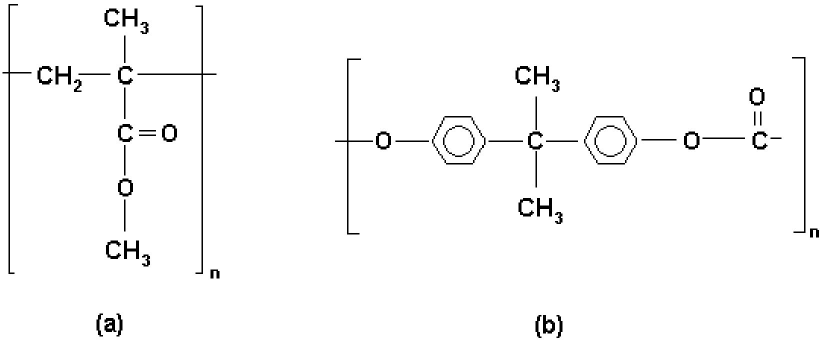
## 2.3 Thermoplasts

Thermoplast is a polymer that softens when temperature rises above its glass transition temperature. Thermoplasts return to their original state after cooling. Polymethyl methacrylate (PMMA) and polycarbonate (PC) are two of the most used thermoplastic polymers in microfluidic applications. The chemical structures of both polymers are presented in figure 2.3.

PMMA has a glass transition temperature of 100-122 °C and it starts to decompose at over 200 °C. It is chemically compatible with diluted acids and bases but it will dissolve into aromatic and chlorine-based solvents. The transparency of PMMA is relatively high. For microfluidic applications PMMA is usually hot embossed but injection molding and laser ablation are also possible [87–89]. PMMA is also generally used as a resist for e-beam lithography [90].

Polycarbonate has a glass transition temperature of 145-148 °C and it starts to decompose at 230 °C. Polycarbonate can be processed by hot embossing, injection molding and laser ablation. Hot embossed and thermally bonded PC microchip with integrated PCR/CE has been presented [91]. The PC microchannels were shown to support EOF but the EOF mobility of the native PC is considerably lower than that of glass at pH 7.The UV-irradiation of polycarbonate was reported to decrease the water contact angle of polycarbonate as well as increase the electro osmotic mobility.

Topas®is a cyclic olefin copolymer (COC) and has excellent physical and chemical properties for lab-on-a-chip applications. It is chemically resistant, has low water absorption and is transparent to UV-light. Glass transition temperature of the material can be tailored between 78 and 178 by changing the content of the norbornene between 60 and 85 wt.%, respectively. Topas has been structured by hot embossing, injection molding [92] and nanoimprint lithography [93]. The main disadvantage of Topas®is its hydrophobicity (contact angle 100°) although preliminary studies show that it can be reduced to less



(a) (b)

**Figure 2.3.** Structure of (a) PMMA and (b) PC

than 10° by plasma treatment [94]. Dynamic coating of the COC channels with hydroxyethyl cellulose has also been proven to enhance hydrophilicity of COC surface making protein separations possible [95].

Zeonor is an other commercial cyclic polyolefin that can be processed by hot embossing (glass transition temperature of Zeonor750R is 70 °). Zeonor750R has very high UV transmission and low autofluorescence.

Several other thermoplastic materials have also been used for microfluidics, including polypropylene, polystyrene and polyurethane [96, 97].

## 2.4 Other polymers

Although the above mentioned polymers are by far the most used ones in microfluidic applications, there are several others that have shown promise in different applications.

Polyimides offers several advantages over more conventional polymers used in microfluidics, such as excellent chemical and thermal stability, low coefficient of thermal expansion (CTE), relatively low contact angle with water and good biocompatibility [98, 99]. Kapton®is a commercially available polyimide material that has been used also for microfluidic applications.

Viton®is a fluoroelastomer which has a low permeability to a wide range of gases. Viton is highly resistant to aliphatic and aromatic hydrocarbons [100]. Other fluoropolymers that has been tested for microfluidic devices include polytetrafluoroethylene (PTFE), fluorinated ethylene propylene copolymer (FEP) and polychlorotetrafluoroethylene (PCTFE) [101]. In general, fluoropolymers have excellent chemical resistance and high resistance to heat and UV-light. They can be embossed or molded to form microstructures.

Poly lactic acid (PLA) [102], poly(lactic-co-glycolic acid) PLGA [103, 104], and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) [105] have been used for the fabrication of tissue engineering scaffolds. The common requirement for all the materials used in scaffolds is biocompatibility and they are usually biodegradable.

**Table 2.1.** Properties of polymers used in microfluidic applications

| Material | $T_g$ [°C] | Melting T /degradation °C | CTE [ppm/°C] | Refractive index (589 nm) | UV-transparency |
|---|---|---|---|---|---|
| PMMA | 100-122 | 250-260 | 70-150 | 1,49 | good |
| PC | 145-148 | 260-270 | 60-70 | 1,58 | poor |
| PDMS (Sylgard 184) | -125 | 400 | 310 | 1,43 | excellent |
| SU-8 | 240 | > 340 | 64 | 1,59 | fair |
| Polyimide | 325-400 | 550-620 | 3-40 | 1,7 | poor |
| COC | 70-155 | 190-320 | 60-80 | 1,53 | excellent |
| Ormocomp | - | 270 | 100-130 | 1,52 | good |

**Table 2.2.** Properties of polymers used in microfluidic applications (continue)

| Material | Contact angle [°] | EO-mobility [$cm^2/Vs$] (pH 7) | Solvent resistance | Acid resistance | References |
|---|---|---|---|---|---|
| PMMA | 73 | $2,07*10^{-4}$ | good | good | [96, 106] |
| PC | 80 | $0,7*10^{-4}$ | good | good | [91, 96, 106] |
| PDMS (Sylgard 184) | > 100 | $1*10^{-4}$ | poor | fair | [53, 64] |
| SU-8 | 85 | $4,5*10^{-4}$ | good | excellent | [73, 75, 84, 107] |
| Polyimide | 50 | N.A. | good | good | [108, 109] |
| COC | 100 | $3,08*10^{-4}$ | excellent | good | [94, 95, 106] |
| ORMOCOMP | 63 | $5,3*10^{-4}$ | good | good | I, II, III, [52] |

# 3. Fabrication of polymer micro and nanostructures

Fabrication methods for polymer micro- and nanostructures have been mainly borrowed from the IC-industry but also from traditional plastic industry manufacturing. Conventional optical lithography is still the most common method to fabricate microfluidic structures. However, due to a broad variety of polymeric materials available, many new fabrication methods have also been invented. When polymer becomes a structural part of the microfluidic system, the layer thickness is much thicker than typical photoresist used for patterning. The aspect ratio of the structures will also increase since the resolution requirements can be in the micron range even with thicker layers. This will bring new challenges to fabrication. In this chapter some of the most common fabrication processes for polymer microfluidic devices have been summarized.

## 3.1 Thick resist lithography

In IC-industry the resist thickness is of the order of micrometers and often less than a micron. In micro electromechanical systems (MEMS), the resist thickness is usually in the order of tens of microns and even up to millimeter [110]. Also when the thickness increases it usually means that the aspect ratio will also increase since the structure sizes in MEMS range from few microns to hundreds of microns. When thick, high aspect ratio structures are processed it sets up new requirements on the photoresist and the exposure tool [111].

One method for thick resist patterning is x-ray lithography (also called deep x-ray lithography (DXRL)) when very thick films (up to 1 mm and over) are patterned. By far the best x-ray source is the synchrotron which emits high flux of usable collimated X-rays allowing short exposure times and high throughput. The availability and high cost of the synchrotrons is however a huge limitation of the process. The fabrication of the masks for the x-ray lithography is very difficult and much more expensive than in conventional lithography. LIGA [112] method is a well known fabrication method where X-ray lithography is needed. LIGA is a German acronym for X-ray lithography, electrodeposition and moulding. In LIGA process a thick resist - from several microns to centimeters - is exposed with X-rays. The resist structures are then filled by metal electroplating after which the resist is removed. This metal structure can be the final product or it can be used as a mould for example in polymer injection moulding. One of the reasons for developing the thick resists for UV-lithography, was to replace the x-ray exposure step of the LIGA process with some more commonly available technique. Technique that utilizes UV instead of X-rays is often called poor-man's LIGA [113].

There are two important properties of photoresist to be suited for ultrathick applications. One is the low molecular weight which allows dissolution into variety of solvents to form very high concentration mixtures. The other thing is the low optical absorption in the near-UV spectrum.

Spin coating of the thick layers is more problematic than thinner ones and many times non-uniform resist surface and layer thickness can occur. For example in the case of SU-8, the fabrication of the layers thicker than 1 mm with single spinning step is not practical. The high viscosity of SU-8 together with slow spin speed will lead to poor photoresist coverage [114]. Spin coating of multiple layers on top of etch other is of course possible but this will lead to even longer processing time. Another problem in spin coating is the build up of the resist to the wafer edges, also called edge bead effect. The edge bead will make good contact between the photomask and the resist impossible. Automatic edge bead removal methods have been developed for industry but in academic research settings it is usually not available. There are, however, some manual tricks that can be used for edge bead removal such as dispensing solvent on the edges of the wafer while spinning it at 500 rpm [115].

One of the main problems is baking of very thick photoresist layers. Solvent evaporation from the thick resist layers has to be done slowly since too fast evaporation can lead to non-uniform films. Thick resist layers are usually baked on a hot plate by slowly ramping the temperature. This will ensure that the solvent will evaporate from the whole layer thickness and not only from the top. Slow cooling of the thick layer is important since too fast cooling can lead to cracking of the resist layer due to stresses in the film. Also leveling the hot plate can be an issue since thick resist layers will flow until the solvent has completely evaporated.

Shrinkage in volume will cause stress which will lead to cracking and loss of adhesion. The same thing can happen if there is a large difference between the thermal expansion coefficient of the resist and that of the substrate. The stress formed in the thick resist film can even cause buckling of the underlying substrate which may be a problem in further processing.

Diffraction of UV-light must also be taken into account especially when very thick resist layers are patterned. Diffraction, and therefore also resolution, depends on the wavelength of light, the resist thickness and the distance between the resist and the photomask. The use of well collimated light is important so that light spreads only little while propagating. With thicker resist the resolution is lower but the distance between resist and mask has to also be considered. Even if contact mode is used, where, in theory, there is no gap, the edge bead effect can cause a significant distance between the photomask and the resist. One interesting idea for reduction of diffraction effect on SU-8 photoresist was introduced in [116]. The air gap between the photomask and the SU-8 was filled with glycerol to eliminate the diffraction problem induced by the air gap. With this method a very straight side walls were produced compared to the non-glycerol filled one.

Developing of the photoresist is typically done by immersion to alkaline solution or organic solvent, depending on the photoresist. Development of thick layers should be done in mild agitation since it will enhance the penetration of the developer into the small structures and that way make the development faster.

## 3.2 Embossing

Embossing, sometimes also called imprinting, is a process where structures of the stamp are transferred to a substrate by pressing them together. The embossing process can be roughly divided into two category; UV- and thermal embossing, depending on the hardening method used. Both micro- and nanosized structures can be fabricated with these methods. All embossing processes are inherently high throughput, because of parallel imprinting. By utilizing embossing methods, high throughput fabrication of nanostructures is thus also possible.

A typical embossing process can be divided into three steps:

- Stamp fabrication
- Replication
- Demolding

One of the main advantages of any embossing technique compared to photolithography, is that it is non-optical and the resolution is thus limited only by the structures that can be fabricated on the stamp. The stamp can be fabricated using methods described in the section 1.2. Depending on the pattern size either UV-lithography (for micro scale and larger) or electron beam lithography (for nanosized) can be used. The selection of stamp material depends on the used embossing method. In the embossing step, the ability of the embossed material to flow is important, so that the embossing process is completed within a practical time frame. Demolding step is commonly the most difficult part of the embossing process. The stamps usually contain some nanoscale roughness on the surface which increases the surface area that contacts the embossed polymer. This will lead to increased adhesion of the embossed polymer to the stamp. Typically stamps are coated with some anti adhesion layer prior embossing to prevent this problem. In the next several paragraphs, different embossing processes are reviewed.

### 3.2.1 Thermal embossing

In thermal embossing processes, a high temperature is used to soften the thermoplastic material where structures of the stamp are replicated. The high temperature sets some additional requirements for the stamp material. There are two commonly used thermal embossing processes called hot embossing and thermal nanoimprint lithography (thermal NIL). In figure 3.1 a typical hot embossing process is presented. First, a thermoplastic polymer is heated above its glass transition temperature under vacuum. At the same time the pressure is increased. When target temperature and pressure are reached, the stamp is kept in contact with the substrate for time $t_2$. Then the temperature is decreased under the glass transition temperature while still keeping the pressure constant. After cooling the pressure is released and the demolding is done.

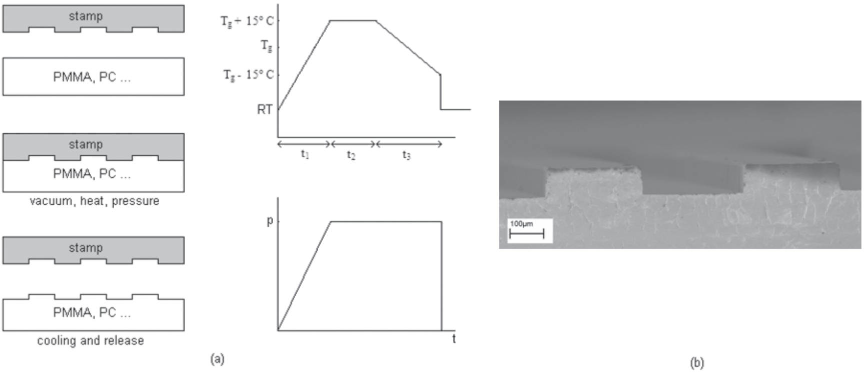


**Figure 3.1.** (a) Schematic of hot embossing process where diagrams present the temperature and the pressure profiles of the hot embossing process (b) Hot embossed PMMA structure

The embossing temperatures for hot embossing are typically 10 to 30 °C above the glass transition temperature of the embossed polymer [117]. The most used substrates for hot embossing are PMMA and PC with glass transition temperatures in the range of 100-122 °C and 145-148 °C, respectively. The applied force and the holding time depend on the structures as well as the viscosity of the embossed polymer.

