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FABRICATION OF SU-8 MICROSTRUCTURES FOR ANALYTICAL MICROFLUIDIC APPLICATIONS

Doctoral Dissertation

Santeri Tuomikoski

Helsinki University of Technology
Department of Electrical and Communications Engineering
Micro and Nanosciences Laboratory

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Dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the Department of Electrical and Communications Engineering for public examination and debate in Large Seminar Hall of Micronova at Helsinki University of Technology (Espoo, Finland) on the 2nd of February, 2007, at 12 noon.

Helsinki University of Technology
Department of Electrical and Communications Engineering
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Name of the dissertation

Fabrication of SU-8 microstructures for analytical microfluidic applications

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Opponent	Fredrik Nikolajeff
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(Instructor)	

Abstract

Miniaturization of analytical devices has been an ongoing trend to improve performance of analytical tools. These systems have been microfabricated originally of silicon and glass, but polymers have become increasingly popular as alternative materials. Polymers are mostly used because the material costs are lower and fabrication processes are easier. However, those facts depend heavily on the fabrication method and particular polymer. In this thesis the usability of epoxy-polymer SU-8 has been studied for analytical microfluidic applications. Lithographically defined SU-8 can provide simpler fabrication processes as comparison to silicon and glass fabrication. In this thesis processes for microdevice fabrication of SU-8 are studied and developed. For many devices the proper microchannel enclosure method becomes the most critical fabrication step. Therefore adhesive bonding using SU-8 has been studied extensively.

A widely applicable microchannel fabrication process has been developed as a combination of lithographic patterning of SU-8 and ultraviolet-cured adhesive bonding. This process enables high yield of microfluidic devices with wide range of channel dimensions both laterally and cross-sectionally. Furthermore, multilevel structures are possible, inlet fabrication is easy and alignment of inlets and other structures like electrodes can be done fully lithographically. Hence the SU-8 fabrication process avoids many limitations of the earlier fluidic chip fabrication processes.

SU-8 microchannels have been used for electrophoretic applications for the very first time. Electroosmotic flow (EOF) mobility was measured in the channels and also first electrophoretic separations were made. SU-8 promises good properties for such applications, because the EOF mobility was higher than with most other untreated polymers. Solid phase extraction was integrated to electrophoresis chips to enable sample purification or concentration before the electrophoretic separation. This was realized by applying high aspect ratio pillars and multilevel SU-8 structures. Detection from the chip can be done by fluorescence detection through the SU-8 cover. To improve detection sensitivity mass spectrometric detection was also studied from the chips using electrospray ionization (ESI) mass spectrometry. SU-8 provided good material properties for the ESI-analysis. Furthermore, SU-8 enables accurate tip fabrication that is difficult with most other microfabrication methods.

Keywords SU-8, Microfluidics, Microfabrication, Adhesive bonding, Mass spectrometry, Electrophoresis

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Tekijä Santeri Tuomikoski

Väitöskirjan nimi

Mikrorakenteiden valmistaminen SU-8 polymeeristä analyyttisiin mikrofluidistisiin sovelluksiin

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Työn valvoja Sami Franssila
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Tiivistelmä

Analyyttisten mikrofluidististen laitteistojen kehitys alkoi 1990-luvun alussa tavoitteena parantaa kemiallisten analyysien tehokkuutta. Laitteistojen miniatyrisointi on perinteisesti toteutettu piin ja lasin mikrovalmistusmenetelmillä. Kuitenkin polymeeriset materiaalit ovat syrjäyttäneet viime aikoina piitä ja lasia monissa sovelluksissa. Polymeeristen komponenttien mikrovalmistus tuo kustannussäästöjä valmistukseen ja myös valmistusprosessit ovat yleensä helpompia. Tässä väitöskirjassa SU-8 epoksi-polymeeriä on käytetty miniatyrisoitujen analyyttisten laitteistojen valmistuksessa. Pii- ja lasimikrosiruihin verrattuna SU-8 käyttö on helppoa, koska rakenteiden kuviointiin tarvitaan vain ultravioletti-litografiaa. SU-8 mikrorakenteiden valmistusta on tutkittu ja kehitetty tavoitteena yleiskäyttöinen mikrofluidististen komponenttien valmistusmenetelmä. Tärkein kehityskohde on ollut kannen liittäminen mikrofluidistisiin rakenteisiin ultravioletti-valolla kuvioidulla adhesiivisella liittämisellä. Väitöstyössä kehitetyllä kanavien valmistusmenetelmällä pystytään toteuttamaan laajasti erilaisia mikrokanavia sekä kohtisuoraan kiekon tasoon että poikkileikkauksen puolesta tarkasteltuna. Kehitetty valmistusmenetelmä mahdollistaa myös monikerroksisten rakenteiden ja mikrokanavien suuaukkojen tarkan määrittelyn litografian avulla. Nämä ovat olleet ongelmallisia aikaisemmin mikrofluidististen kanavien valmistuksessa

SU-8 mikrokanavia on käytetty ensimmäistä kertaa miniatyrisoituihin elektroforeesi-sovelluksiin. Elektro-osmoottisen virtauksen nopeus on tutkittu kanavissa ja ensimmäiset erotukset on tehty. SU-8 toimivuus elektroforeesissa oli hyvä, koska elektro-osmoottisen virtauksen nopeus SU-8 kanavissa on suurempi kuin yleisesti muokkaamattomilla polymeereillä. Elektroforeesi-kanaviin liitettiin myös kiinteäfaasiuuttoallas, jolla voidaan suorittaa esierotuksia tai konsentroida näytteitä. Kiinteäfaasiuutto toteutettiin korkeilla kapeilla pilareilla ja monikerroksisella kanavarakenteella, joiden valmistaminen muilla polymeerivalmistusmenetelmillä on vaikeaa. Näytteiden detektio voidaan suorittaa SU-8 kanavasta kannen läpi fluoresoivilla näytteillä. Toisena vaihtoehtona tutkittiin mikrokanavien käyttöä sähkösumutus-ionisointiin, jolloin detektio tehtiin massaspektrometrillä. SU-8 soveltui materiaalina hyvin myös tähän tarkoitukseen. Materiaali on stabiili ja näin ollen kanavamateriaali ei aiheuta häiriötä mittaukseen. Väitöskirjassa kehitetty valmistusprosessi terävän SU-8 kärjen tekemiseksi sähkösumutus-ionisointiin on ainutlaatuinen, koska yleisesti terävien kärkien mikrovalmistus on vaikeaa.

Asiasanat SU-8, Mikrofluidistiikka, Mikrovalmistus, Adhesiivinen liittäminen, Massaspektrometria,

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Preface

The work for this thesis has been carried out in Microelectronics Centre at Helsinki University of Technology. The research has been done in projects: *Integrated polymer microsystems for fluidics* funded by Academy of Finland (project no. 211019) and *High speed micro analytical system (HISMIS)* funded by Finnish National Technology Agency, Orion Pharma Oyj, Perkin-Elmer Life Sciences Wallac Oyj, Perlos Oyj and Hormos Medical Oyj. Besides these I would like to acknowledge Helsinki Graduate School of Electrical and Communications Engineering and Graduate School of Chemical Sensors and Microanalytical Systems (CHEMSEM) for financial support of my research.

I would like to thank all the people who have helped and supported me during the research and writing of this thesis. First of all I'm most grateful to Sami Franssila for supervising this thesis, letting me carry out the research and letting me to realize all the ideas I have had along the way. In addition I would like to thank all the co-workers at Microelectronics Centre for their support and thank them for creating great working environment. Especially I would like to acknowledge the technical staff at Microelectronics Centre for keeping day-to-day routines working like keeping the cleanroom equipment running during my research.

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I want to thank my mother for her support throughout my studies. Finally very warm thanks go to all my friends for giving me other things to do and think after working hours. It has been very crucial for finishing this thesis.

Espoo, January, 2007

Santeri Tuomikoski

Contents:

List of papers

The following peer-reviewed papers are forming the basis of this thesis. Papers are hereafter referred by their Roman numerals:

I. Tuomikoski, S., Franssila, S., Wafer-level bonding of MEMS structures with SU-8 epoxy photoresist, *Physica Scripta,* **T114,** (2004), 223-226

II. Tuomikoski, S., Franssila, S., Free-standing SU-8 microfluidic chips by adhesive bonding and release etching, *Sens. Actuators A,* **120**, (2005), 2, 408-415

III. Sikanen, T., Tuomikoski, S., Ketola, R., Kostiainen, R., Franssila S., Kotiaho, T., Characterization of SU-8 for electrokinetic microfluidic applications, *Lab on Chip,* **5**, (2005), 888-896

IV. Sikanen, T., Korpisalo, I., Tuomikoski, S., Ketola, R., Kostiainen, R., Franssila, S., Kotiaho, T., Characterization of SU-8 microchannels for electrophoretic separations, 9th international Conference on Miniaturized Chemical and Biochemical Analysis Systems, MICROTAS 2005, Boston, USA, 1349-1351

V. Tuomikoski, S., Virkkala, N., Rovio, S., Hokkanen, A., Siren, H., Franssila, S., Design and fabrication of integrated sold phase extraction – zone electrophoresis microchip, *J. Chromatogr A.* **1111,** (2006), 258-266.

VI. Tuomikoski, S., Sikanen, T., Ketola, R., Kostiainen, R., Kotiaho, T., Franssila S., Fabrication of enclosed SU-8 tips for electrospray ionization mass spectrometry, *Electrophoresis*, **26**, (2005), 4691-4702

VII. Tuomikoski, S., Sikanen, T., Ketola, R., Kostiainen, R., Kotiaho, T., Franssila, S., Fabrication and optimization of enclosed SU-8 tip structures electrospray ionization mass spectrometry, 9th international Conference on Miniaturized Chemical and Biochemical Analysis Systems, MICROTAS 2005, Boston, USA, 982-984

Unpublished data: Samples for Figure 11 author has fabricated in collaboration with Louisiana State University Center for Advanced Microstructures and Devices (CAMD)

Author's contribution to the papers included in the thesis:

Paper I:

Planning of the experiments, all the experimental work and writing of the article.

Paper II:

Planning of the experiments, all the experimental work and writing of the article.

Paper III:

Part of planning the experiments and all the microfabrication work. Article was written by T. Sikanen with contribution from the author

Paper IV:

Part of planning the experiments and all the microfabrication work. Article was written by T. Sikanen with contribution from the author

Paper V:

Part of planning the experiments and all the microfabrication work. Most of the article writing with the help of co-authors.

Paper VI:

Part of planning the experiments and all the microfabrication work. Article was written by author with contribution of T. Sikanen

Paper VII:

Part of planning the experiments and all the microfabrication work. Article was written by author with contribution of T. Sikanen

Abbreviations

μ-TAS	micro total analysis systems
APCI	atmospheric pressure chemical ionization
BCB	benzocyclobutene
CE	capillary electrophoresis
COC	cyclo olefin copolymers
CTE	coefficient of thermal expansion
CVD	chemical vapor deposition
DIOS	desorption/ionization on silicon
DRIE	Deep reactive ion etching
EOF	electroosmotic flow
ESI	electrospray ionization
FTIR	Fourier transform infra red
GC	gas chromatography
IC	integrated circuit
IR	infra red
LC	liquid chromatography
LIF	laser induced fluorescence
LIGA	lithographie galvanoformung und abformung
MALDI	matrix assisted laser desorption/ionization
MEMS	micro-electro-mechanical systems
MS	mass spectrometer
M_w	molecular weight
NIL	nanoimprint lithography
PC	polycarbonate
PCB	printed circuit board
PCR	polymerase chain reaction
PDMS	poly(dimethylsiloxane)
PET	poly(ethylene terephtalate)
PI	polyimide
PMMA	Poly(methylmetacrylate)
PS	polystyrene
RIE	reactive ion etching
SPE	solid phase extraction
T_g	glass transition temperature
TIC	total ion current
UV	ultraviolet
ZE	zone electrophoresis

1 Introduction

Microfluidics has been around us already for some time, but we don't recognize it. The most familiar microfluidic application is inkjet printing that revolutionized home printing in late 80's. Ink droplets are shot by microfabricated actuation method through microfabricated array of holes. The device fulfills all the requirements of microfluidics. Fluids are handled and the channel/nozzle dimensions of the fluid manipulation device are in the range of micrometers. Other commonly used microfluidic applications are also based on microfabricated nozzles. One example is fuel injection that improves efficiency of fuel driven machines. In most areas the microfabricated devices for fluidics are still in the development stage. For analytical applications increasing number of microfluidic systems are commercially available, but those have not yet entered our everyday life. However, the development is going towards easy to use microfluidic devices that could be used by laypersons.

Despite relatively small number of commercial products, history of analytical microfluidic devices is already long. The first microfabricated analytical system was introduced nearly three decades ago. A gas chromatograph was realized on silicon wafer using microfabrication techniques [1]. However, after the first publications 10 years passed without major milestones in this field. In 1990 Manz et. al. introduced the concept of miniaturized total analysis systems [2] that later has changed into shorter form of micro total analysis systems (μ-TAS). The concept has a general idea of integrating fluidic components for chemical analysis to smaller scale and with this procedure to improve performance of analysis. The μ-TAS concept integrates on a same microchip controlled sample introduction, preparation and transportation together with separation and detection. Depending on the application there can be also other functions like sample concentration or mixing and sample recovery stages. This conceptual idea exploded the development of microfluidic devices and the last decade has been fast development towards μ-TAS or lab-on-a-chip devices.

The driving force for miniaturization is improvement in performance. In microscale new features become prominent in fluid handling and microfluidics take advantage of these effects. Benefits from miniaturization depend on the application but few general advantages are mentioned here. Surface to volume ratio is high in miniaturized systems improving several processes like separations and reactions due to increased contact area. Fluidic volumes can be controlled precisely enabling accurate sample injection and detection volumes. By miniaturization separation times are also reduced, diffusion paths are shorter, reactions occur with higher efficiency. Furthermore, microfabrication techniques enable device fabrication with minimized dead volumes that gives significant improvement in device performance. Most microchip fabrication techniques enable also fabrication of parallel systems that allow high throughput applications. Reduced fluid volumes lead to smaller consumption of chemicals. This gives cost advantage due to reduced consumption of expensive analytes, but it also enables analysis from smaller sample volumes. For example DNA analysis could be done at the crime scene faster and from minimal traces or health monitoring or drug tests can be done from single drop of blood. In some applications also the smaller device itself can be advantageous, for example miniaturization could enable personal portable health monitoring systems.

Although microfluidics is still rather new and not fully-characterized area the development has gone in some applications already beyond microfluidics to nanofluidics. In nanofluidics some features like surface to volume ratio is further increased and new special properties for fluid interactions start to exist. On the other hand downscaling laws are not necessarily working anymore and therefore some fluidic properties have to be reassessed. For example electroosmotic flow (EOF) that plays major role in microfluidics cannot be used similarly in nanometer scale channels. Even the basic term microfluidic chip is not unambiguous. Fully integrated system for DNA separation [3] can be called as microfluidic chip as well as an array of microfabricated vials [4]. In static microfluidics like in the array applications samples can be brought manually with pipette without fluidic transportation on the chip. In these applications advantage of microfabrication is for example due to controlled volumes or controlled surface properties. DNA and antibody arrays are examples of static microfluidics. Static microfluidic and nanofluidic chips are not discussed in detail in this thesis, which concentrates on the fabrication of analytical microfluidic chips that are based on fluid transportation in microchannels with typical dimensions in the range from few μm to few hundred μm.

The fabrication of microfluidics has become a subspecialty of micro-electro-mechanical systems (MEMS). Fabrication processes were adopted and modified to be suitable for fabrication of fluidic channels. Silicon microfabrication was well-established already in integrated circuit (IC) processes and therefore first microfluidic systems applied silicon as a material for the devices [1, 5]. Glass has been the traditional material for chemical analysis and reactions; therefore it was natural choice also for microchips. Known surface chemistry simplified the development of miniaturized fluidic structures [6]. However, microfabrication in glass substrates is not as simple and well developed as fabrication with silicon substrates.

Polymer microfabrication techniques were developed later to reduce fabrication time and cost. Cost reduction comes mostly from reduction of bulk material price, but in most cases also fabrication cost is lower, especially in large volume products. In analytical microfluidic applications disposable devices are often required to improve certainty of the analysis. Disposable devices are dramatically increasing fabrication volumes and this requires reduction in single device fabrication cost. Wide selection of polymeric materials and fabrication techniques gives suitable methods and materials for most applications. However, polymeric microfluidic devices have been around for 10 years only and therefore the fabrication processes and polymer properties in fluidic applications are not yet fully analyzed. In this thesis interrelations between microfabrication, polymeric materials and applications are described. This thesis gives an overview of the fabrication techniques and how polymer microfluidic chips have been used. The chips discussed are applied as analytical microfluidic devices, leaving a large number of mechanical fluidic components like pumps or valves aside, even those are important in integrated microfluidic systems. Emphasis of the thesis is in lithographic patterning of microfluidic structures from epoxy polymer SU-8.

2 Microfluidic structures and applications

In the applications described in this thesis the microchannels form the basis of the microfluidic chip. Even though most separation channels are straight, different functions for a device can be accomplished by more complex channels. For example crossings in the channels can be used to realize sample injection, labeling, sorting and concentration. Depending on the application also different cross-sectional shapes of the microchannels are needed. Electrically or pressure driven flows are more stable if the channels are symmetrical (round or square). On the other hand in chromatographic separations large microchannel wall area compared to the channel volume improves performance of the device. Besides the cross-sectional shape of the channels also surface roughness has remarkable effect when the channel dimensions are scaled down [7]. In most analytical applications open channels are not useful and therefore also channel enclosure by some method has to be done. Hence accurate microfabrication techniques for enclosed microchannels are crucial in analytical fluidic applications.

Inlet fabrication to the microchips is normally done by drilling or by through-wafer etching which are relatively difficult or time consuming methods. Lately powder blasting has become one of the key techniques for inlet fabrication especially for glass substrates. It provides faster and more robust method for making the fluidic inlets. After the inlet fabrication connection to the outer world has to be realized by some technique. World-to-microchip and microchip-to-world interfaces have become an important field of investigation. Early microfluidic chips used open reservoirs made of pipette tips glued on drilled inlets [6]. This can be simple and good enough approach for many low pressure applications especially for using EOF for liquid pumping. Another possibility is to glue capillaries directly to microchips forming closed microfluidic chips suitable also for pressure driven flows [V]. There are also few commercial solutions to do the fluidic connections that enable connections with relatively easy way [8, 9]. These inlets are normally compatible with high pressures, but they have problems with large size compared to microchips and also their price is high. Expensive and large connection system can remove some advantages achieved by microfabrication. No connection system is suitable for every fluidic chip, but the choice of connection mechanism is application specific. In some applications the easiest way to produce fluidic connections is to microfabricate dedicated connector for that device [10].