Other thermal embossing method is thermal nanoimprint lithography, which, as name indicates, is commonly used for the replication of nanosized features [118]. A typical nanoimprinting process is presented in figure 3.2. In thermal NIL, the thermoplast is typically spun on top of the substrate after which the replication is done as in the case of hot embossing. The embossing temperature is commonly higher in NIL than in hot embossing process, around 70 to 90 °C above the glass transition temperature of the thermoplast. The use of higher temperature is possible due to a substrate that is most typically silicon. Higher temperature reduces the viscosity of the thermoplast making the imprinting process faster. Most often the thermally imprinted resist is then used as an etch mask in further processing. The etching properties of the resist material must also be considered since the residual layer has to be removed while at the same time resist has to withstand the subsequent substrate etching.

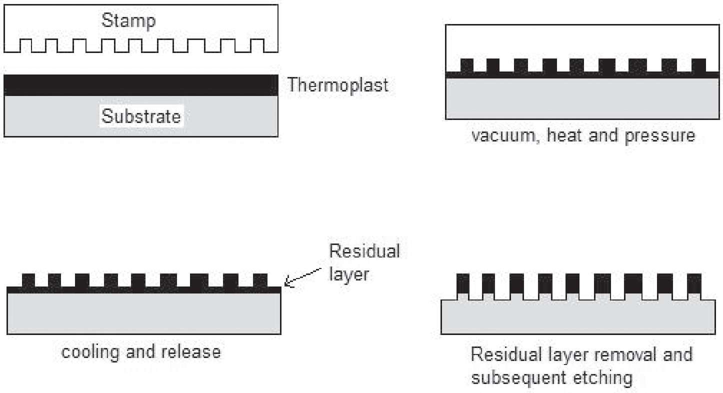


**Figure 3.2.** Schematics of typical thermal nanoimprint lithography process

Due to a relatively high temperatures used in the thermal embossing processes, the mismatch of thermal expansion coefficient between substrate and stamp has to be considered. The large thermal stress may appear and induce fractures to the polymer micro and nanostructures. The stamps for hot embossing are often made of nickel by using the LIGA method [117] but laborious fabrication has led to investigation of other stamp materials such as silicon [119], SU-8 [120] and even PDMS [121, 122]. In thermal nanoimprint, silicon, silicon dioxide, silicon nitride and metals are commonly used. In nanoimprint lithography, hard stamps are commonly preferred since pattern deformation happens when soft stamps are used.

### 3.2.2 UV-embossing

In UV-embossing process the idea is the same as in hot embossing process but UV-light is used to cure the polymer layer. Either the substrate or the stamp has to be made of transparent material in order to make UV curing possible. The most commonly used UV-embossed materials are based on free radical polymerization of acrylic and methacrylic monomers because of their high reactivity. The acrylate based UV imprint resist has also a large shrinkage upon curing (ca. 10 %) which can be an issue in some processes although this can also facilitate the release of the stamp. One benefit of the UV-embossing process is that since heating is not usually done, the mismatch of thermal expansion coefficient is not an issue. Also the process time is potentially shorter since long heating and cooling times are omitted. The embossing pressures used in UV-embossing are typically much lower than in hot embossing process.

UV-nanoimprint lithography is similar to thermal NIL, but UV curable resins are used instead of thermoplastics. One of the requirement for the UV-NIL is the use of hard stamp. The use of hard stamp is essential when accurate transfer of nanoscale features is wanted because patterns on the stamp should not deform, buckle or collapse during imprinting. However, in UV-embossing, when micro scale structure are produced the use of elastomeric, PDMS stamp has it advantages. PDMS stamps can be easily fabricated using the casting method described previously. PDMS has low surface energy which together with elasticity makes it easy to release from the embossed substrate. Since no anti-adhesion coating is done, the PDMS stamp can be used several times. PDMS is also UV transparent, which makes the UV-curing through the stamp possible. In figure 3.3 Ormocomp microstructures fabricated by UV-embossing with PDMS stamp are presented.

Due to the simplicity of the UV-embossing process, several variations aiming for high throughput manufacturing have been presented. In step and flash imprint lithography (SFIL), an organic transfer layer is spin-coated on a substrate. The stamp is then brought into close proximity after which a low viscosity, photocurable organosilicon solution is introduced into the gap between the stamp and the transfer layer. The polymer fills the gap by capillary actions. The stamp is then pressed into contact with the transfer layer and UV exposure through the transparent stamp is made. After curing, the stamp is released and subsequent processing can be done as already discussed in NIL process. [123, 124]. Another high throughput manufacturing method is roll-to-roll embossing where, as the

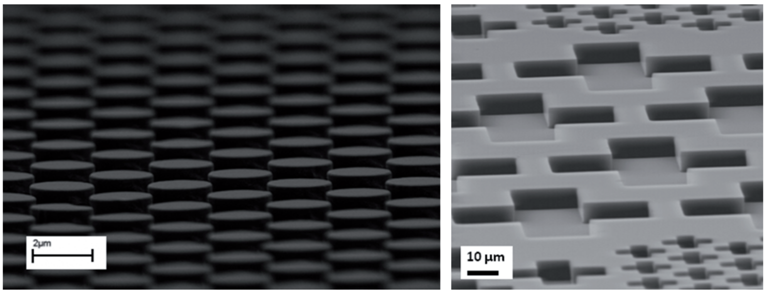


**Figure 3.3.** UV-embossed Ormocomp microstructures [I]

name indicates, the polymer layer is patterned by using a rolling stamp. Both UV- and thermally curable polymers can be patterned but in both cases the resist has to be easily printed with low pressure and the curing should be relatively fast [125].

### 3.3 Micromolding in capillaries (MIMIC)

Micro molding in capillaries was first presented by Kim et. al. [126]. The mold is fabricated by using a common molding process. The process starts by placing the mold on top of a substrate. The liquid polymer is then applied and the polymer is drawn to the channels of the mold by capillary action. The polymer is then cured by UV or thermal curing and finally the mold is removed. An ideal MIMIC process flow is presented in figure 3.4.

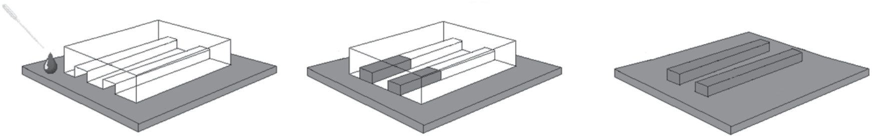


**Figure 3.4.** Micro molding in capillaries; The polymer is applied at the orifice of the channels and drawn in by capillary forces. The PDMS mold is removed after the filling is complited and the structures are cured.

The MIMIC technique is extremely simple, allowing fabrication of structures in broad range of materials and dimensions. Compared to embossing techniques, the advantage of the MIMIC is the lack of residual layer after processing since it is often unwanted. There are however some limitations, since MIMIC requires a connected network of capillaries and formation of isolated patterns is thus impossible. Also, the capillary filling in short distances (ca. 1 cm) may be sufficiently fast but the rate of filling decreases significantly over longer distances. The rate of filling is also proportional to channel cross section which means that filling of the nanochannels may be difficult. If the channels are of the different size, wider ones will fill faster and can even block the ends of the narrower channels and that way incomplete filling of the structures may occur.

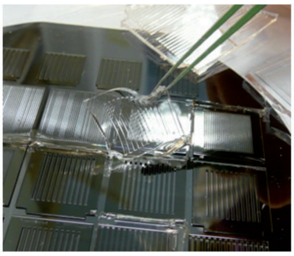
In our own research, micro molding in capillaries have been applied to the fabrication of tissue engineering scaffolds. Methacrylated poly($\epsilon$-caprolactone) and poly($\epsilon$-caprolactone-co-D,L-lactide) oligomers were used for scaffold fabrication [127]. The used polymers are photo curable and biodegradable. A complete fabrication process is presented in figure 3.5. First, a master is fabricated of SU-8 and PDMS is casted on the master. After curing, the PDMS molds are peeled off the SU-8 and attached to PMMA substrate. The polymer is applied to the openings of the microchannels. The microchannels are 500 $\mu$m high and wide and 1 cm long. After filling the mold, the polymer is partially cured after which the PDMS mold is removed. Partially cured polymer layer can then be detached from the PMMA substrate and stacked together. Scaffolds consisting of up to 20 layers have been fabricated.

## 3.4 Microstereolithography

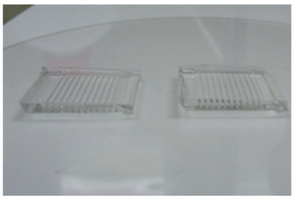
Microstereolithography ($\mu$SL) is a direct development from the laser stereolithography used in industry for rapid prototyping of 3D objects [128]. In $\mu$SL system the elevator platform where structure is build is moving downwards in a container with UV curable resin. By using an accurately controlled elevator a layer thickness of less than 10 $\mu$m can be achieved. The UV-laser is used to draw the structures on each layer. After one layer is solidified, the elevator moves downward by the thickness of the next layer and the new layer is again solidified. Since sequential scanning of the laser beam is relatively slow even for small structures the resist material used should be rapidly curable. Also, the resist should not require a soft bake and the curing should happen immediately after the UV exposure without the need for post exposure bake.

## 3.5 Other fabrication methods for polymers

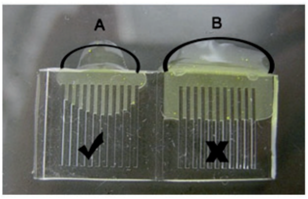
**Micro injection molding** Injection molding is a well known method to fabricate macroscopic plastic parts. When brought into a micro scale, term micro injection molding [129] is generally used. Micro injection molding is probably one of the most promising technique for the fabrication of non-electronic micro devices [130]. Injection molding is most



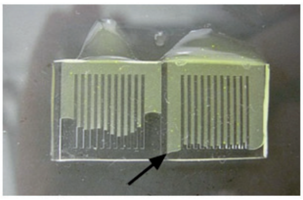
PDMS molds fabricated on a SU-8 master



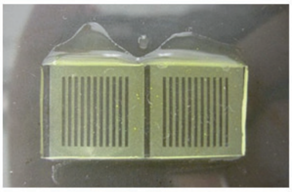
PDMS molds on top of PMMA substrate



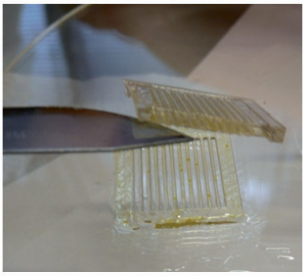
Polymer applied to the inlets



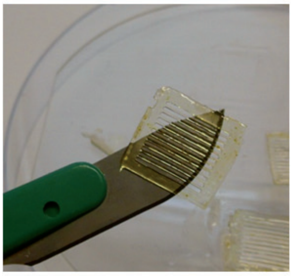
Molds are filled by capillary action



Completely filled molds



Peeling off the PDMS mold



Single polymer layer detached from the PMMA

Complete scaffold by stacking several polymer layers together

**Figure 3.5.** Fabrication of tissue engineering scaffold by using micromolding in capillaries

suitable for the fabrication of large batches, since fabrication of the mold is usually laborious.

Schematic injection molding process is presented in figure 3.6. The mold is first closed, evacuated and heated above the glass transition temperature of the injectable polymers. The polymer itself is also heated up and then injected into the mold. The mold is cooled down below the glass transition temperature and the part is demolded. The main process parameters include mold temperature, injection speed and pressure, holding time and holding pressure. The main advantage over the hot embossing method is the shorter cycle time of the process.

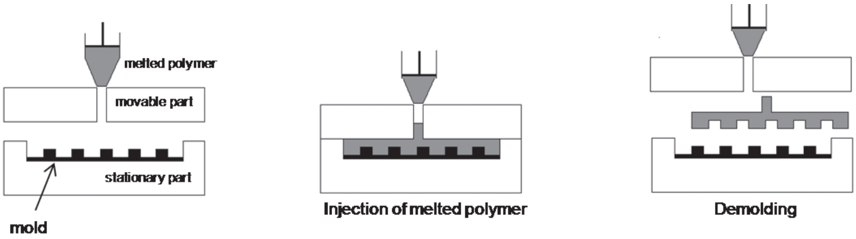


**Figure 3.6.** Schematics of micro injection molding process

**Microthermoforming** Thermoforming is used in macroworld for the low cost production of the thin walled plastic objects like yoghurt cups. Microthermoforming is its microworld counterpart and it is used to form micro sized structures into thin thermoplastic polymer films. Thermoforming process is presented in figure 3.7. First, the polymer film is stacked in between two mold parts. The mold is then evacuated, heated to about 10 degrees over the glass transition temperature of the polymer film. The film is then pressed into the microstructures by using gas, the mold is cooled down to about 20 degrees below $T_g$, and the ready polymer part is demolded from the mold.

Truckenmüller et. al. [131, 132] presented a fabrication of low cost capillary electrophoresis chip by using microthermoforming. They were able to integrate electrodes on the CE reservoirs by first patterning the metal by shadow masking on the polymer thin film and then positioning the film on the mold so that the electrodes were aligned correctly.

Although microthermoforming provides a relatively simple and fast fabrication there are some limitations. The temperature that can be used is limited since the polymer will become more permeable to gases when temperature rises. The fabrication of high aspect ratio structures and sharp corners is thus difficult.

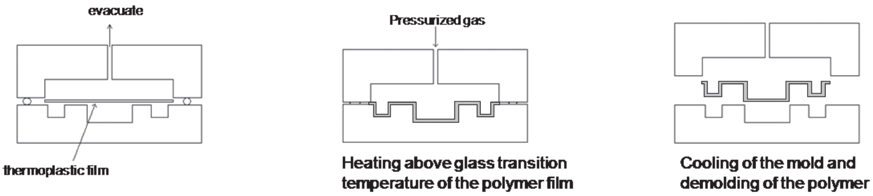


**Figure 3.7.** Schematics of microthermoforming process

**Laser ablation** Laser ablation was first used by Roberts et. al. [15] to produce polymer microfluidic channels. In laser ablation UV laser is used to draw the structures on a polymer substrate. The polymer decomposition is due to either photodegradation or thermal degradation or combination of the two. The ablation can be done by using mask or by maskless process where substrate is placed on movable stage. Many different polymers can be patterned using laser ablation.

## 3.6 Fabricating enclosed polymer channels

Most of the microfabrication methods for polymers produce open microfluidic structures. However, in many applications enclosed channels are needed. In microfluidic applications, the situation where all the walls of the microchannel are of the same material is usually optimum, especially when electro-osmotic flow is used to drive the fluidic in the device. Enclosed polymer microchannels can be fabricated with several different techniques depending on the used polymer.

### 3.6.1 Bonding of PDMS

One major advantage of PDMS is easy bonding. PDMS can be bonded irreversible or reversible not just to it itself but also to many other materials. Reversible bonding can be done to basically any clean surface and even though the bond does not tolerate high pressures this method can be used in many applications. By using reversible bonding, it is also easier to clean and reuse the channels. However, if stronger bonding is needed, PDMS can be activated by oxygen plasma treatment (figure 3.8). The formation of bridging, covalent siloxane (Si-O-Si) bonds by a condensation reaction will form the irreversible bonding between two PDMS substrates [14]. Formation of irreversible bonding between several plastics and PDMS has also been presented [133]. The surface of the PDMS will however regenerate back to hydrophobic when in contact with air which is due to a diffusion of the hydrophobic residues into the surface [65]. For this reason the bonding of the PDMS have to be done less than one hour after the plasma exposure.

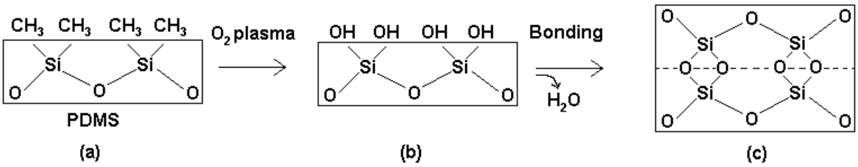


**Figure 3.8.** (a) native PDMS surface (b) PDMS surface after oxygen plasma treatment (simplified image) (c) PDMS to PDMS bonding

### 3.6.2 Bonding of thermoplastic polymers

Bonding of thermoplastic polymers for microfluidics has been recently reviewed [106]. PMMA and PC structures can be bonded by using thermal fusion bonding [91]. The bonding is done by heating the hot embossed substrate as well as a blank PMMA wafer near or over the glass transition temperature and applying pressure [134]. This type of direct thermal fusion bonding has been extensively used due to its simplicity and relatively high bond strength. The main problem with thermal bonding is that the increased temperature can deform the channels because of the bulk polymer flow. Important bonding parameters for thermal bonding are temperature, time and force.

To decrease the temperature used in thermal bonding, different kinds of activation methods have been tested, such as the application of UV/ozone surface treatment for low temperature bonding of PMMA and COC substrates [135]. The set of UV/ozone treated chips showed significantly improved bond strength when compared with the untreated ones bonded at the same temperature.

Other solution to decrease the bonding temperature is to use surface modification assisted bonding methods such as solvent bonding [136, 137]. In solvent bonding, a solvent or mixture of solvents is used to soften the surface of the polymer and in that way make the bonding possible. The solubility parameters of the polymer and selected solvent should be dissimilar to prevent excess solvent uptake which can lead to channel deformation. Wide range of thermoplastic polymers can be solvent bonded but in practice only few have been reported. Solvent bonding of PMMA by using capillarity and short term contact with acetone has been presented [138]. Acetone was introduced into the microchannel that was formed by placing two PMMA sheets together, one of them containing the microchannel. Quantity of solvent, its water content and flow rate as well as residence time in the channel, all have impact on the bond quality. Due to a very short residence time, acetone did not deform the channel features. This bonding method, however, requires relatively complicated assembly and can only be done for one channel at the time. It was also observed that the drop of solvent left in the channel for longer than 2-3s dissolved the plastic and resulted in channel deformation.

Other solvent bonding method presented for PMMA is thermally actuated solvent bonding [139]. In this method, the solvent bonding is done by using a solvent, in this case isopropanol, which does not dissolve the polymer, or dissolves very little, at room temperature. When temperature is increase, the solubility will also increase making the bond formation possible. Temperatures from 25 to 80 °C were tested of which 70 °C was found to be the best. This is still well below the temperatures used in thermal bonding, but the benefits compared to room temperature solvent bonding is not as clear.

Since the deformation and clogging of the microchannels is a common problem, a solvent bonding method where a sacrificial material was used to fill the channels prior to bonding has been studied [140]. After the solvent bonding, the device is heated above the melting temperature of the sacrificial material and the material is flushed out as a liquid. Although the solvent bonding is mostly used for PMMA, other thermoplasts have also been solvent bonded such as COC [141].

Another possible bonding method for thermoplast is localized welding [106]. Localized welding can be done by using ultrasounds, microwaves or IR-laser. Common for all these methods is that the bonding can be done locally for specific region. Localized welding is not however widely adopted for microfluidics since it requires a special chip design (for ultrasonic welding), additional metal patterns (microwaves) or has special material requirements (IR-laser).

Adhesive bonding of thermoplastic polymers is also possible. The same methods can be used as already discussed in section 1.2. All the benefits and disadvantages apply also when two polymer substrates are joint together.

### 3.6.3 Fabrication of enclosed SU-8 structures

Since SU-8 is a much used polymer for microfluidic applications, several different methods for the fabrication of SU-8 channels have been developed. In figure 3.9 (a) a filling material is used to work as a sacrificial layer in the bonding process. The benefit of this method is that the clogging of the channels is not a problem. The main disadvantage is the difficulty of the filling process and the selection of the filling material. Also the dissolution of the filling material after bonding is limited by diffusion which makes the fabrication of long channels impossible. In figure 3.9 (b), the intermediate metal layer is used to block the UV-light [142]. The problems are the deposition of the metal layer and its removal, since usually quite harsh chemicals are needed to etch metals. In figure 3.9

(c) a SU-8 laminate is used to bond the structures. The process is relatively simple but some optimization of the bonding temperature is required. Bonding temperatures from 48 °C to 75 °C has been tested [16]. With the higher temperatures, the clogging of the channels can be a problem. When using lower temperatures (below the glass transition temperature of 64 °C, although $T_g$ as low as 55 °C has been reported for unexposed SU-8 [114]) the channel clogging is usually not a problem since the viscosity of the bonding layer is high but some pressure has to be used for successful bonding. The bond strength is not as high as can be achieved when using higher temperatures. Somewhat optimum bonding temperature was found to be 68 °C [16]. In figure 3.9 (d) a proton beam is used to partially expose the roof of the channel [143]. Even though proton beam can achieve good dosage control, it is not a popular source and this method is thus not widely used. Also the removal of the uncured SU-8 inside the microchannel can be a problem.

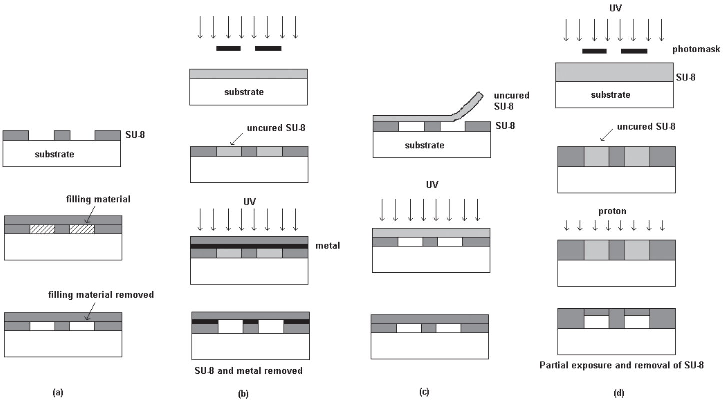


**Figure 3.9.** Fabrication of embedded SU-8 channels (a) by using sacrificial material (b) by using intermediate metal layer (c) by using SU-8 laminate (d) by proton beam exposure

# 4. Fabrication of Ormocomp micro- and nanostructures

Since ORMOCERs have been primarily used in dental and coating applications, the microfabrication methods for ORMOCERs are not well established. In our research, a commercially available ORMOCER material, Ormocomp, has been used for the fabrication of micro- and nanosized structures for microfluidic and bioapplications. Ormocomp is sensitive to UV-light and can thus be patterned by using common microfabrication methods such as UV-lithography and UV-embossing. For microfluidic applications, the fabrication of enclosed structures is important and for that reason, developing the bonding process for Ormocomp has been one of the key issues in this thesis.

## 4.1 UV-lithography

Ormocomp works like negative type of photoresist that can be crosslinked by using UV-light. Since Ormocomp is fairly unknown material in microfluidics, its lithography process is here compared to much used negative photoresist, SU-8 (see figure 4.1). Both materials are spun on top of the substrate, most commonly silicon or glass, although, in case of Ormocomp any (cheap) polymer substrate can be used, since Ormocomp does not contain solvent. In figure 4.2 the spincurves for SU-8 50 and Ormocomp are presented. The thickness of the Ormocomp layer is limited to about 70 $\mu$m whereas 100 $\mu$m can be achieved with the single spinning step by using SU-8 50. If SU-8 100 is used the layer thickness up to 500 $\mu$m is possible to achieve.

Since Ormocomp is solvent-free, the purpose of the soft bake is to remove air bubbles from the film and is done in a few minutes. In case of SU-8, the soft bake time depends on the film thickness and can be several hours when thicker films are processed. The soft bake of SU-8 is usually done at two different temperatures, 65 °C and 95 °C, with ramping between them, to reduce stress from the SU-8 layer.

In case of Ormocomp, UV-exposure has to be done in proximity mode since Ormocomp

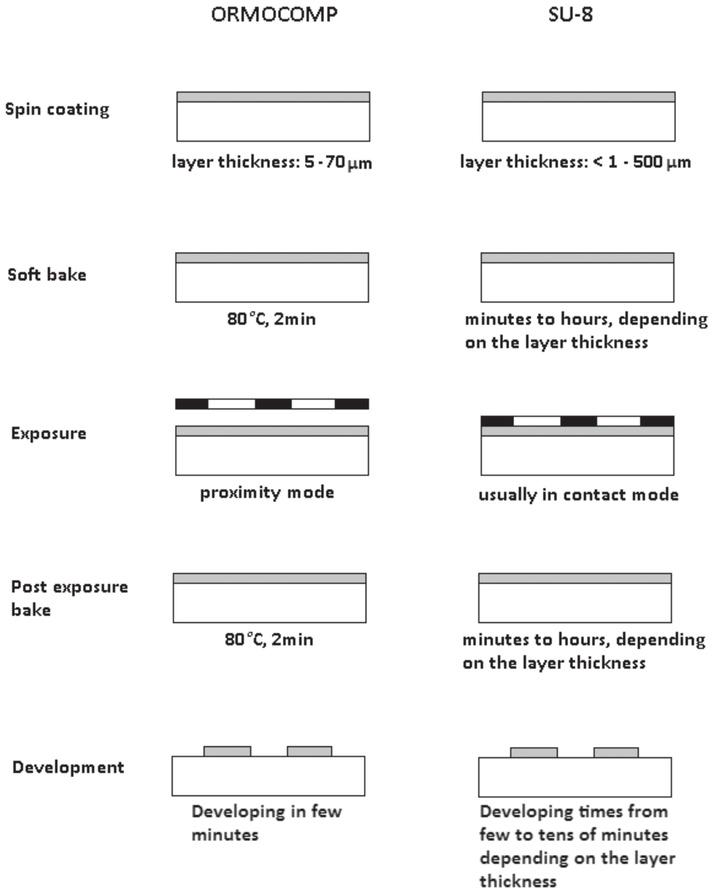


**Figure 4.1.** Comparison of Ormocomp and SU-8 processing

is wet after the soft bake. In contrast, since evaporation of solvent takes place in the soft bake of SU-8, it is solid after soft bake, making the use of contact mode possible. The use of proximity mode in the case of wet resists can be a problem since the gap between the mask and the resist layer should be as small as possible to ensure good resolution but if the gap is not big enough the resist will come in contact with the mask. The edge bead can make the problem even more difficult, since even with relatively large gap, the edges of the wafer can come in contact with the mask.