After finishing the microfabrication and fluidic connection the first application step in many μ-TAS devices is purification or concentration of the samples. For example most biological samples contain large number of compounds and therefore require some preseparation or concentration. A conventional way to bring preseparations to microchip world is to use off-line solid phase extraction (SPE) and to analyze the eluate with a microchip [11]. In integrated microfluidic chips the SPE is often realized by extension of macroscale world to microfluidics. This is done by coating or packing standard capillaries with the SPE-matrix and connecting those to the microchip [12]. However, connection of capillaries is laborious and leaves easily large dead-volumes.

Direct microchip purifications or concentrations have been done by C18-coated channels [13], but most microfabricated chips use beads [14-17, V]. Beads and SPE sorbents increase surface-to-volume ratio giving advances in separations and still exploiting the benefits of microfabrication techniques. Beads can be used also to introduce for example bio-molecules to ready-made chips. This is advantageous if desired molecules do not tolerate the conditions of chip fabrication: e.g. fabrication

temperatures are too high or fabrication chemicals are incompatible with the molecules. There are several bead stopping mechanisms on microchips; reservoirs used for mechanical stopping of beads [15, V] or other forces like chemical binding [17] or special flow profiles [16]. Depending on the realization, capability for bead stopping does not necessarily require additional process steps, although it often does. An example of microfabricated SPE reservoir with pillars for mechanical bead stopping is shown in Figure 1. Pillars are done in the same process step together with channels. The whole structure is made of SU-8. With DNA-samples, besides possible preseparation or concentration, the weak signal is usually amplified before separation and detection. It is done by polymerase chain reaction (PCR). PCR-microchips have been reviewed comprehensively in [18, 19].

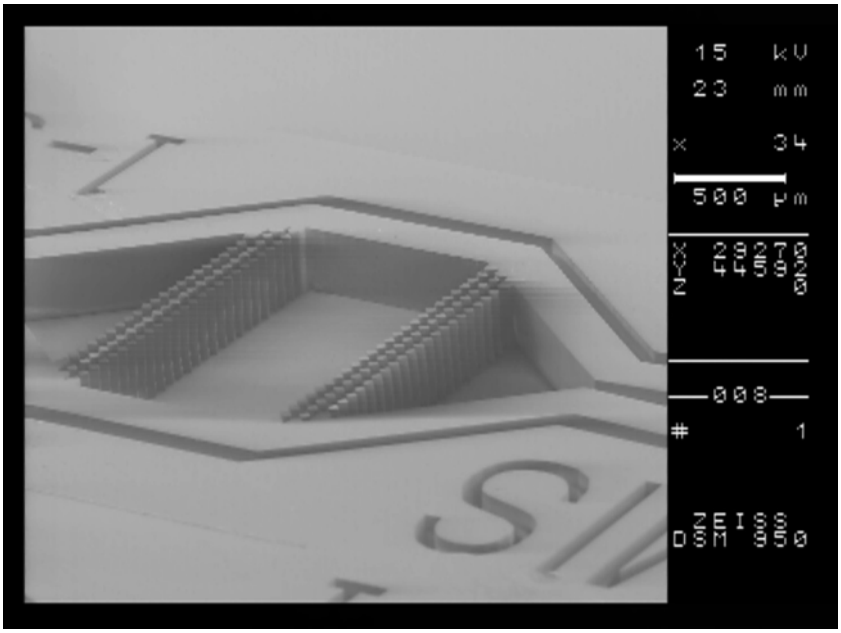


Figure 1 Microfabricated SPE reservoir with bead stopping pillars. Structure is fabricated in one step lithography process of SU-8

Chromatographic and electrophoretic separations form the basis of most separation techniques in microchips. In chromatography the separation is based on physicochemical properties of analytes. Analytes are transported in moving phase and the separation is based on retention caused by stationary phase in the microchannel. The stationary phase has to be selected so that retention times for analytes are different. The method for the retention can be various, for example it can be based on size or affinity. Both liquid chromatography (LC) [5] and gas chromatography (GC) [1] were demonstrated in microchip format already at the early stages of analytical microfluidic device fabrication. In chromatography the fluid transportation is normally done with pressure driven flow but in electrochromatography electric field is used for liquid transportation [20]. The stationary phase in microchip chromatographic systems can be formed during or after the channel fabrication. If stationary phase is done during the channel fabrication it can be open tubular channel without additional structures [5] or for example pillars are etched to the substrate during the channel formation [21]. The stationary phase can be done after channel fabrication with porous monolith materials [22], sol gels [23] or by filling the channels with beads [20, 24].

Electrophoresis is the other widely used separation method in miniature scale. In the basic format the separation uses high electric field (100-1000 V /cm) in microchannel to separate the samples based on their electrophoretic mobility. The mobility is affected by the molecule charge and size. Liquid transportation in the channels is done by electric field induced electroosmotic flow (EOF) without need for external pumps. EOF mobility is mainly affected by the buffer and by the channel material. Uniform flow is achieved only if surface charge density of the channel walls is even. Electrophoresis in microfabricated channels was demonstrated for the first time in 1992 [25]. The electrophoretic separations were performed initially in microchannels fabricated in chemically glass-like materials [6, 25, 26], since the surface chemistry is similar to silica commonly used in capillary electrophoresis (CE). Cross-shaped channel geometry with well defined injection volume and miniaturized channel size improve the separation efficiency as comparison to conventional CE. Electrophoretic separations have been demonstrated in the timescale less than millisecond [26], but normal separation times are seconds in comparison to minutes in conventional CE. Besides standard electrophoresis in a microfabricated channel, other forms of electrophoresis has been realized in microchip format as well; for example isotachophoresis [27, 11], gel-electrophoresis [28] and free-flow electrophoresis [29].

Detection in the analytical microfluidic chips is as important as good separation performance. Without detection there is no knowledge what has been separated. The variety of detection methods is wide and therefore in-depth study of those is excluded from this review. For further reading there are several reviews published about detection in microchips [30-34]. Optical detection is normally the easiest approach to realize detection in microfluidic chips. Different methods based on absorbance, fluorescence, or luminescence have been reviewed for example in [30]. The ease of the optical detection holds true from fabrication point of view only if the detection is done with detector outside the microchip. If looking the usability side an integrated detector is important. Microfabrication techniques enable also the integration of the optical detection on the microchip, but it increases dramatically chip complexity. Simple one lithography channel fabrication process can expand to 13 lithography level process if detector is integrated to the same chip [35]. In some applications detectors are made on separate reusable wafers that can be used together with simple disposable microchannels. Laser induced fluorescence (LIF) is very sensitive detection method being highly suitable for microscale separations with small amounts of analytes. Drawback in fluorescence detection techniques is the need for labeling of the analytes. Recently a similar method based on thermal lens detection is introduced. It is an optical detection method without need for labeling [36]. Also detection at infrared (IR) range can be done without labeling. However, channel walls are normally not completely transparent in IR wavelengths and therefore expensive solutions with detection windows have to be made [37].

Mechanical sensors are based on beams, membranes and cantilevers. A detailed review about cantilever sensors has been published [38]. Vibrating cantilevers can measure mass change deposited on top of the cantilever from the frequency change of the cantilever. Respectively static cantilevers measure surface stress change caused by the deposited material. These methods have been applied mostly in biological applications for selective binding of molecules on top of the cantilevers.

Electrical detection systems require accurate positioning of electrodes to the microchip, which can be easily realized by many microfabrication techniques. Detection possibilities are based on amperometric, conductivity or potentiometric detection. The electrical detection techniques are reviewed for example in [31].

Microchip coupling to mass spectrometer (MS) is one of the key detection methods in microfluidics because MS provides high sensitivity and specific detection of analytes. Different approaches have been described for coupling miniaturized systems to MS [30, 34]. Schematic pictures of three main coupling techniques are shown in Figure 2. Laser desorption/ionization is effective method for sample ionization and these methods have been used in microfluidics to some extent. In matrix assisted laser desorption/ionization (MALDI) problems remain in crystallization of analytes with matrix compounds. This is time consuming and therefore continuous systems based on this method are difficult to realize. However, microfluidic systems connected with a dispenser have been used to spot samples to MALDI plates [39]. New approaches with laser desorption/ionization on silicon (DIOS) [40, 41] does not require additional matrix compounds and could be easier to integrate in fluidic systems. So far the laser ionization methods have been used rarely in integrated fluidic devices.