The polymerization of Ormocomp is based on free radical polymerization whereas in the case of SU-8, the polymerization reaction is cationic. Due to the polymerization method Ormocomp is extremely sensitive to UV-light, making the exposure time selection problematic. If high resolution is needed, the exposure time has to be optimized carefully. Too long exposure will result in joint structures and too short exposure will lead to adhesion

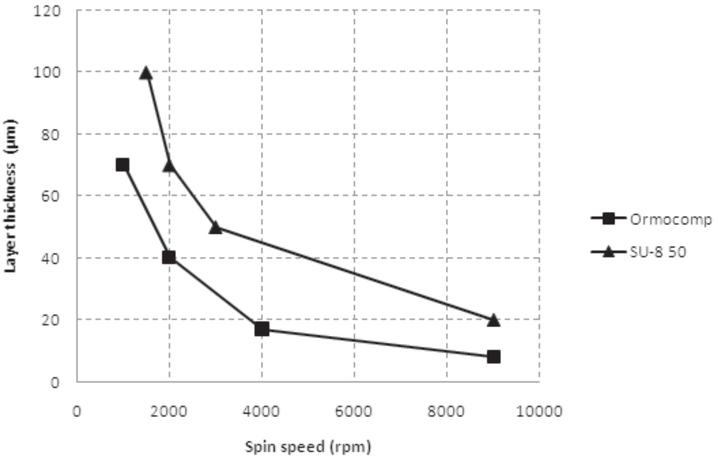


**Figure 4.2.** Spincurves for Ormocomp and SU-8 50

failure between Ormocomp and the substrate. Usually it is recommended that a flood exposure of the structures is done after the development to ensure good adhesion. The adhesion promoter for Ormocomp, Ormoprime, should be used especially when glass is used as a substrate.

Free radical polymerization is also sensitive to oxygen, which inhibits the curing of the top few microns of the Ormocomp layer [144]. In many cases this reaction is unwanted and several methods have been considered to overcome the problem [145].

In case of SU-8, the exposure dose is less critical although for high resolution patterns, optimization of exposure time is needed. Exposure time is also layer thickness dependent, so longer exposure times are needed for thicker layer. The exposure times range from few to tens of seconds. Too short exposure times lead to adhesion failures where as too long times will cause widening of the exposed parts.

After exposure, SU-8 has to be post exposure baked to finalize the curing, since in cationic systems, the UV-light only initiates the polymerization reactions. For Ormocomp, post exposure bake is not required, which makes it a suitable material also for stereolithography.

The development of SU-8 is done by using an organic solvent, 1-methoxy-2-propylacetate (PGMEA). The development time depends on the thickness of the layer, as well as the density of the structures. Ormocomp is developed by using Ormodev, which is a methylisobutylketone/isopropyl alcohol mixture (1:1). The development of Ormocomp structures is significantly faster that SU-8 ones. The development time for Ormocomp is from few seconds to minutes. In both cases, over development is not usually a problem, since structures are stable after curing.

## 4.2 UV-embossing of micro and nanostructures

For Ormocomp, the UV-embossing offers a simpler way of patterning than UV-lithography. This is due to two reasons; first, since Ormocomp is still viscous after the soft bake, the exposure using photomask is difficult. Second, since the resolution in UV-embossing is defined by the structures of the stamp, the exposure dose is less critical. Embossing of structures from few hundred nanometer to few hundred micrometers have been tested. In principal the UV-embossing of Ormocomp is done as already explained in 3.2.2, but there are some issues especially in stamp selection that should be taken into account.

### 4.2.1 Stamps

In our own studies, four different stamps have been used: a two-level SU-8/glass stamp with integrated metal structures [146], hard silicon stamp, soft PDMS stamp [III] and soft h-PDMS/PDMS composite stamp [II]. In the next paragraphs the pros and cons of these stamps are reviewed.

**Two-level SU-8/glass stamp with integrated metal patterns** We have tested a hard stamp fabricated of two-level SU-8 on glass substrate with integrated metal patterns [146]. By using a two-level stamp the fabrication of bottom and the channels of the chip at the same time is possible omitting one extra spinning step. The integrated metal parts make the fabrication of through vias possible if the chip is released from the substrate after processing. Glass was selected as a substrate due to its transparency, making exposure through the stamp possible. Also, the integration of metallic pattern on glass is easier than for example on some polymer, like PMMA. Embossing by using SU-8 hard stamp with integrated metal patterns as well as the photography of the stamp is presented in figure 4.3. The use of SU-8 makes the fabrication of two-level structures simple.

Hard stamps are generally more complex to fabricate but they do not suffer from deformation of the structures that is typical for elastomeric stamps. The biggest problem with the hard stamp fabricated of two or several different materials is that the adhesion between the structures and substrate fails easily during demolding, decreasing the lifetime of the stamp. Also the use of anti-adhesion coating is mandatory for facile release but degradation of the coating can also lead to short lifetime of the stamp.

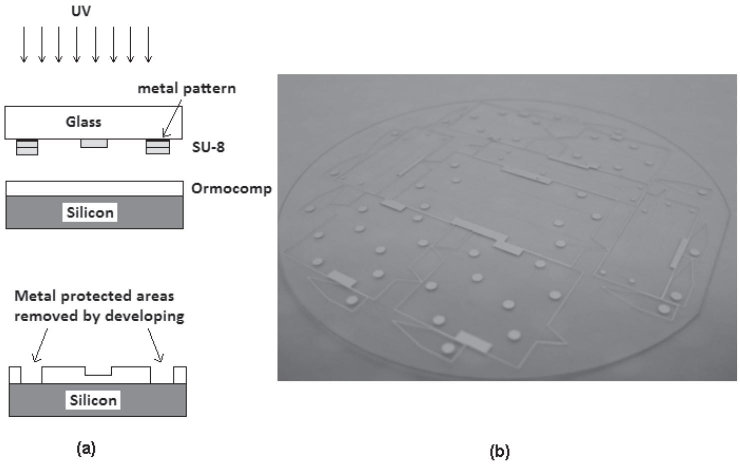


**Figure 4.3.** (a) Embossing using multi-level SU-8 stamp with integrated metal patterns (b) Photograph of two-level SU-8 stamp on glass with integrated metal patterns [146]

**Silicon stamp** As already mentioned, the main problem with integrated SU-8/glass stamp is the adhesion failure between the glass and SU-8 which happens especially during demolding. This can be avoided by fabricating the structures directly on silicon or glass substrate. In case of glass the microfabrication is somewhat laborious, especially if multilevel structures are wanted. The microfabrication of silicon is, however, relatively easy, but the brittleness of silicon makes the release of the stamp difficult. Also since silicon is non-transparent to UV-light, the exposure has to be done through the substrate (see figure 4.4). Thus the use of transparent substrate is mandatory and the integration of metal patterns is not feasible.

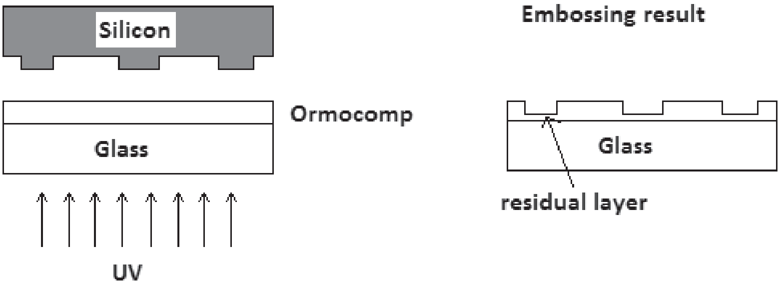


**Figure 4.4.** UV-embossing by using silicon stamp

**PDMS and h-PDMS/PDMS composite stamp** Elastomeric, PDMS stamps are easy to fabricate and for that reason has been most widely used in our own research. The stamp master, usually done out of SU-8 or silicon, can be used many times and the fabrication of SU-8 master is simple and relatively fast. After the master fabrication, the stamp is fabricated by using a simple casting process. PDMS stamps are simple to use, since the embossing can be done on a hot plate and the demolding is easy due to elasticity and the low surface energy of the PDMS. No additional anti-adhesion coatings are needed when PDMS is used. The main problem associated with elastomeric stamp is the mechanical deformation of the stamp during embossing. This can be overcome, by using thicker stamps or by using an additional support at the back of the stamp. The elasticity of PDMS also limits the aspect ratio of the structures. Aspect ratio of 2 has been considered to be the upper limit [21]. In figure 4.5 the embossing process using PDMS is presented, along with the photograph of the PDMS stamp.

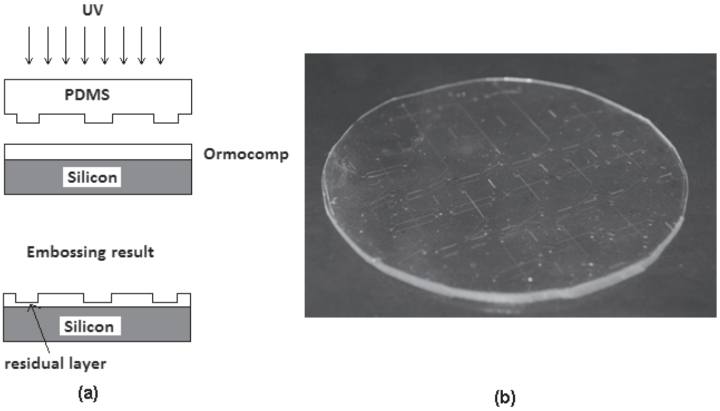


**Figure 4.5.** (a) UV-embossing by using PDMS stamp (b) Photograph of soft PDMS stamp [method used in I, III, IV]

Another type of elastomeric stamp is h-PDMS/PDMS composite stamp that is used for embossing of nanostructures. When nanostructures are replicated, the use of common PDMS is no longer possible as can be seen in figure 4.6 (c), since common PDMS can not replicate nanostructures precisely enough [21]. In publication II, a fast and simple fabrication method for Ormocomp nanopillars is presented. The master for h-PDMS/PDMS composite stamp is fabricated using inductively coupled plasma - reactive ion etching (ICP-RIE) and relatively well known black silicon process [147, 148]. The black silicon nanostructures (figure 4.6 (a)) are then transferred to h-PDMS and common PDMS works as a structural support (figure 4.6 (b)). This stamp is then used to emboss Ormocomp in similar manner as in the case of microstructures (figure 4.6 (d)). Since PDMS is used as a stamp, the lateral deformation of the structures happens but since nanopillars are used for surface modification, it does not cause a problem.

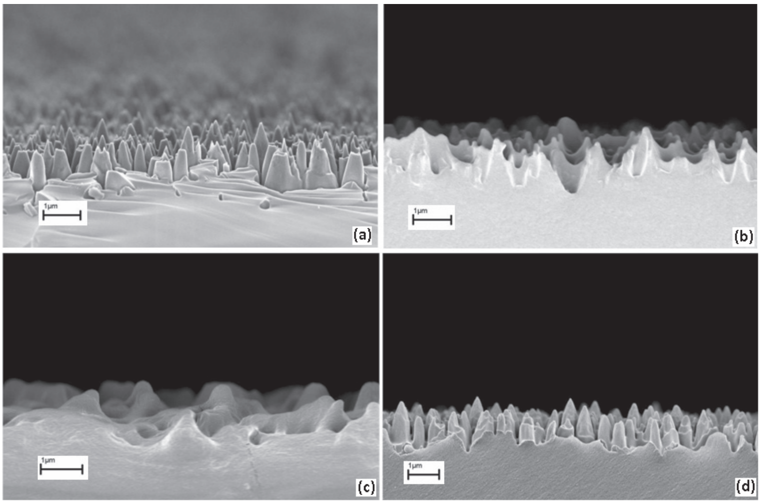


**Figure 4.6.** (a) Black silicon master (b) composite PDMS/h-PDMS stamp (c) common Sylgard 184 PDMS stamp (d) replicated Ormocomp nanopillars using composite stamp [II]

### 4.2.2 Embossing process

In UV-embossing, the Ormocomp is spun on top of the substrate with the spin speed that depends on the desired structure height. The layer thickness is not as critical in UV-embossing as in UV-lithography since the height of the structures is defined by the stamp and not the layer thickness itself. The embossing can be done either manually on a hot plate or in a bondering machine under vacuum. When stamping is done on a hot plate, a PDMS stamps should be used, since air bubbles have to be squeezed out between the stamp and the Ormocomp layer. Furthermore, air bubbles are easier to remove if relatively thin PDMS stamp is used since only little force is needed to push the bubbles out. Thin PDMS stamp is however more easily deformed than thicker one. Even if very thin PDMS stamp is used, air bubbles are still quite easily trapped on some structures. In figure 4.7 (a) a perfectly imprinted channel crossing is presented. In figure 4.7 (b) air bubbles are trapped between two channels.

To overcome the problem with the air bubbles, the embossing can be done under vacuum. The AML AWB-04 bonding machine has been tested for embossing process. The substrate that contains the Ormocomp is placed at the bottom electrode and the stamp on the top one. The chamber is evacuated and the electrodes are brought together. The stamp is kept in contact to let the Ormocomp flow and fill all the structures. Contact times between few minutes to half an hour have been tested. After that, the stamp is

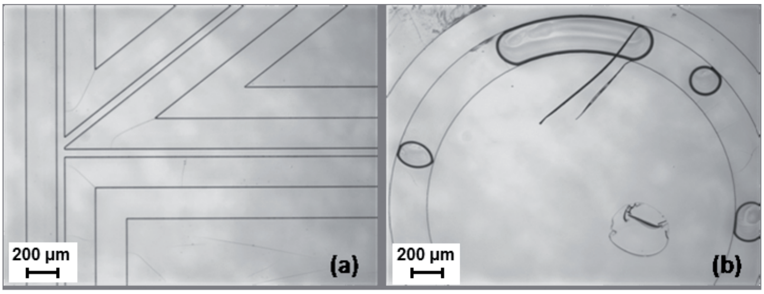


**Figure 4.7.** (a) PDMS stamped channel crossing (b) Air bubbles are easily trapped between structures

released from the upper electrode and the chamber is ventilated. The substrate-stamp stack is then moved to a mask aligner where exposure through the stamp is done.