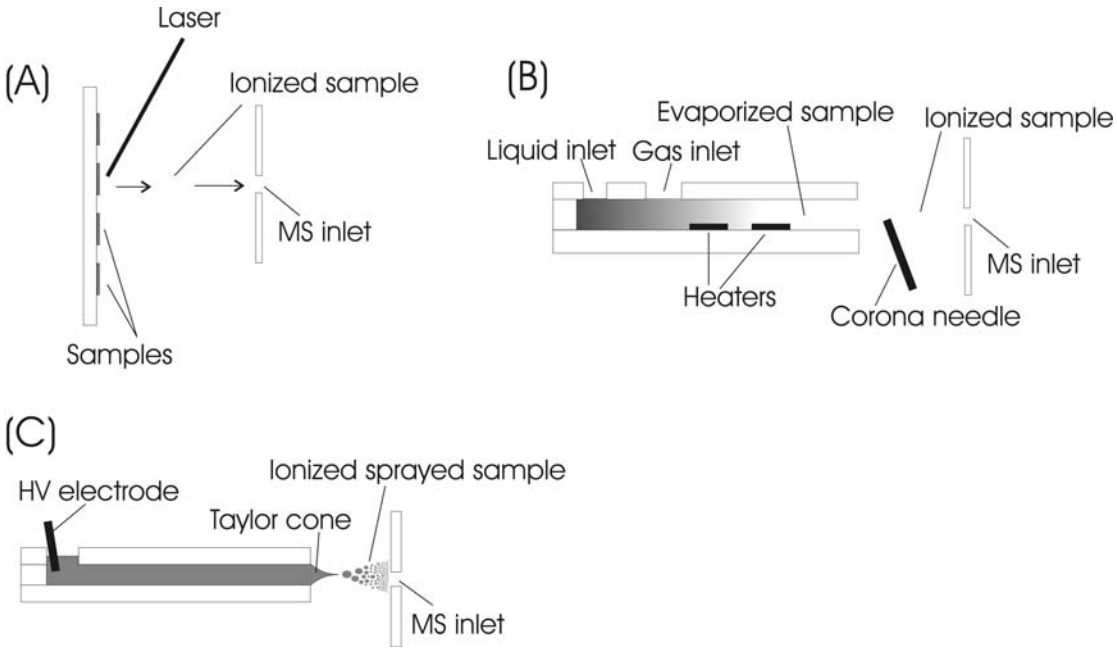


Figure 2 Three main detection methods for microchip coupling with MS: (A) Laser desorption/ionization, (B) atmospheric pressure chemical ionization and (C) Electrospray ionization

Lately chemical ionization has been miniaturized and connected to macroscopic separation systems [42-44], but it has potential to be integrated also with miniaturized separation systems. In atmospheric pressure chemical ionization (APCI) a nebulizer chip evaporates the sample, which is then ionized in the gas phase by corona discharge [42] or by photoionization [43]. This method has recently been miniaturized for the first time and therefore it has not been in wider use. Electrospray ionization (ESI) is a widely applied technique in analytical chemistry and it can be easily used with microchips because the flow rates in microfluidics are similar to the flow rates optimal for ESI-MS. High electric field (in the range of kV potentials) at the ESI tip creates fine spray transporting ionized compounds to MS. An example of spray from microchip is shown

in Figure 3. Different designs of ESI microchips have been reported [45, 46] and ESI has been integrated with microseparation techniques; with electrophoresis [47, 48] and with chromatography [49 50]. Electrospray itself works well in microsystems and the method has lot of potential for integrated devices, but so far only few integrated systems have been demonstrated. Limitation comes mostly from the fabrication. Accurate ESI nozzles and separation systems are difficult to realize with microfabrication techniques to the same microchip. Therefore nozzles have often been replaced with manually assembled capillaries. Manual assembly naturally reduces the benefits achieved by microfabrication techniques.

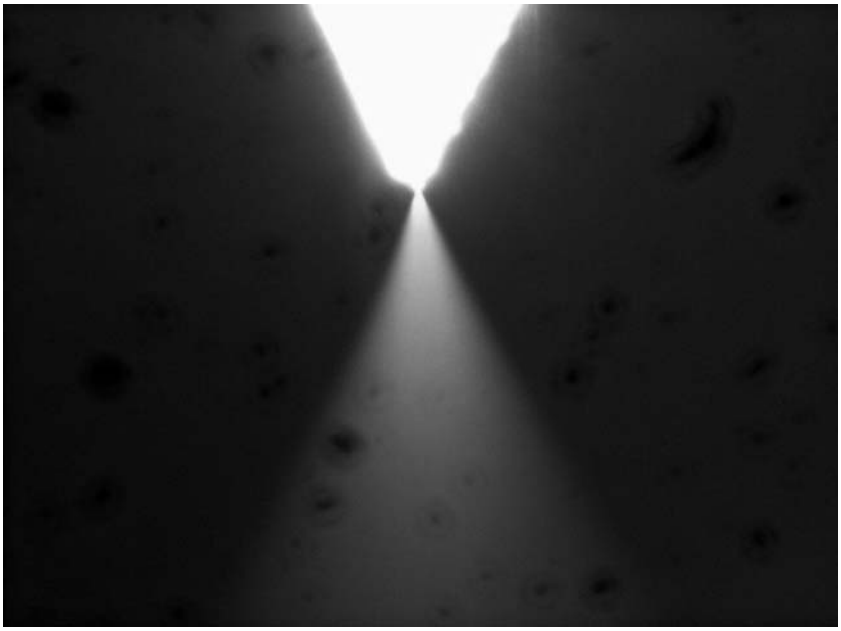


Figure 3 Electrospray generated from a SU-8 microchip [VI]

During the last few years biological microfluidic devices like chips for cell culturing, handling and analysis have gained a lot of research interest. These devices have their own requirements for the materials and structures. Channel shapes are not as crucial as in separation devices mostly described in this thesis but material issues like biocompatibility become more emphasized.

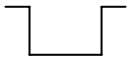
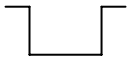
3 Microfabrication methods

The first reported microfluidic devices were made of glass or silicon. Different etching schemes for those are available and etching can produce wide range of channel shapes. Simplified overview of achievable microchannel cross-sectional shapes is shown in Table 1. Similar channels can be produced also with other methods like powder blasting [51] or laser ablation [52]. These methods are not widely used with silicon because silicon etching is simple. In the case of glass processing especially powder blasting has become a popular method.

Open channels are rarely useful for any fluidic applications due to intolerance to pressure and fast evaporation of samples. Therefore channels have to be enclosed. The channel enclosure is normally done with some bonding method. For silicon and glass the selection of different bonding techniques contain thermal bonding methods [53], electrically assisted methods like anodic bonding [5], chemically assisted methods [54]

or adhesive bonding with polymers [55]. Besides bonding methods enclosed channels can be realized by other micromachining techniques like optimized thin film deposition [56] or sacrificial etching techniques [57]. Moving of single microdroplets on flat surfaces has also been demonstrated without need for channel enclosure [58, 59]. These devices have been applied for droplet manipulation, but more complex microfluidic systems have not been demonstrated.

Table 1 Shapes achieved in silicon or in glass by different etching techniques

Fabrication method	Material	Etchant	Depth	Width on top	Sub-structures	Channel shape
Wet etching anisotropic	Silicon	Potassium hydroxide	Any	1,42*depth	Some	
Wet etching isotropic	Silicon	Hydrofluoric acid+nitric acid+acetic acid	Any	2*depth	No	
Reactive ion etching isotropic	Silicon	Sulphur hexafluoride+ oxygen	Any	2*depth	No	
Reactive ion etching anisotropic	Silicon	Sulphur hexafluoride+ oxygen	Any	Any	Yes	
Wet etching	Glass	Hydrofluoric acid	determined by etching mask	2*depth	No	
Reactive ion etching	Glass	Carbon tetrafluoride+ oxygen	~100 μm	Any	Yes	

Glass is an ideal material for microfluidic chips from the historical perspective. All chemical reactions and separations have been made in some kind of glass systems and therefore material properties are well known. However, the micropatterning of glass is difficult compared to silicon microfabrication. Wet etching produces only round structures and the masking of the etching is difficult. Furthermore, smaller features in the channels (defined as sub-structures in the Table 1) are difficult to fabricate. Reactive ion etching (RIE) of glass requires expensive and sophisticated equipment and is limited in height even in deep reactive ion etchers [60]. Fluidic inlet drilling to fragile glass wafers easily breaks the substrate. Nowadays powder blasting is increasingly used for making fluidic inlets. With that technique all the inlets can be fabricated simultaneously and glass breaking is not as a big risk. The selection of patternable fluidic structures to glass is limited and therefore polymers have gained popularity. Wet etched semi-spherical channel profile in glass is not optimal shape for most fluidic applications. However, almost circular channels can be made by bonding two similar chips together, but it requires very accurate alignment for bonding [61].

Silicon processes are well-established and surface chemistry similar to glass can be obtained with oxidized silicon. Numerous microfluidic systems have been demonstrated with silicon microchips [62]. However, application of silicon in microfluidics has few drawbacks; optical detection from the chips is difficult due to opaque substrate material and application of high electric field requires thick insulation layers. Thermal growth of silicon oxide on top of the channels can take weeks for high voltage applications [63]. In addition to limitations caused by the materials both silicon and glass processing are

relatively time consuming and expensive methods. Channel enclosure requires high temperatures limiting application of thermally sensitive materials and inlet fabrication through substrates is difficult. Polymers are used increasingly to meet the price requirements of the chips. In many cases they can also provide good properties for fluidic chips in addition to fabrication issues like fewer process steps and shorter throughput time.

Polymer microfabrication is relatively new field in microfabrication. Lately the fabrication processes have been developed with accelerating speed meeting the demands of microfluidic chips. Material properties of polymers support the microfluidic chip fabrication. High voltage, required for many applications, can be applied because polymers are insulating materials excluding few conductive polymer types. Transparency of most polymers enables optical detection. However, autofluorescence from the polymeric materials sets its limitations [64, 65]. The selection of polymers is wide and all polymers are not suitable for every application. For example chemical stability of polymers might not be suitable for all applications; also thermal requirements of some applications limit the use of low melting point polymers [10]. To compensate non-stabilities surface modifications and coatings have been investigated to tailor polymer surfaces for fluidic operations [66, 67]. Polymer properties for microfluidic applications have been scantly published. Therefore comparison of polymer properties is rather difficult based on published data. Some fluidic properties of commonly used polymers are collected to Table 3 at the end of this thesis

Polymer fabrication processes can be divided in various ways. Here the processes are divided into two main groups. Replication techniques are methods that apply some kind of master to reproduce the structures to polymers. These techniques are taken from polymeric fabrication of macroscopic structures and the processes have been adapted for micropatterning. The other group is direct fabrication. These methods fabricate the structures directly to polymers by using techniques based on silicon and glass microfabrication. In the following chapters polymer microfabrication techniques are described and examples of polymeric fluidic structures are shown.