When using a bonder machine, the use hard stamps or thick PDMS stamps is also possible, which decrease or prevents the mechanical deformation of the structures. The stamps should, however, be even in thickness, since uneven stamps results in uneven embossing. This can be a problem when thick PDMS stamp are fabricated.

In table 4.1 the embossing conditions on a hot plate and in bonder machine are compared. When using the bonder machine several different process parameters have been tested. The tested temperature range is between the room temperature and 80 °C and the embossing force range is from 150 N up to 1 kN. Even though several parameter combinations were tested, no parameters where found that could produce channels with as good profiles as was possible when embossing was done on a hot plate with PDMS stamp. The rounded corner profile seen in figure 4.8 was typical when hard stamps were used. This might be due to too short embossing time or too low embossing force. The embossing temperature was also varied to increase the flowing ability of the Ormocomp. This however let to overflow of the Ormocomp and contamination of the bondering machine. Also since SU-8/glass stamps were mostly used, the adhesion failures between the SU-8 and glass limited the lifetime of the stamps. For that reason UV-embossing on a hot plate by using PDMS or h-PDMS/PDMS composite stamp was used to fabricate all the microchips presented on the next chapter.

Use of PDMS stamp also opens up another interesting possibility; the alignment of the embossed structures to the already existing structures on the wafer. In figure 4.9, a microreactor site is aligned to a platinum heater element which is fabricated on the backside of the wafer prior to embossing. The embossing is done on a hot plate and the alignment of the structures can be done first by naked eye and then by using optical microscope to get more accurate positioning. When PDMS stamp is used the accuracy

**Table 4.1.** Process conditions for Ormocomp UV-embossing on a hot plate and in bonder machine

| | Hot plate | Bonder machine |
|---|---|---|
| Vacuum | none | $10^{-4}$ mbar |
| Embossing temperature | 80 °C | RT to 80 °C |
| Force | Manual by tweezers non-uniform | 150 N - 1 kN Stamp and substrate pressed together, uniform |
| Embossing time | about 10 min | 2 min to 30 min |
| Stamp material | thin PDMS | PDMS, hard stamps (silicon, glass) |

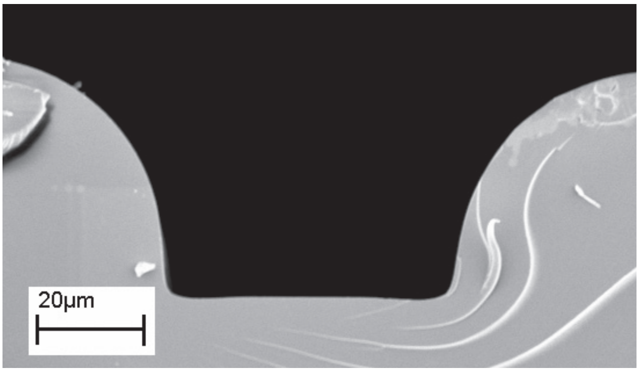


**Figure 4.8.** Profile of the channel embossed with the hard stamp

is limited since the PDMS stamp is deformed during embossing. The aligned embossing with PDMS stamp is thus possible only when the alignment accuracy is not critical and structures are relatively large.

## 4.3 Bonding of Ormocomp

Enclosed Ormocomp microchannels have been created either by using PDMS [I] or Ormocomp as a top layer [III,IV]. The Ormocomp to PDMS bonding is simple; the planar PDMS is exposed to oxygen plasma after which it is pressed in contact with Ormocomp that contains the channel structures. The inlets are punctured in the PDMS layer. However, as already discussed earlier, for analytical devices it is preferable to have all the walls of the channel made of the same material. For that reason the Ormocomp-Ormocomp bonding has been developed.

Ormocomp to Ormocomp bonding starts by spin coating Ormocomp on top of a transparency that is attached to a carrier wafer with double sided tape (figure 4.10). After 2

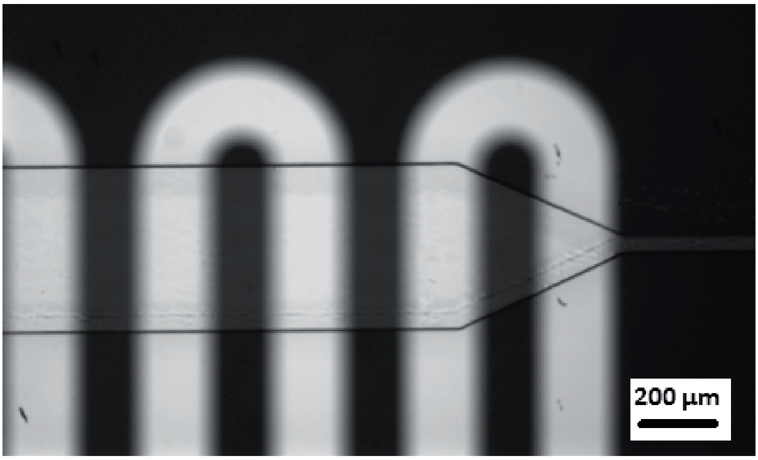


**Figure 4.9.** Channel is aligned to a platinum heater patterned on the backside of the wafer

min soft bake, the Ormocomp layer is exposed to UV-light for 15 s. During UV-exposure, the Ormocomp layer is cured, except the top few micro meters that remain uncured due to oxygen inhibition reactions. This uncured layer is used as glue in Ormocomp-Ormocomp bonding. After UV-exposure, the transparency is detached from the carrier wafer and bonding is done on a hot plate. Due to a very thin uncured layer, clogging of channels is usually not a problem. UV-exposure through the transparency is done to harden the bond, after which the transparency is easily peeled off. The bonded Ormocomp channel is presented in figure 4.11 (a).

The Ormocomp-Ormocomp bonding can be considered as adhesive bonding but since the bonding layer is only a few micrometers, the bond quality is extremely sensitive to any particle on the surfaces as can be seen in Figure 4.11 (b). To overcome this problem, the channel wafer should be carefully rinsed with isopropanol prior to bonding. If the particles are not removed they can affect the performance of the CE channels.

Another thing to consider is patterning of the bonded layer. Since the exposure is done prior to bonding the patterns should be formed at the same time. This, however, makes the bonding itself more problematic since aligned bonding would be needed. This type of bonding requires the use of special aligning and bonding tools. If the structures are not needed, the bonding is quite straightforward.

One more thing to consider is the curing of the roof of the channel. Since Ormocomp-Ormocomp bonding is based on the oxygen inhibition reaction, the question arises whether the area facing the channel is really cured after bonding is completed. Although this question remains unanswered, it is shown that even if the thin layer on the roof of the channel is uncured, it does not cause any problems when testing the channels. The chan-

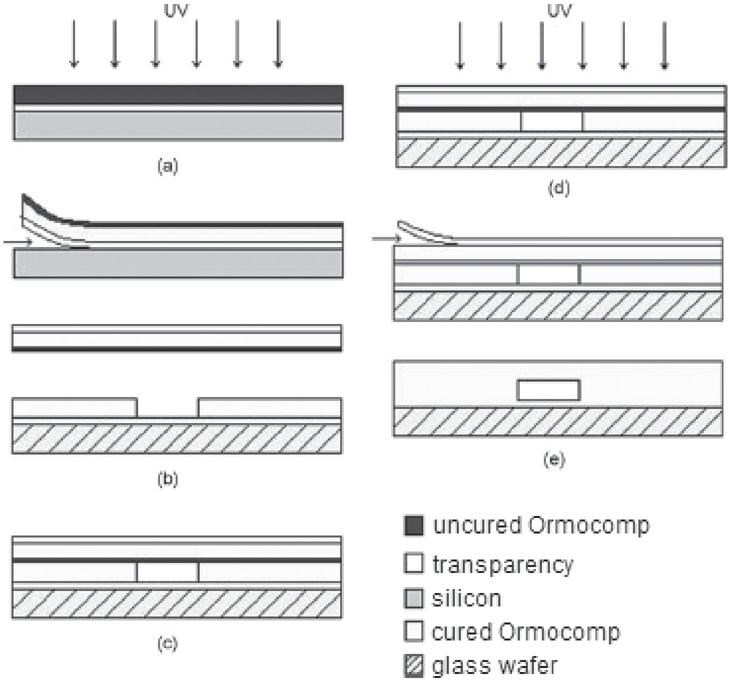


**Figure 4.10.** Schematics of the Ormocomp-Ormocomp bonding (i) Ormocomp is spun on transparency and flood exposured (ii) Transparency is detached from the carrier wafer and brought together (iii) The bonding is done on a hotplate by pressing with the tweezers (iv) UV-exposure through the transparency is done to finalize the bonding (v) The transparency is peeled off and the bonding is completed

nels are rinsed prior to experiments which washes away any possible uncured Ormocomp left in the channels.

## 4.4 Pore formation in Ormocomp

The possibility to introduce pores to the material surface opens up several interesting application opportunities where high surface areas are needed. Such applications include biosensors [149] and drug delivery [150] and separation devices [151]. Mostly used porous materials are porous silicon [152] and anodic alumina [153] which are formed by electrochemical etching. Porous polymer monoliths have also been integrated into microfluidic channels [154].

Porous Ormocomp, on the other hand, can be formed by simply exposing Ormocomp to oxygen plasma [155],[V]. The pores are formed since oxygen plasma preferentially etches the organic parts of the Ormocomp, leaving inorganic backbone behind. By changing the

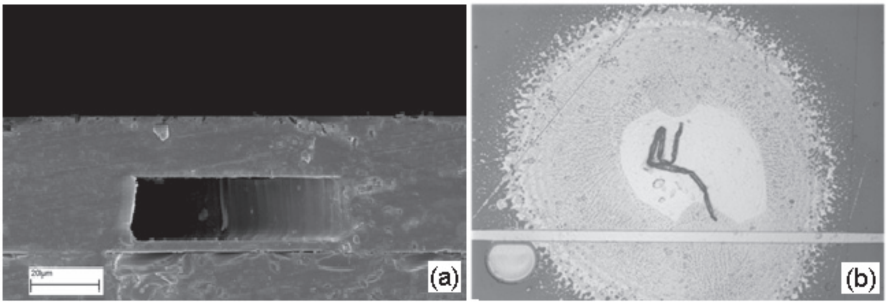


**Figure 4.11.** (a) Bonded Ormocomp-Ormocomp channel (b) Particles can cause defects on bonding

plasma parameters, different type of pores can be formed (figure 4.12). In our experiments, the variable parameters were plasma power and time. Other parameters were chamber pressure and oxygen and argon flow rates, which were kept constant at 250 mTorr, 45 sccm and 5 sccm, respectively (see table 4.2).

**Table 4.2.** Process conditions for porous Ormocomp formation

| | power [W] | time [min] | Pressure [mTorr] | Oxygen flow [sccm] | Argon flow [sccm] |
|---|---|---|---|---|---|
| Sample a | 100 | 1 | 250 | 45 | 5 |
| Sample b | 100 | 10 | 250 | 45 | 5 |
| Sample c | 200 | 1 | 250 | 45 | 5 |
| Sample d | 200 | 10 | 250 | 45 | 5 |

The characterization of porous Ormocomp has been done by using x-ray photoelectron spectroscopy (XPS), Rutherford backscattering spectrometry (RBS) and time-of-flight elastic recoil detection analysis (TOF-ERDA) [V]. In table 4.3 the XPS measurements for untreated and oxygen plasma treated sample are presented. Both of the samples contain carbon, silicon and oxygen, but the carbon to silicon ratio is significantly lower in oxygen plasma treated sample. This is a good indication that carbon is depleted in the process.

**Table 4.3.** Elemental composition as obtained by XPS before and after plasma treatment

| | Carbon (%) | Silicon (%) | Oxygen (%) | C:Si (%) |
|---|---|---|---|---|
| Untreated | 69 | 5 | 26 | 14.7 |
| Plasma treated (200 W, 10 min) | 10 | 32 | 58 | 0.3 |

In figure 4.13, the RBS measurements for planar and porous Ormocomp samples are presented. All the samples were coated with 6 nm thick TaN layer by using atomic layer deposition technique. Tantalum peaks are prominently resolved and the expected silicon,

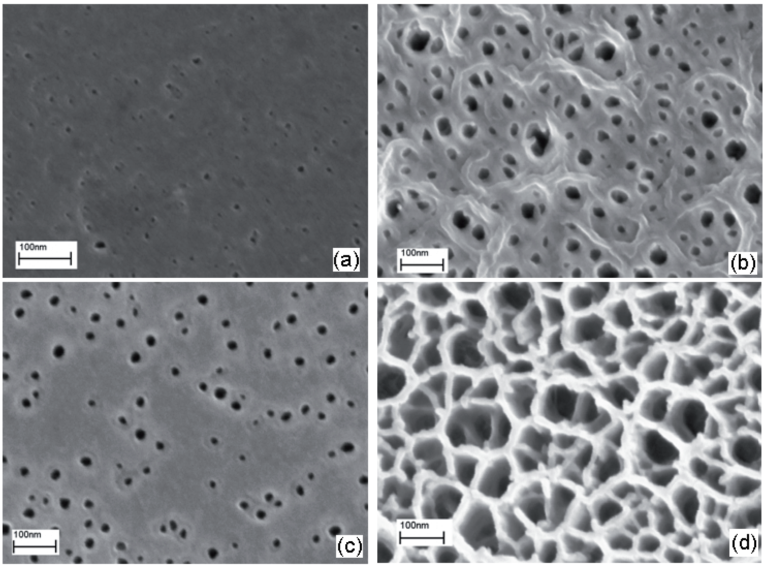


**Figure 4.12.** Porous Ormocomp by using different plasma parameters (a) 100 W, 1 min (b) 100 W, 10 min (c) 200 W, 1 min (d) 200 W, 10 min

oxygen and carbon peaks are also found. From the spectra, it can be calculated that the surface area of the tantalum peak is 9.8, 5.6, 2.8 and 1.2 times larger for porous samples a to b (see table 4.2), respectively, than for the planar sample. This is in good correlation when compared with the SEM images shown in figure 4.12.