4 Direct polymer microfabrication for microfluidics

Direct polymer microfabrication is an extensive group of techniques with wide range of possible structure shapes. Most of the techniques use some kind of radiation to pattern structures directly to the polymers. Structures can be realized by polymerizing or by selectively destroying polymer with radiation. This group contains lithographic patterning, mechanical and laser micromachining as well as selective etching of polymer layers. Fabrication techniques are originally from silicon or glass microfabrication areas. This gives the possibility to utilize silicon and glass substrates for the structures and to apply processing methods developed for those together with polymer microfabrication. Methods are clearly microfabrication-oriented and this enables larger freedom for the integration of various functions on a same microchip. Direct polymer microfabrication techniques are new and the techniques are not fully researched. However, those can provide alternative fabrication techniques for most silicon or glass fabrication. Direct polymer microfabrication techniques include also methods that can be combined with silicon or glass fluidic components to prepare certain part. One example is selective UV-polymerization of monolith matrix inside a microchannel to increase surface to volume ratio [22].

4.1 Lithography

Optical lithographic patterning has its history in the early stages of IC-fabrication. Radiation sensitive layer is deposited on top of a substrate and exposed with ultra violet (UV) light through a mask. The exposed image is developed and the mask image becomes visible on the substrate either in positive (exposed areas are developed away) or in negative (exposed areas stay on the wafer) mode. Besides the standard UV-exposure, the radiation can be X-rays, electrons or ions. UV- or X-ray exposures are done through a mask that is opaque to the applied radiation. Masks in UV-exposures are normally glass or quartz plates with about 100 nm layer of chromium to define non-exposed parts. In rapid prototyping of fluidic components printed transparency masks have become popular to reduce cost and to speed up the design cycle [68]. The disadvantages of using transparency masks are rougher channel walls and not as accurately defined structure sizes as with glass masks. Furthermore the minimum linewidth depends on the resolution of the printer. In X-ray exposures thicker (in the range of 10 μm) electroplated metal structures are required to define non-exposed areas increasing the price of mask fabrication. Electron or ion beam lithography uses computer controlled exposure pixel by pixel or larger areas at a time in modern techniques eliminating mask altogether.

In fabrication of fluidic structures with lithography there are several important requirements for the materials in comparison with normal lithography. Adequate mechanical strength, possibility to apply and expose uniformly thick layers of polymers are the basic requirements for thick photoresist layer patterning. Additionally we have to consider microfluidistic properties of the materials; compatibility with chemicals, hydrophobicity, surface charge and so on. These requirements are dependent on the application. Material strength requirements are best achieved by tight network of polymers achieved by polymerization of monomers or by cross-linking of oligomers in the case of negative photoresists. Positive photoresists normally lack the mechanical, thermal and chemical durability required in fluidic applications.

Lithographic patterning produces structures with nearly vertical walls. Verticality of the walls depends mostly on the UV-transparency and reaction kinetics of the material. With good materials fluidic channels with square cross-section can be done in fast process by exposure and development of thick photoresist film. Depth of the channels is defined by resist layer thickness and the width by the mask. Based on applied materials the lithographic patterning of fluidic components is divided in to two groups in this review: patterning of photoresists and liquid photopolymerization. Photoresist patterning is the method adopted from MEMS fabrication using more traditional photoresists [69, 70]. It is limited to the materials known to behave like photoresists; suitable for spinning, baking and contact exposure in a mask aligner. There are a limited number of materials suitable for this kind of processing. In liquid photopolymerization the material selection is wider, but method is rarely used for microfluidics [71-73].

Photoresist patterning with lithographic techniques for fluidic applications has gained popularity within last few years, but it is still rather little characterized and applied. Epoxy photoresist SU-8 is the most widely used material, but also polyimides (PI), benzocyclobutene (BCB) and few others have been proposed for fluidic structures. SU-8 is a negative photoresist developed by IBM [74]. The oligomers in SU-8 have high density of epoxy-groups that can form tightly cross-linked structure during curing. Initially SU-8 was applied as a standard photoresist for high resolution applications

[75], but its best properties were found to be in thick film photoresist applications; as an etching or electroplating mask [69, 76]. However, unlike many other thick photoresists, SU-8 is suitable also as a structural material for fluidic components. It is mechanically strong and optically transparent for wavelengths above 350 nm [69]. These properties enable patterning of thick layers with high aspect ratios and vertical walls. Layer thicknesses over 2 mm have been demonstrated [77] and with proper optimization aspect ratios up to 190:1 can be obtained with UV-exposure of SU-8 [78]. Hence structures similar to silicon deep reactive ion etching (DRIE) can be fabricated with simple lithography process. Furthermore, multiple layers can be patterned and aligned to each other by standard UV mask aligner [V]. SU-8 can be patterned with X-ray and electron or ion beam exposures as well [79, 75, 80].

SU-8 has been shown to be biocompatible [81, 82]. It is chemically stable; no background has been noticed from the material itself in analytical applications [VI] and SU-8 has been shown to be compatible with most chemicals applied in analytical applications [VII]. Transparency of the material enables optical detection. However, some fluorescence signal is emitted from the SU-8 itself [65]. Drawbacks of the material are relatively high coefficient of thermal expansion (CTE) [83] that may cause stresses to wafers if SU-8 is used with materials with widely different CTE values at elevated temperatures. SU-8 cross-links tightly making mechanically strong structures possible, but negative aspect of high cross-linking density is difficult removal of the material. However, good patternability of SU-8 enables wafer-level batch fabrication of accurately defined microfluidic components. Depending on the microchip area several microfluidic devices can be fabricated on one wafer and several wafers can be fabricated at the same time. An example of parallel fluidic channels made of SU-8 on a silicon wafer is shown in Figure 4.

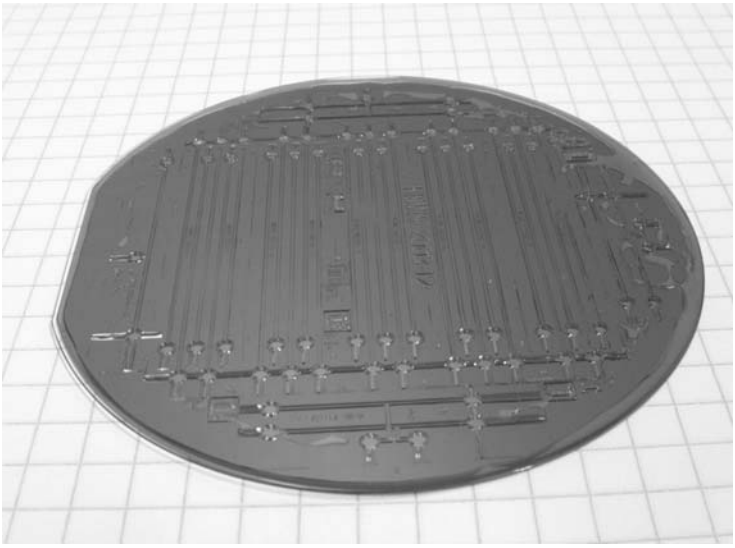


Figure 4 Parallel microfluidic channels fabricated of SU-8 on top of four inch silicon wafer.

First SU-8 microchannels were proposed already in 1997 [84], but only lately has SU-8 become one of the material choices for microfluidics. Fabrication of open channels is fast and straightforward process, but lack of proper enclosure techniques for making fully SU-8 channels has limited applications earlier. Enclosure of the microchannels is a problem with every polymeric material. This is discussed in chapter 7 in more detail. Due to difficulties in fabricating fully SU-8 channels the material has been applied

mostly as one part of the microfluidic chips. Depending on the application this might cause non-uniform properties for the device and might be critical for device functioning. In certain applications like in cell growth and handling different materials do not cause difficulties as long as all materials are biocompatible [85].

In structures made partially of SU-8 the material has been used mostly to define only channel walls or larger fluidic areas, because thick layers can be patterned accurately and rapidly on top of any substrate. There is large number of such publications. For example [85-95] have used SU-8 for formation of channel walls. Application areas for such structures vary from separation devices like dielectrophoresis [88, 89] and field flow fractionation devices [90, 91] to enzymatic microreactors [87]. One important contribution of microfabrication is to bring new functionalities to conventional systems. One example of such structures is fabrication of detection window for Fourier transform infra red (FTIR) detection for conventional CE. It has been realized with CaF_2 windows constructed with SU-8 walls [92].

Several papers have lately reported fabrication processes for microchannels fully made of SU-8 [84, 96-104, II]. However, analytical results using Fully SU-8 channels have been rather limited. Electrophoresis chips have been proposed of SU-8 in many references [101-104], but earlier only detection of single molecule with FTIR in the channels has been reported without separation or values for EOF-mobility [37]. However, we have recently measured the electroosmotic flow mobility in fully SU-8 microchannels [III]. Electroosmotic flow in SU-8 channels is high compared to other unmodified polymer samples and the direction of flow can be inversed with simple channel flushing without need for channel coating. This promises fast separations and enables new types of fluidic devices. Electrophoretic separations have been demonstrated in similar free-standing fully SU-8 microchips with fluorescence detection [IV, 65]. An example of fully SU-8 microchannel for electrophoretic separations is shown in Figure 5. High aspect ratio patterning of SU-8 has been applied to produce SPE reservoirs with integrated electrophoresis channel. This enables concentrations and purifications before sample introduction to electrophoresis channel. LIF detection was used through the cover to visualize SPE-reservoir and electrophoresis channel [V].