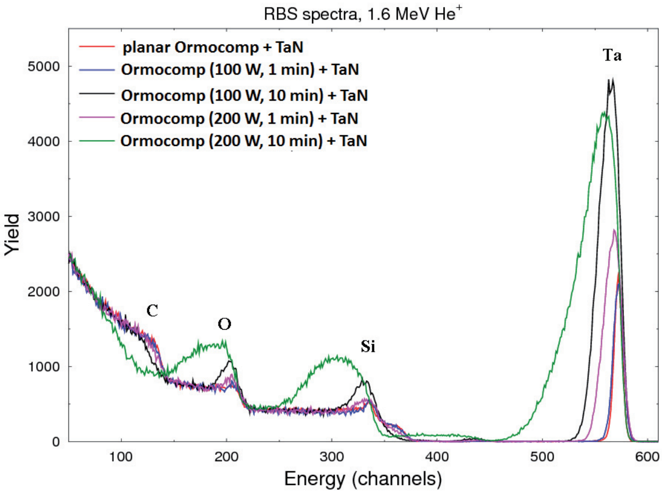


**Figure 4.13.** RBS measurements on planar and porous Ormocomp samples with thin TaN layer coated by atomic layer deposition (ALD)

One of the advantages of porous Ormocomp formation process is that pores can be also introduced on the selected areas. This can be done either by using shadow mask or metallic hard mask, such as aluminum. Shadow masks are metallic plates that contain through holes and can be directly placed on top of the substrate to be patterned. In figure 4.14 (a) the porous and non porous areas done by using shadow mask is presented. As can be seen the edges of the lines are blurred which is due to oxygen plasma penetration under the shadow mask. In figure 4.14 (b) the square patterns done by using aluminum as a mask can be seen. The squares are well defined although some rounding of the edges can be seen. Also in this case oxygen plasma penetrates under the mask since the size of the squares and gaps between them should be similar in size. By using aluminum as a mask, relatively well defined porous and non-porous areas can however be produced. These porous and non-porous areas have been used for self-alignment of microchip by using adhesive droplets [156].

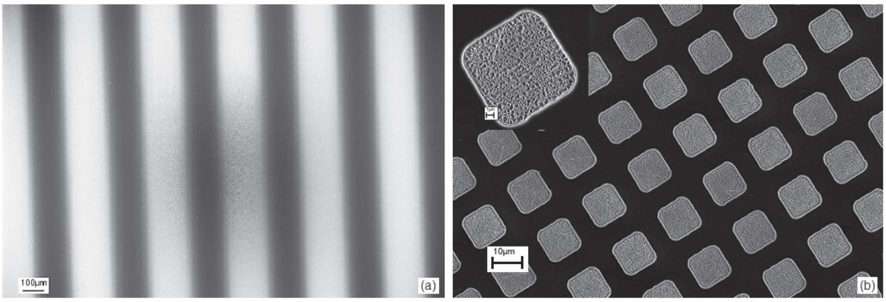


**Figure 4.14.** Patterned porous/non-porous areas (a) by using shadow mask (b) by using aluminum as a mask

Another interesting possibility is to form pores on already existing structures like pillars. In figure 4.15 (a) and (b) a porous Ormocomp micropillar is presented. As can be seen on the pictures, the pores are evenly distributed on top and on the sidewalls of the micropillars. With this method, a surface to volume ratio can be drastically increased and pillars could be used as a stationary phase in liquid chromatography as has been done with porous silicon pillars [151]. In figure 4.15 (c) a porous Ormocomp nanopillars are presented. These pillars have been used as a surface assisted laser desorption ionization (SALDI) plates [II], [157].

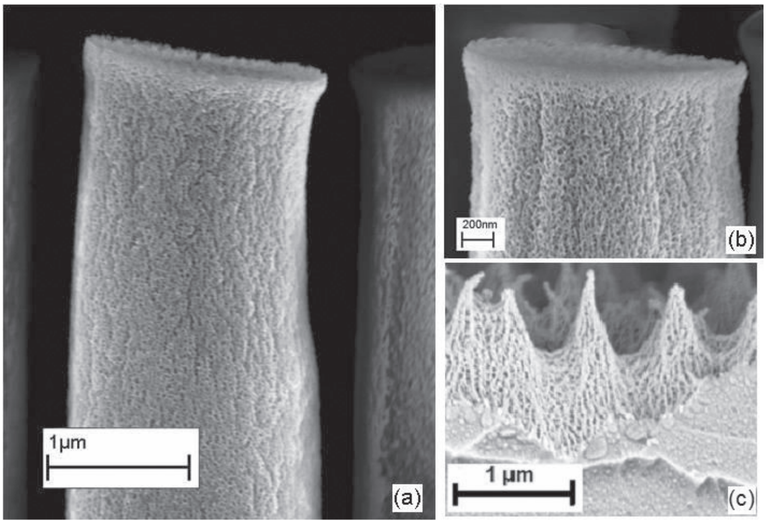


**Figure 4.15.** (a) Porous Ormocomp pillar (pillar distorted due to charging) (b) Top of the porous pillar (c) Side of the porous pillar

# 5. Microfluidic applications for Ormocomp

There is wide variety of different types of ORMOCER materials available due to flexibility in the formation process of the material. For that reason the application range is also relatively wide, such as abrasion resistant coatings, filling material in dentistry and optical devices. This chapter will however, concentrate on the application of Ormocomp in different types of microfluidic applications, mainly capillary electrophoresis, electrospray ionization and as substrate plates for surface assisted laser desorption ionization mass spectrometry.

## 5.1 Microchip capillary electrophoresis

Capillary electrophoresis is one of the most popular separation technique for miniaturization [158]. In capillary electrophoresis, voltage is applied across the capillary that contains a conductive solution (referred to as buffer or background electrolyte (BGE)). When voltage is applied, the solution starts to move toward the cathode, giving rise to electro osmotic flow (EOF). The electro osmotic flow moves the analytes inside the capillary and the analytes are separated based on their size and net charge. The electro osmotic flow has plug-like flow profile. This is the reason why better resolving power (higher number of theoretical separation plates) can be achieved with CE than with pressure driven chromatographic separations which show parabolic flow profile.

The use of electro osmotic flow as a mean of fluidic actuation on a microchip sets some requirements for the microchip material. Because high voltages are used, the material has to be electrically insulating. Other general requirements are compatibility with the buffer solutions, established microfabrication methods and good optical transparency if UV- or fluorescence detection is to be used. The main requirements (and probably the most difficult to meet) are that material should support a stable electro osmotic flow and that adsorption of the target analytes to the channel surface is negligible.

Microchip capillary electrophoresis has been used for separation of amino acids, peptides and proteins, as well as DNA, drugs and ions [158–160].

### 5.1.1 Fluidic design

In figure 5.1 (a) a typical layout for microchip capillary electrophoresis channel design is presented. The chip consist of a separation channel and four inlets; one for sample introduction, one for separation buffer and two for waste. The channels are typically 15 - 50 $\mu$m in depth and 30-100 $\mu$m wide. The length of the separation channels should be as long as possible for efficient separations but is usually limited to few centimeters due to space limitation if straight channels are wanted. By using a serpentine geometry, channels over 10 cm in length have been produced [8]. The problem with introduction of turns is however that the migration path at the inner and outer perimeter of the turn are different, resulting in sample dispersion. The turn-induced band broadening can be reduced by narrowing the separation channel width before the start of the turn and widening it back after the turn [161] (see figures 5.1 (d) and 5.1 (e)).

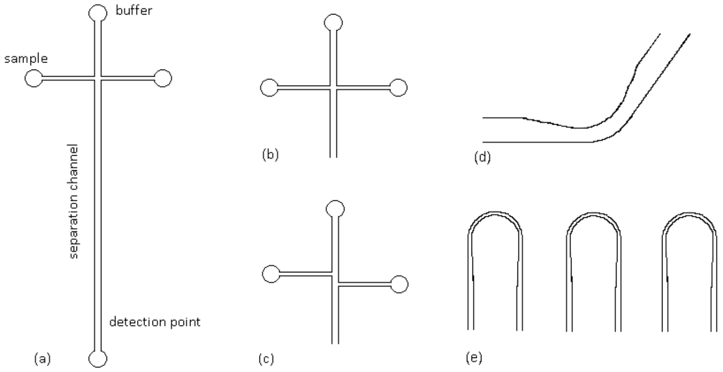


**Figure 5.1.** (a) Typical microchip capillary electrophoresis design, channels typically 15 to 50 $\mu$m deep and 30 to 100 $\mu$m wide (b) simple injection cross (c) double-T injection cross (d) tapering of the channel turn (used in [IV](e) tapering of the meandering channels

### 5.1.2 Effect of surface properties

The surface properties play a significant role in capillary electrophoresis separations. The electro osmotic flow is induced by the movement of counterions in an electric field. For example, if channel surface is negatively charged, positively charged cations induce the EOF. The electro osmotic mobility inside the microchannel depends on the surface charge of the material at given pH. In table 5.1, the electro osmotic mobilities for Ormocomp/PDMS, glass and SU-8 microchannels are given at different pH values. The EOF rate is important since too rapid flow rate may result in elution of analytes before separation has occurred. At low flow rates, the hydrodynamic pressure affects the flow, particularly if the fluidic resistance of the microchannel is low. Also with low flow rates, the adsorption of positively charged species to negatively charged channel wall through Coulombic interactions is more pronounced. As can be seen in table 5.1, the EO mobilities of Ormocer/PDMS microchannels are similar to those of glass and SU-8, although in the case of SU-8, the effect of low pH is more pronounced since the direction of EOF changes at low pH [162]. The EO mobilities in Ormocer/PDMS channels reached values up to 5 x $10^{-4}$ cm$^2$ V$^{-1}$s$^{-1}$ which is higher than generally reported to fully PDMS microchannels (typically 1 x $10^{-4}$ cm$^2$ V$^{-1}$s$^{-1}$) [64].

**Table 5.1.** Comparison of electro-osmotic mobilities (x$10^{-4}cm^2V^{-1}s^{-1}$) between Ormocomp/PDMS, glass and SU-8 channels

| Buffer solution (20 mM) (pH) | Ormocomp/PDMS [I] | Glass [I] | SU-8 [162] |
|---|---|---|---|
| Phosphate (2) | 0.86±0.04 | 2.36±0.08 | -1.10±0.22 |
| Citrate (3) | 0.91±0.05 | 2.71±0.10 | negligible |
| Ammonium formate (4) | 0.94±0.02 | 0.95±0.07 | negligible |
| Ammonium acetate (5) | 2.41±0.03 | 1.61±0.03 | 2.98±0.04 |
| Sodium citrate (6) | 2.18±0.04 | 3.79±0.14 | 3.11±0.56 |
| Sodium phosphate (7) | 4.73±0.15 | 4.86±0.06 | 3.08±0.31 |
| Tris-borate (8) | 5.26±0.03 | 5.59±0.15 | 4.57±0.15 |
| Sodium borate (10) | 4.37±0.51 | 3.82±0.14 | 4.17±0.14 |
| Sodium carbonate (11) | 3.19±0.04 | 5.40±0.06 | 4.36±0.13 |
| Sodium phosphate (12) | 3.41±0.02 | 4.88±0.03 | 3.78±0.10 |

In addition to electro osmotic mobility, the adsorption of analytes, or more precisely the material ability to inhibit analyte adsorption is an important material property in separation devices. Analyte adsorption will lead to loss of sample and/or poor performance. In figure 5.2 (a), the protein (bovine serum albumin, BSA) adsorption measurements for PDMS, glass and Ormocomp are presented. BSA is a high molecular weight (66kDa) protein which is known to easily adhere to most plastic surfaces. The measurements are done by using a fully automated tensiometer based on Wilhelmy plate method in which the material is dipped in to a concentrated protein solution [I]. The dynamic contact angle is monitored during the dip cycles and the reduction in hysteresis during repeated cycles can be attributed to protein adsorption [163]. The greater the change in hysteresis after multiple cycles, the greater the extend of protein adsorption on the surface. From figure 5.2 (a), it can be concluded that the protein adsorption on Ormocomp surface is similar to that of glass and significantly lower than that of PDMS surface. This is reasonable since protein adsorption on PDMS surface is a known problem [164]. The low protein adsorption to Ormocomp surface has also been verified in context of microchip capillary electrophoresis [III, IV]. In figure 5.2 (b), the MCE analyses of FITC-labeled BSA (2.5 mg/mL) in 18 mM sodium borate (pH 10) using Ormocomp and glass microchips are presented. The peak profile (width and symmetry) are perfectly comparable between repeated runs, suggesting negligible interaction between proteins and Ormocomp surface.