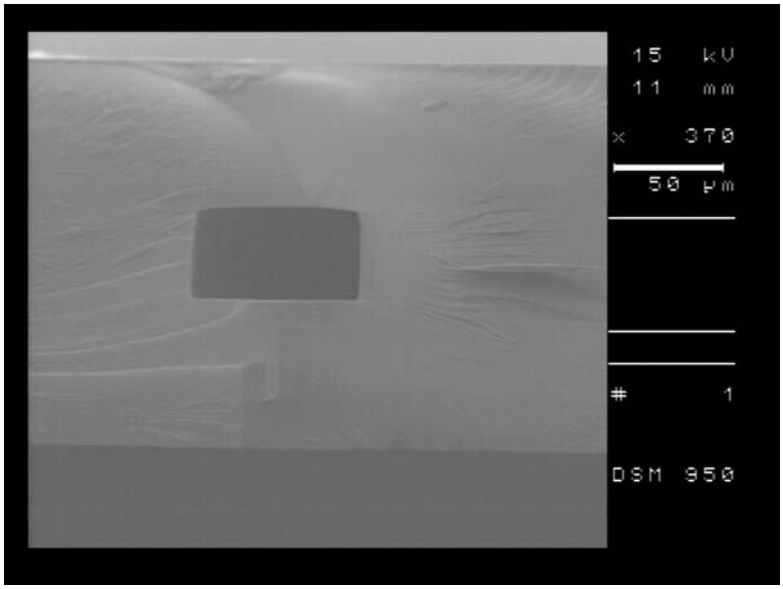


Figure 5 Cross-sectional view of electrophoresis channel made fully of SU-8

Chromatographic columns have been fabricated in SU-8 with UV-cured monolith as the stationary phase. Separation has been demonstrated using commercial nanospray capillary connected to the microchip for MS detection [105]. This detection technique requires laborious manual assembly after the microchip fabrication. To avoid need for manual assembly, ESI-emitters have been fabricated of SU-8 and applied for MS-measurements. SU-8 enables fabrication of accurately defined free-standing tips for ESI. The tips show good stability and ionization performance without background from the material [VI]. SU-8 ESI interfaces have been demonstrated both as open versions [106-108] and as fully enclosed microchips [VI, VII]. The open SU-8 ESI-tip versions have been integrated with SU-8 chromatographic columns mentioned earlier, but separations with on-line MS detection have not been demonstrated with integrated systems [109]. An example of enclosed ESI-tip made fully of SU-8 is shown in Figure 6.

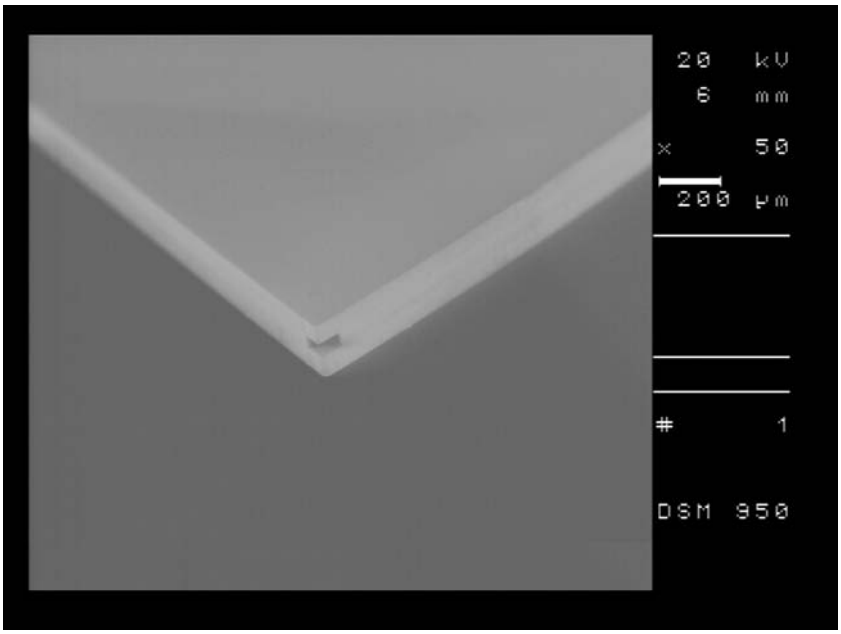


Figure 6 Fully enclosed ESI tip fabricated of SU-8. Spraying end of the channel is seen at the end of the tip.

Due to good optical properties of SU-8 material optical detection can be done through the cover layer of the chip [IV, V]. Different strategies have been also described for integration of optical waveguides to SU-8 microfluidic chips [110-113]. All publications with waveguides have concentrated on fabrication and optical characterization, rather than analytics. Cantilever sensors for biomolecule detection have been fabricated of SU-8 and they show response when molecules are attached to the cantilever surface [114-116]. However, timescales for the cantilever stabilization are rather long compared to timescales in normal microfluidic devices. Cantilevers function normally in timescales of tens of minutes whereas normal fluidic chips work in seconds. Therefore cantilevers are normally used in autonomous sensor chips rather than in connection with fluidic systems.

Another thick negative photoresist similar to SU-8 is THB-series from JSR. It is rarely used in microfluidics but suitable for thick layer patterning similarly to SU-8. Up to millimeter thick layers and aspect ratios around 10:1 have been demonstrated [117]. In microfluidics THB-photoresist has been used to construct electrophoresis microchips

with integrated electrochemical detection [118]. In the construction Poly(methylmetacrylate) (PMMA)-plates were used as bottom and top plates and THB formed only the walls of the chip. The electrophoretic or other fluidic properties of the pure THB resist channels have not been measured.

PI is a group of negative photoresists used for fabrication of structural components for MEMS and for fluidics. This group contains several kinds of materials with different features and therefore the properties are not as well characterized as properties of SU-8. Mechanical material properties of few different PI materials have been collected in [119]. Both photopolymerizable and non-photopolymerizable PI have been used in microfluidics [70, 120]. Non-photodefinable PI is patterned by polymer etching described in chapter 4.4. The photopatterning of PI is limited compared to SU-8 patterning; aspect ratios are low and layer thicknesses are in the range of tens of micrometers. These features are enough for many basic fluidic components, but might become limiting in special applications.

Polyimides have also good biocompatibility and resistance to analytical chemicals [119]. PI is thermally stable and the material can be selected so that thermal expansion coefficient is suitable for other materials on the chip [119]. This makes the electrode integration easy. Despite good features of polyimide it has been used little in microfluidic applications. PI microchannels with integrated electrodes have been reported without specific applications both on top of substrates and as flexible released microfluidic chips [70, 121, 122]. On the application side dielectrophoresis with PI walls and poly(dimethylsiloxane) PDMS cover has been demonstrated [88]. Moreover PI has been used to make implantable microchips for recording electrical activity of neuro-cells and for drug delivery and monitoring [119, 123, 124].

Photodefinable BCB and Teflon-like polymers have been used for fabricating fluidic structures in few articles [125-128]. These materials are mostly used as bonding material in wafer bonding and those can also be patterned either by etching or directly with lithography to form the channels. Photopatternability is limited and only relatively thin layers and low aspect ratios have been demonstrated. These materials are mostly used to fabricate channels in combination with other materials. Fully lithographically made channels like with SU-8 and PI have not been demonstrated. Also the material properties for fluidic applications have not been well characterized. However, materials are biocompatible and implantable drug delivery needle has been demonstrated of BCB [128].

Dry film laminated photoresists that were originally developed for printed circuit board (PCB) patterning, have been used for fluidic applications. The photoresists are laminated with hot-roll-lamination system on top of the substrates and patterned as normal photoresists by UV. Dry film resists have very uniform thickness and therefore the enclosure becomes easier compared to spincoated photoresists. However, the layer thickness and patternability are rather limited. Thicknesses are in the range of 20 μm [129, 85] and aspect ratios are around 1:1. Dry film photoresists have been used to fabricate fluidic channels and applied for example for electrophoresis [130], dielectrophoresis [129] and for cell culturing [85]. However, the channels have been only with two or three walls made of dry film photoresist.

PMMA is an exception in lithographically defined microchannel materials. It behaves as positive photoresist, but its properties are still suitable for fluidic applications. However,

patterning cannot be done by standard UV-exposure. X-ray, electron and ion exposures have been used to define microchannels to PMMA [131, 132]. These methods are rather expensive and require specialized equipment. Methods are also complicated as a comparison to other methods for patterning PMMA (mostly hot embossing described in chapter 5.2) and therefore rarely used. However, direct fabrication techniques for patterning PMMA enable high aspect ratio structures unlike hot embossing. Electrophoresis has been demonstrated in PMMA channels made with X-ray exposure [132].

4.1.1 Liquid photopolymerization

A special case of lithographic patterning is liquid photopolymerization. A cavity or a mold is placed on top of substrate and filled with monomer solution. The solution in the cavity is photopolymerized through a photomask like in lithography. Material selection for liquid photopolymerization is wide. In comparison with lithography material requirements set by spinning are not important and therefore the method is suitable generally for any monomer solution that can be cured selectively by UV-exposure. Despite principally wide material selection the liquid photopolymerization method is rarely used technique in fabrication of microfluidics.