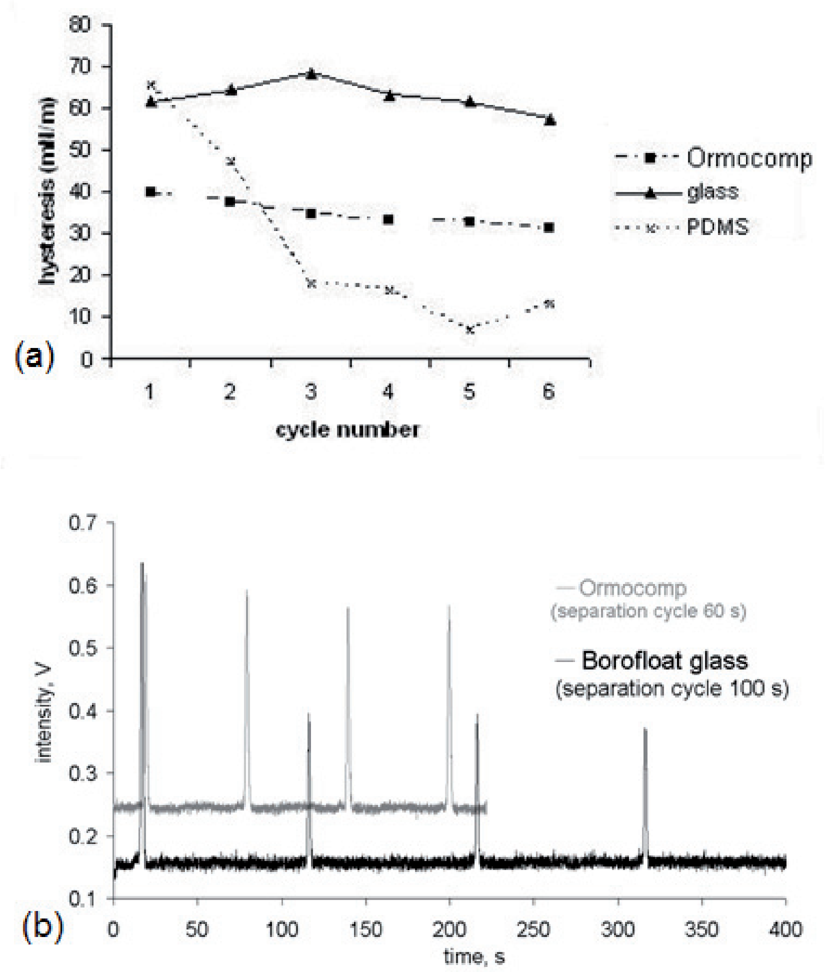


**Figure 5.2.** (a) Protein (BSA) adsorption measurements for PDMS, glass and Ormocomp (b) Four repeated FITC-labeled BSA injections in native (=no added surfactants) Ormocomp (peak width 3.8 s) and glass (peak width 3.4 s) [I]

### 5.1.3 Separation of amino acids, peptides and protein digests by microchip capillary electrophoresis

Amino acids are the basic building blocks of peptides and proteins, making their separation and detection important in clinical diagnostics and life sciences. Amino acid separations on microchip have therefore been extensively demonstrated [7, 165, 166][III]. In glass and many of the plastic microchips the separation of the amino acids mixture have been performed without any surface coating [7], but in many other cases coating of the channels is required for successful separation. Nonionic surfactant (Tween 20) as dynamic coating in PDMS channel has been presented for electrophoresis of amino acids [166]. With these channels the separation of four amino acids was possible by improving the stability of EOF and by reducing the amino acid adsorption to the PDMS surface. By using micellar electrokinetic chromatography (MEKC), the separation of 19 amino acids has been demonstrated on a microchip [167].

We have presented amino acid separation in Ormocomp/Ormocomp microchannels [III]. The separation of fourteen FITC-labeled L-amino acid on Ormocomp/Ormocomp microchannels is presented in figure 5.3. No coating was needed to perform the separation.

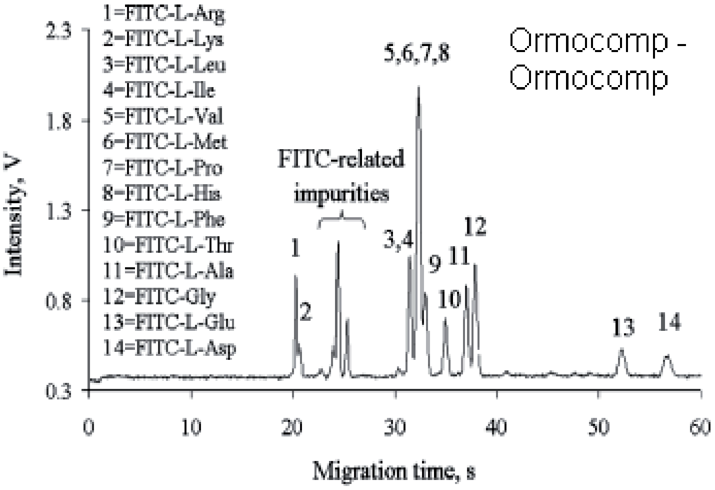


**Figure 5.3.** MCE separation of FITC-labeled L-amino acids (all 50 $\mu$M) in 36 mM sodium borate (pH 10) at 720 Vcm$^{-1}$ by using Ormocomp-Ormocomp microchannel. The distance of detection was 4.5 cm. [III]

The peptide and protein digestion separations on microchips have also been widely presented. Separation of peptides and especially the separation of protein digests are important for protein identifications. Typically the protein is digested via enzymatic digestion and chromatographic or electrophoretic separation of the resulting fragments is done. The protein digestion have been done off-chip [168] and on-chip [169].

In our own studies both Ormocomp/PDMS, glass and Ormocomp/Ormo-comp microchannels have been tested for peptide separations. When PDMS was used as a cover in CE channels, the separation peaks were wider than in the case of Ormocomp or glass microchips. Especially in the case of substance-P the difference between the Ormocomp/PDMS and glass channels is easily seen by looking at the figure 5.4. This is due to a long stretch of hydrophobic amino acids in the middle of the substance-P amino acid sequence. The same behavior was observed in other study [170], where the concentration of the substance-P was increased so much that the surface of the PDMS was overloaded by it, and only after that the analyte could be detected. The analyte was, however, slowly leaching from the channel surface which could be seen as a drifting baseline. Also when separation of peptides in Ormocomp/PDMS and glass CE devices (figure 5.4) are compared, one can see that the migration order of the peptides is different because of the interactions between the substance-P and PDMS surface.

The peptide separation in Ormocomp/Ormocomp microchannel is presented in figure 5.5. Fast (< 60 s) and highly efficient peptide separation is demonstrated with record breaking plate numbers 2.6-3.2 x $10^4$ plates per separation length of 4 cm, corresponding to 6.5 - 8.0 x $10^5$ plates per meter. This is generally better than those previously reported for microchips, for example 1.3-1.8 x $10^5$ for SU-8 [162] and 3.3 x $10^4$ for polyacrylamide coated PDMS chips [171]. The direct comparison is, however, not possible due to differences in the experimental conditions and fluorescent labels.

### 5.1.4 Protein separations by microchip capillary electrophoresis

As has already been shown in the previous paragraphs, the analyte adsorption on the surface of the microchip channel is a major drawback of microchip CE in many plastic channels. This is most pronounced in separation of proteins. The adsorption of analytes can lead to local variations in EOF, the loss of resolving power and poor reproducibility. Recent developments in protein separation on microchip electrophoresis are reviewed by Tran et. al. [172].

Due to analyte adsorption, the separation of proteins is usually facilitated by coating the surface of the microchannel. In [173] it was reported that without coating of the glass channels, a broad tailing peptide peaks were seen while no protein peaks were detected due to significant adsorption on the channel wall. By coating the channels either with linear polyacrylamide or polyvinyl alcohol (PVA), mixture of four proteins could be separated and detected. Several other polymer microchips have also been used for protein separations. In terms of fabrication, PDMS microchips are most attractive but the use

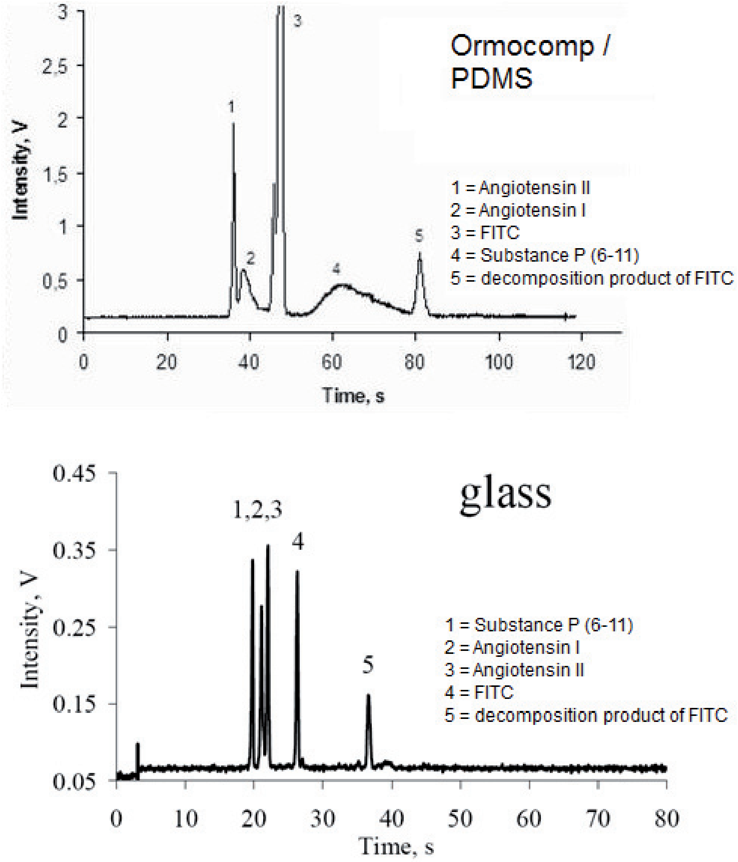


**Figure 5.4.** MCE separation of FITC-labeled substance-P (6-11), angiotensin I and angiotensin II (all 100 $\mu$M) in 20 mM sodium borate (pH 10) at 700 Vcm$^{-1}$. The distance of detection was 7 cm. [I]

of some coating, like polyacrylamide [171], is required for successful protein separations. PMMA [174] and SU-8 [162] microchips have been used for protein separations by applying a dynamic coating. The dynamic coatings can be used by simply adding a suitable surface active ingredient to the buffer solution. Since coating of the microfluidic channel is almost always necessary for effective protein separations, several review articles on different channel coating methods have been published [175, 176].

In our own studies, we have used Ormocomp microchips for intact protein separations without any surface coating [IV]. The Ormocomp microchannels show native resistance to analyte adsorption, making Ormocomp highly suitable material for analytical microchips (see figure 5.2).

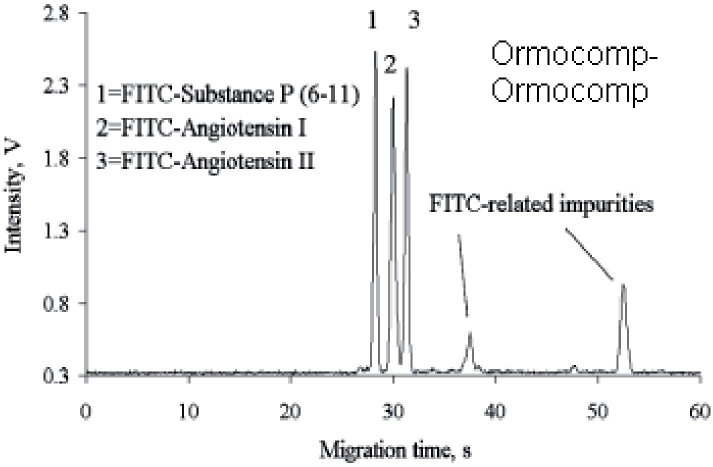


**Figure 5.5.** MCE separation of FITC-labeled substance-P (6-11), angiotensin I and angiotensin II (all 200 $\mu$M) in 18 mM sodium borate (pH 10) at 300 $\mathrm{Vcm}^{-1}$ by using Ormocomp-Ormocomp microchannel. The distance of detection was 4 cm. [III]

### 5.1.5 Detection methods

Different detection methods, used with microchip capillary electrophoresis, include UV/Vis [177], fluorescence [I], laser-induced fluorescence (LIF) [III], mass spectrometry [178][IV] and electrochemical detection [179]. The detection method for microfabricated separation devices has to be sensitive due to the extremely small injection volumes. Although, the UV/Vis detection is most popular one in conventional chromatographic and electrophoretic separation systems, it is still not widely used in microchip applications because of the sensitivity and reliability problems caused by the small size of the channels [177]. Fluorescence detection has been the most popular one due to its selectivity and sensitivity, laser induced fluorescence being the most easily adapted to the dimensions of microchips. The large size of the detection system limits the benefits gained from the microchip miniaturization. Electrochemical methods can be used to address this issue. In electrochemical detection, electrodes are microfabricated on the chip [180].

Classical fluorescence detection requires the labeling of the analyte with some fluorescent molecule, like dyes. Although it is relatively straightforward process, it still adds an additional step to analysis and may even be unwanted, e.g. for studies of proteins in their native state. The more attractive alternative is to use native fluorescence of molecules but it requires the use of deep-UV excitation which sets some addition requirements for the optical transparency of the microchip material (see figure 5.6). Also only some of the analytes, like pharmaceuticals, exhibit native fluorescence when excited between 200 and

400 nm.

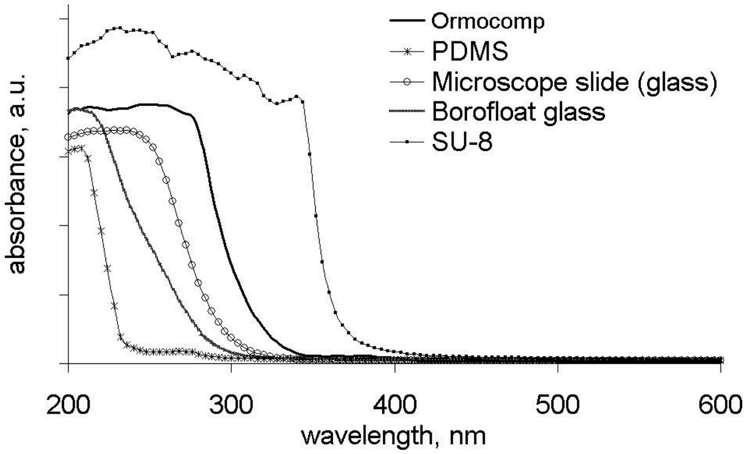


**Figure 5.6.** UV-Vis absorbance spectra for different chip materials [I]

Mass spectrometry (MS) is a popular detection method due to its high sensitivity and specificity. Integration of microfluidic separation device into a MS offers several advantages over conventional methods, such as low analyte and reagent consumption and possibility for high throughput system. For microdevice integration to MS, electrospray ionization (ESI) offers the best ionization method due to its atmospheric operation conditions and similar liquid flow rates compared to microfluidic systems.

## 5.2 Integrated capillary electrophoresis electrospray ionization

Several different methods have been tested for introduction of the sample from capillary electrophoresis to mass spectrometry. One of the simplest methods is to spray the sample directly from the edge of the microchip [181] or at the corner [182]. Droplet formation can however be a problem and to overcome the problem a capillary tube can be inserted at the end of the separation channel [183]. This usually requires gluing of the capillary which can cause sample contamination or clogging of the tip. The most sophisticated method is to integrate the electrospray tip to the chip already at the fabrication stage [81] but this requires some additional effort from fabrication. There are several review articles available on microdevices coupled to mass spectrometry [13] and especially to electrospray ionization mass spectrometry [178, 184].

Our own Ormocomp/Ormocomp microchip CE-ESI is presented in figure 5.7. The design of the chip is similar to that presented by Mellors et.al. [182]. The chip is fabricated on top of a silicon wafer by using embossing and bonding techniques presented in previous chapter. The electrospray tip of the chip is defined by sawing and the chips are then mechanically released from the silicon substrate. The chips have to be released since silicon is not a suitable substrate for electrically driven applications but even if glass was used as a substrate the released chips are still favored. This is because the glass substrate will increase the total thickness of the chip and the three dimensional shape of the tip is lost.

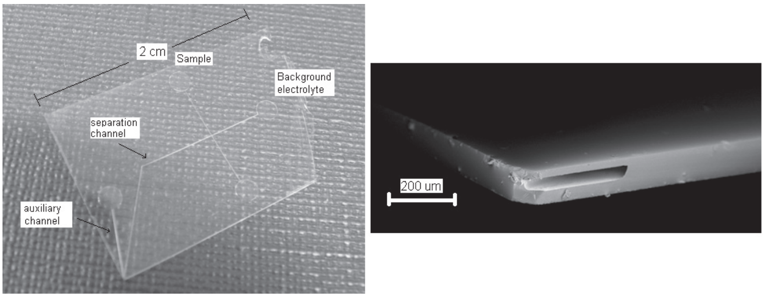


**Figure 5.7.** Ormocomp capillary electrophoresis electrospray ionization chip [IV]

There are some review articles available also for the integration of capillary electrophoresis and electrospray ionization for the analysis of intact proteins [185] but intact protein separations on integrated microchip CE-ESI is still quite limited [173, 182][IV]. In figure 5.8, the CE/ESI-MS analysis of five protein standards with different size (MW), charge (pI) and hydrophobicity is presented. Since cytochrome C and ubiquitin are hydrophilic proteins, a minor peak tailing is considered to originate from the presence of both native and denaturated protein isoforms, which slightly separate from each other in the analysis.

## 5.3 Surface assisted laser desorption ionization substrate

Surface assisted laser desorption ionization (SALDI) is an ionization technique where the analyte is directly ionized from the surface upon exposure to laser light [186]. It is similar to the much better known matrix assisted laser desorption ionization (MALDI).

In MALDI, the analyte is mixed with the matrix and they are then co-crystallized together on the surface. MALDI has been a popular ionization method due to its high sensitivity, tolerance to salts, applicability to complex mixtures and throughput [187]. There are, however, some problems associated in the use of the matrix. The selection of suitable matrix is often difficult and the trial-error method is often necessary. The

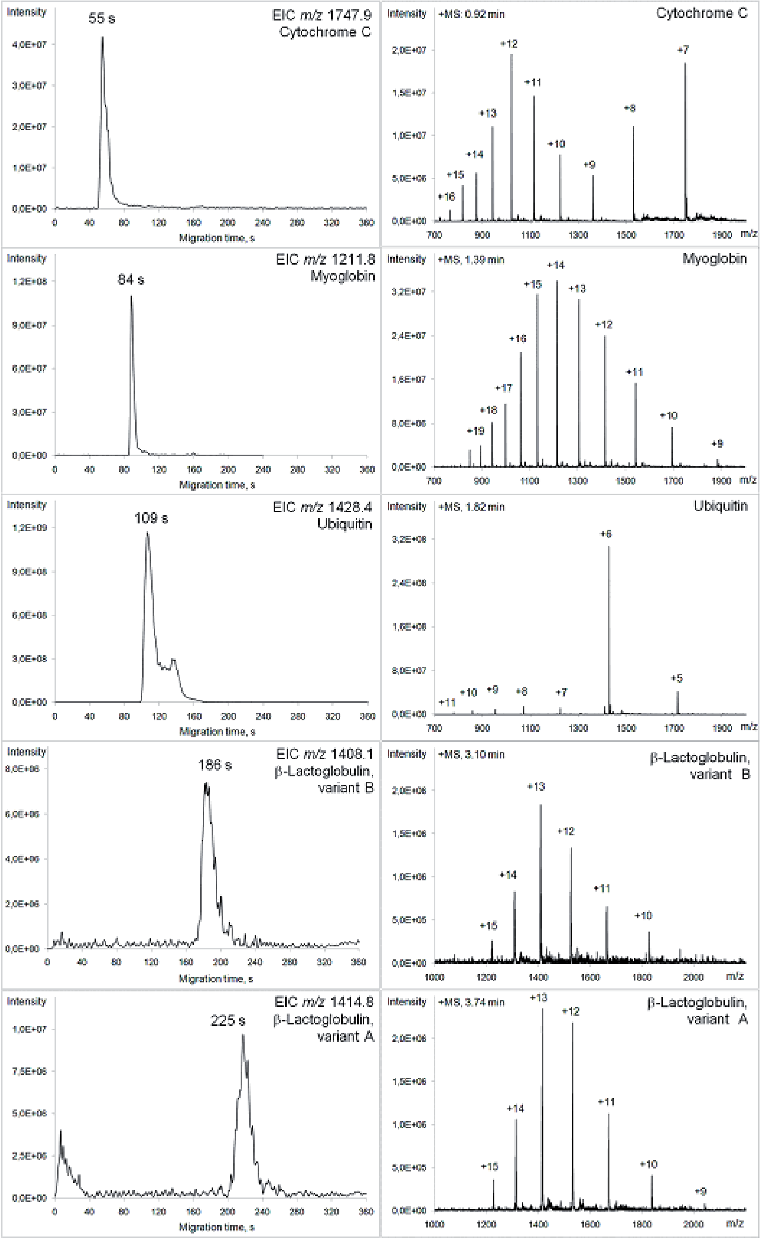


**Figure 5.8.** CE/ESI-MS analysis of cytochrome C, myoglobin and ubiquitin (each 5$\mu$g/$\mu$L), and $\beta$-lactoglodulin variants A and B (total of 5 $\mu$g/$\mu$L) [IV]

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co-crystallization is often non-uniform producing so called hot spots; areas where the concentration of analytes is higher than would be in uniform sample. The analysis of low molecular weight analytes (m/z < 500) is difficult since the matrix interferes with the analysis.

All the above mentioned problems have led to development of matrix free methods, often called SALDI. One of the most studied SALDI method is the desorption/ionization on silicon (DIOS) [188]. In this method porous silicon is used as a substrate where the analyte is directly deposited. Although the method is often called matrix-free due to a lack of any chemical matrix, the porous silicon can actually work as a matrix for desorption/ionization. Other substrates than silicon can also show SALDI activity. The porous structures of alumina and polyethylene promoted ionization of angiotensin and insulin molecules when the surface was coated with metals [187].

In our own studies, we have tested the suitability of porous Ormocomp as SALDI substrate [II], [157]. Planar porous Ormocomp, nanostuctured porous Ormocomp as well as micropillar porous Ormocomp substrates were tested. All the samples were coated with 80 nm of amorphous silicon. Amorphous silicon was found to produce best quality analyte signal among the tested coatings, which good be because it combines the favorable surface chemistry to sufficient UV-absoptivity [157]. The planar porous and nanostructured porous Ormocomp gave a strong signal when the sample with 10 pmol of des-arg-bradykinin was analyzed. This is in good agreement with the previous studies where submicrometer structures and roughness were found to be essential for efficient ionization [187, 189]. The substrate with micropillars was significantly worse than the other two, which could be due to an uneven distribution of the analyte on the bottom, wall and top parts of the micropillars. Uneven distribution of the laser light due to a shadowing of the micropillars might also explain the results.

In figure 5.9 a mass spectra obtained by SALDI-MS (amorphous silicon coated porous planar Ormocomp) and MALDI-MS are presented. Mass spectra are obtained from the mixture of five different peptides.

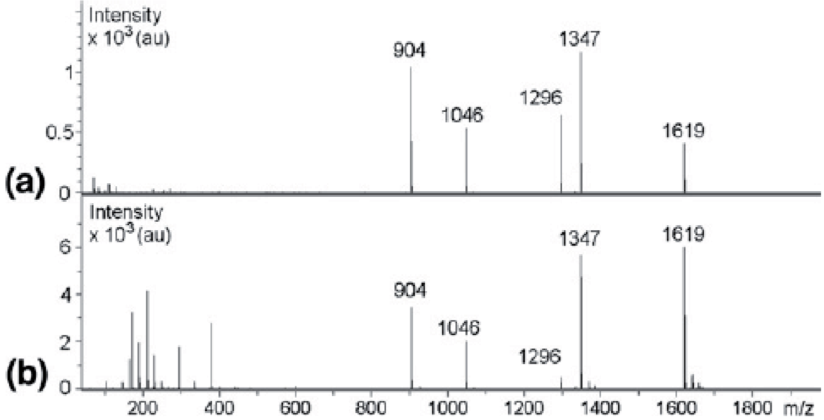

**Figure 5.9.** Peptide mass spectra obtained by (a) SALDI-MS and (b) MALDI-MS. Note the lack of background in SALDI.

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# 6. Conclusions and outlook

Since 1990s the research in the field of microfluidics has grown tremendously. The science community has waited for some killer application that would really bring microfluidics as a part of our everyday life. Although there are some products on the market, this killer application is yet to be found. There are several reasons for this and one of them is the extremely challenging working environment. From the beginning, one of the application fields has been the miniaturization of analytical devices, but to really get these devices into a laboratory use would require validation of material and fabrication methods, calibration, quality control and many other regulatory procedures. Probably even more complex field is the diagnostic or point-of-care devices that are other major application area for microfluidic devices.

Another challenge is the connection of the microchip in to a macroworld. Even though the small size improves microchip performance, the connection of fluidic and gas tubes can be problematic. The usability of the microchips has generally not been very well addressed in the scientific publications.

One further challenge, that has been addressed also in this thesis, is the selection of material for microchip. Even though silicon and glass are suitable for many applications, the use of some polymeric material is nowadays often preferred. Although polymer substrates can be as expensive as silicon or glass, the real saving comes from the fabrication costs. Polymers can often be processed without the need of many expensive clean room equipment and their fabrication methods are suitable for mass production.

There are many polymers available but only a few of them have really been characterized for use in microfluidic devices. When new material is being evaluated for microfluidic devices, often new microfabrication methods or at least optimization of the existing ones have to be made. This is one reason why new materials are not easily taken into use.

In this thesis it was shown that a new type of inorganic-organic polymer, Ormocomp, could fullfil many of the requirements for microfluidics. Micro and nanofabrication strategies were developed for fluidic applications. Ormocomp-Ormocomp bonding method that utilizes the thin uncured layer typical for acrylate resins, is something that has not been presented previously. The developed embossing methods enable the micro and nano patterning of Ormocomp. The easy pore formation process by oxygen plasma is something that has not been shown for any other polymer, and could open up new application possibilities.

The fabrication of microchip capillary electrophoresis, microchip capillary electrophoresis with integrated electrospray ionization and surface assisted laser desorption ionization plate were demonstrated. Ormocomp showed superior performance compared to PDMS in microchip capillary electrophoresis separations. The unique property of Ormocomp to resist biofouling makes it highly suitable for separation devices; even separation of intact proteins was demonstrated.

Although many things have been solved in this thesis, there are still areas that would need more attention. The fabrication of the micro- and nanostructures by using embossing equipment would be one step closer to real mass production. Also further study of the pore formation process and the integration of porous structures to fluidic devices is something that could open up new application possibilities.

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# Errata

### Publication II

Caption for Fig. 6 should be: Fig 6. Droplet on (a) non-treated (b) oxygen plasma treated (c) teflon coated hybrid nanopillar surfaces. Contact angles 120 °, < 5 ° and 160 °, respectively

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