Liquid phase photopolymerization has been used in few applications as a rapid prototyping technique to make microfluidic structures. Single and multilayer fluidic structures have been patterned applying hydrogels. These have been applied for various basic fluidic operations by the group of Beebe et al. [71, 133]. Fluidic structure fabrication without specific applications has been presented for other polymers as well [72, 73]. The use of cavity instead of standard spinning for applying the material limits the structure fabrication to some extent. Accurate cavity thickness control is needed and cavities are difficult to produce and apply for thin layers. Therefore only relatively thick layers have been fabricated. Patternability of these polymer materials has also been clearly worse than with normal photoresists. This is mostly due to lower viscosity of the materials which enables faster diffusion. Diffusion after UV-exposure enlarges the structures. Therefore structures with this technique are mainly in the size-range of 100 micrometers and up, both in lateral and horizontal dimensions [72].

4.1.2 3D lithographic fabrication

Normally lithography is used to fabricate structures that vary in two dimensions. With some modifications lithographic techniques are suitable for three-dimensional patterning of polymers, but depending on the technique possible structures are limited in shapes. Inclined exposures are done with standard UV exposures and photoresists to fabricate structures at certain angle. For example fluidic filters or channels with non-vertical walls have been made with inclined exposures [134, 135]. The method is principally as easy as standard lithography. However, the method is suitable only for structures at certain angle. Gray tone lithography uses shades of gray to define round shapes or multilevel structures in single exposure [80]. The method is similar to standard lithography, but the photomasks are using all shades of gray instead of black and white only. The method suffers from high price of gray scale photomasks. It has been mostly used for fabrication of round lens-type structures and it has so far no applications in microchannel fabrication.

Lasers are well suited as light sources for lithography because of good collimation of the light. Lasers are expensive and exposure is time consuming compared to standard

lithography. Therefore standard two dimensional structures are not patterned with lasers. However, laser light can be used for some special purposes to define three dimensional structures. Holographic lithography uses lasers to create interference patterns on the substrate. Symmetrical three-dimensional mesh structure can be created with four lasers or with one laser and refracting prism [136, 137]. Structures are mostly used as 3D photonic crystals, but they have the potential to be applied in microfluidics. The method can produce a mesh structure with large surface area required in many fluidic applications.

Stereolithography can be done either by standard UV-lamp or by laser. The method enables more freedom for fabrication of 3D lithographic structures than holographic or gray tone lithography. Structures can be constructed layer-by-layer [138] or with direct 3D writing one spot at a time [139-142]. Layer-by-layer method applies focused light beam directly or UV-exposure through actively changeable mask. Exposure and new photoresist application is automated in a rapid manner. Only thin top-layer is exposed in repeated exposures and all layers are developed simultaneously. The structures have often rough surfaces because of stepwise exposure. In active mask systems smallest definable features are larger than in normal contact lithography; in the size range of 5 μm [138].

Stereolithography by direct 3D writing at one spot at a time is done with lasers based on two-photon polymerization [139, 140]. This method requires accurately focused laser beam that can be moved in all X, Y and Z directions. The method can be used for direct writing of nearly arbitrary shapes with sub-micrometer resolution. Laser spot is moved with computer controlled XYZ-table in polymer without need for repeated resist application [141, 142]. Both stereolithography methods are slow and expensive compared to two dimensional lithographic patterning, Due to expensive and rare tools those have been used only little in fluidic applications.

4.2 Laser ablation

Laser ablation applies high energy laser light to remove material from the bulk material. Material removal is based on evaporation and transporting of melted material from ablated spots and therefore further processing like development is not required as in lithography. Laser light decomposes polymer material by breaking bonds in bulk polymer both with light-induced reactions and with thermal breaking. Short segments are volatile and longer ones turn into melted polymer by the heat of the laser. The method is not limited to polymer microfabrication, but hard materials, even diamond [143], can be ablated by lasers.

Normally the patterning in laser ablation is done by keeping the laser fixed and wafer is moved by a computer controlled XY-table. As a drawback of the method the cost of laser is high and also the controlling system has to be precise to be able to fabricate accurate structures. Furthermore, the fabrication is time consuming and therefore expensive. Microfluidic channels can be ablated directly after drawing the design without mask or master fabrication in between. The first chips can be accomplished and tested immediately after finishing the design. Therefore the method is mostly used for prototyping of devices, but rarely used for fabrication of larger batches of microchips. Structure shapes are limited to rather simple structures. For example integration of other structures like electrodes is more difficult than with lithographically fabricated microchannels.

Wide range of different lasers has been used for polymer laser ablation. Normally excimer lasers working in UV-range are used [144, 145], but also IR-lasers have been employed [146] Laser energies and pulse lengths can vary over large scale. Normally the scale goes from few nJ pulse energies up to nearly 1 J. Correspondingly the duration of the pulses vary from few femtoseconds up to 0,1 s [52, 144 146]. The exposure is done through an aperture that defines the size of the ablated spot. Spot size can be from sub micrometer up to millimeters. The speed of fabrication depends among others on the laser type, depth requirements and ablated area [144, 146]. Material removal per pulse depends on the laser system, but for example material removal rates of 100-400 nm / pulse have been reported [144].

Laser ablation of polymers has been known since 1982 [147] and the first polymer microfluidic devices made with laser ablation were introduced in 1997 [144]. The method benefits from wide selection of patternable polymeric materials. Microfluidic devices have been fabricated for example to PMMA, polystyrene (PS), polycarbonate (PC), cellulose acetate, Poly(ethylene terephtalate) (PET) and PI [52, 49, 144, 145]. Wide range of materials enables material properties to be suitable for different fluidic applications. However, the surface properties of ablated polymers are not similar to bulk polymers. This is due to mechanical and chemical changes caused by the lasers. For example surface roughness of the laser ablated channels is higher than in structures fabricated by other methods. In some fluidic applications the ablated surface might be an advantage. As an example ablation causes more charged walls than other patterning methods supporting EOF better than normal polymer surfaces [144].

In laser ablation microfluidic structures are normally fabricated on the surface of polymers followed by enclosure of the channels with some method. The channel enclosure becomes problematic because of heating effects during the ablation. Laser causes transportation of melted polymer which makes the top surface of the polymer uneven. Furthermore heating by the laser can also cause warping of the substrate resulting in poor contact with bonded wafers. These problems have been controlled by reducing pulse length to femtosecond range that respectively reduces heating effects [52].

With highly focused laser beam it is possible to fabricate the channels directly inside polymers without necessity of channel enclosure afterwards [146]. Microchannels with sub micrometer cross-section were fabricated inside PMMA sheet with highly focused femtosecond laser. The method was also shown to be suitable for fabrication of 3D channels. The method has limitation in channel length, because decomposed material transport from the channel is diffusion-limited and therefore becomes slow in long channels. Fabrication was demonstrated with channels with the length of 100 μm. Most of the laser ablation articles have concentrated on basic channel fabrication and characterization. Applications using laser ablated polymer channels have been reviewed in [148]. Most applications are for electrophoresis [144, 149] or for dielectrophoresis [145] on microchip. Integrated LC-ESI microchips have been fabricated by laser ablation of Kapton and are commercially available [49, 150].

4.3 Polymer surface microfabrication

Parylene, poly(paraxylene), is polymeric material originally used as an insulator and as a protective coating in electronic devices. The main advantage of the material is deposition process resembling standard IC-fabrication. Parylene layers are deposited

with chemical vapor deposition (CVD). Parylene has been used for some micromechanical structures and lately it has been also used for fabrication of microfluidic devices. There are different formulations of parylene of which parylene C is the material mainly used for mechanical and fluidic applications. Parylene is mechanically strong and deposited layers have low stresses. Therefore it is well suited to overhanging structures like electrospray emitters for mass spectrometry [151, 152] and even for free-standing fluidic channels [153]. Parylene has also been shown to be biocompatible [157, 158]. For special applications functionalized parylenes have been directly CVD deposited to open microchannels [154]. By this method it is possible to achieve directly functionalized surfaces for fluidic components.

The fluidic chip fabrication of parylene is based on silicon surface micromachining processes followed by coating with parylene. Proven fabrication process gives large degree of freedom for designing highly integrated fluidic structures. For example electrophoresis channels with integrated fluorescence detection [35] and chromatographic channels both for GC [155] and LC [24, 156] have been fabricated from parylene. Integrated LC-ESI system has been also realized and demonstrated [50]. In biological applications parylene devices have been used for example for cell culturing [157] and as neural probes [158]. Enclosed channel fabrication is normally done by sacrificial enclosure method described in chapter 7.2. The removal of sacrificial material is very time consuming in the case of long microchannels [35]. Besides long fabrication time the cost of fabrication also becomes easily high, because additional processes with sophisticated equipment are required.

Similarly to parylene deposition, other polymer coatings have also been used for microfluidic devices. The range of different polymer coating is wide, but the most widely applied are super-hydrophobic coatings with Teflon-like materials. Teflon-like polymers can be chemically bound, spincoated, spraycoated or deposited by RIE on top of microfluidic chips [58, 159]. The actual fluidic structures under the coating can use the whole range of microfabrication techniques and materials. Therefore variety of possible structure shapes and applications is wide.

4.4 Polymer etching

Microchannels can also be realized by etching the channels to polymers. Oxygen plasma etches all organic materials. Etch rate depends on the polymeric material as well as on the etching tool used. Low molecular weight polymers can be etched relatively fast and correspondingly highly cross-linked polymers are etched slowly. Masking materials for etching are normally sputtered or evaporated metals. Etching profile is usually isotropic (semispherical) similar to glass wet etching. Plasma etching of polymers is rarely used for microchannel fabrication. The process becomes easily complex compared to other polymer microfabrication techniques and also increases the price of the fabricated microfluidic chips. Plasma etching to make microfluidic chips has been mostly used for PI and parylene [160, 148]. Application of polymer etching for microchip fabrication has been reviewed in [148]. The main application of plasma etching of PI films has been the fabrication of ESI source. PI channels for ESI are integrated with desalting membrane made of poly(vinylidene difluoride) [160, 161]. In another version of polymer etching pure argon is used for ion beam etching. This method has been used to pattern microstructures to Teflon-based materials [162].

DRIE is the only possible method for producing high aspect ratio structures to silicon or to glass. For polymers many other routes are possible for such structures. However, few groups have developed high aspect ratio etching for polymers [163, 164]. These methods are similar to silicon Bosch etching, based on alternating passivation and etching pulses. DRIE has been done for parylene, PMMA and for some photoresists. The method has its benefit in producing vertical walls to the polymer structures. In the case of PMMA aspect ratios of 20:1 have been reported [164]. However, the method is time consuming and there are alternative methods that result in similar structure shapes with easier process.

4.5 Other direct fabrication methods

One of the conventional ways for polymer processing is mechanical micromachining. It is suitable only for relatively large structures, but it can be used for fabrication of prototypes of microfluidic devices [165, 166]. For actual microchannel fabrication this method has been rarely used, but mechanical micromachining is standard method for master fabrication to polymer replication techniques.

Two different printing techniques have been demonstrated for making microfluidic devices. A 3D microfluidic network has been realized by direct-write technique using inkjet printing type of fabrication [167]. Network has been fabricated by inkjet printing and then the structure is completely covered with epoxy resin. The resin is cured followed by sacrificial removal of the ink from the channels. After removal of the ink, the desired fluidic channel paths have been created by in-situ UV-polymerization to block other routes. Functioning of the device was demonstrated in mixing applications. A xerographic process, applying laser printing for the microfluidic channel walls to polyester films has also been described [168]. Two printed films were bonded together forming enclosed channels. Electrophoretic separations were demonstrated with the device.

5 Replication techniques

Replication of microstructures to polymers has become very popular group of techniques for making microfluidic chips. Replication techniques have all the same principal idea about copying counterparts of the master to polymers. Main methods for replicating microfluidic chips are: casting, hot embossing and injection molding. Techniques differ dramatically from each other based on the processing conditions, polymer behavior, master material requirements, replication tools and fabrication time. For the fabrication time two different time concepts have to be defined: (1) the complete throughput time from design to ready chip and (2) chip replication time after finishing the master. Replication materials and methods have not been specifically developed for microfabrication, but are adopted from the field of macrofabrication. However, patternability of the standard polymeric materials is mostly good in the size range needed for microfabrication [169]. The most commonly used polymers in replication are PDMS, PMMA, PC and PS, but wide range of other polymers have been used as well.

Replication methods can produce large number of similar microchips fast and cost effectively. This becomes highly desirable if commercial fabrication of disposable microfluidic chips is planned. For academic research purposes usually the number of similar chips is relatively small and therefore replication methods, like injection molding or hot embossing, will loose their best benefits. Casting is instead popular method for fast prototyping of new microchip designs. With short throughput time it is

suitable for research of phenomena in microfluidics. Fast replication time of the techniques corresponds easily to simpler chips. Therefore fabrication of highly integrated microfluidic chips becomes difficult as comparison to direct fabrication techniques. Several comprehensive reviews have already been published about the polymer replication techniques and applications [169-171]. Therefore replication methods are only briefly described here to give an overview of possible polymer patterning techniques. The principal idea of the replication methods is shown in Figure 7. The process in the figure is taken from hot embossing, but all replication methods use a master to copy counterparts of it in to polymers. After the first cycle, the process can be started again and the master is used for second replication.

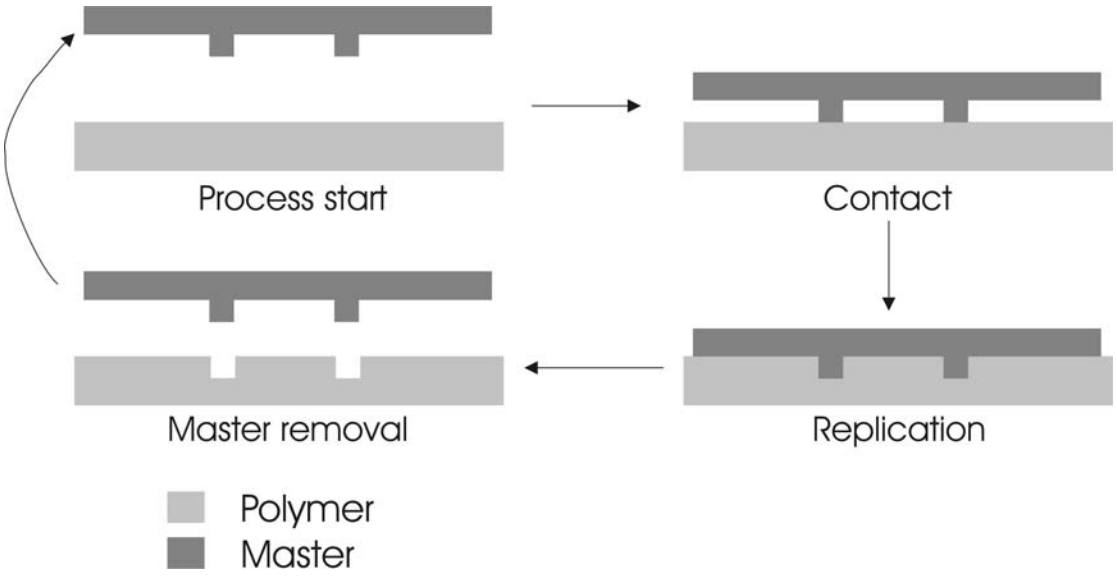


Figure 7 Schematic view of replication process for polymer microfabrication.

5.1 Casting

Casting (also called as molding) is widely used replication method shown in Figure 8. The main principle of casting is to pour either monomer or low molecular weight (M_w) polymer solution on top of a previously made master. Monomer solution is polymerized or low M_w polymer solution is cross-linked by curing. The master is released and the hardened polymer maintains the shape of the master counterpart. After the first cycle the master can be reused for next casting. Curing of the polymer is normally done by heating. In principle light-induced curing is possible, but not used for fabrication of microfluidics. Microfluidic structures fabricated with casting technique are especially done of PDMS [68, 172]. Processing conditions in casting are mild and therefore there is no need for special tooling. Normally curing is done simply on a hot plate. There is no force applied to the master and temperatures for curing are moderate (normally below 100 °C), therefore the masters for casting can be made of various materials.

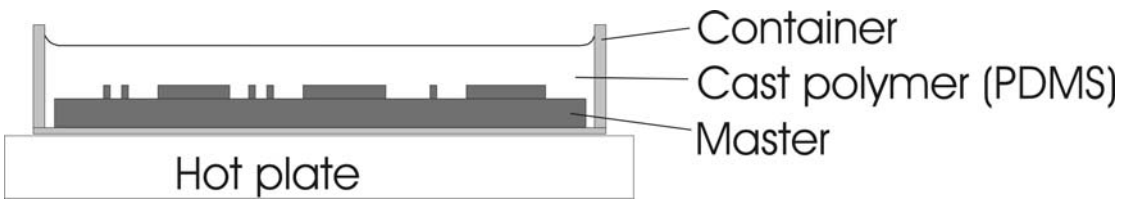


Figure 8 Principle of casting. Master is kept at the bottom of a container and polymer is cast on top of it. Curing is done on a hot plate.

Casting of PDMS has become one of the most popular methods in academic community to produce polymeric microfluidic devices and it has also been shown to be suitable for highly integrated devices [173]. Fabrication has become popular mainly because of easy fabrication process, but PDMS has also other good properties. First miniaturized electrophoresis device made of PDMS was published already in 1997. Replication was made with etched silicon master [172]. The use of PDMS became popular after presentation of rapid prototyping with lithographically made master [68]. Several types of photoresists have been used as a master because they enable faster and non-geometry dependent process for fabrication of PDMS-devices as contrast to wet etched silicon masters. Especially SU-8 has been used because of easy patterning, strength of the material and wider range of thicknesses than with most other resists. Lithographically defined SU-8 structures on silicon substrate can be used as masters for PDMS casting directly without any additional process steps. Uncoated SU-8 master has a limited lifetime, but it is suitable for testing of a new fluidic device design. Lifetime of SU-8 master can be prolonged with coatings that aid removal of SU-8 from PDMS like fluorosilanes [68] or diamond-like carbon [174]. PDMS has been patterned with other masters as well. For example etched silicon masters [172], electroplated metal structures [175] and PCB [176] have been used. These materials have longer lifetime than lithographically made masters, but are more expensive and might stick to PDMS. Coatings like chlorosilanes have been used to ease the releasing process [172].

PDMS is soft silicone type of polymer constructed of silicon-oxygen backbone. The material is elastomeric polymer, but hardness can be tailored to some extent with degree of cross-linking [177]. In casting the advantageous property of PDMS is that it is easily removable. PDMS adheres only reversibly to most flat surfaces (including PDMS itself). This also makes enclosure of the channels easy. Channel enclosure issues are handled in more detail in chapter 7. Optical features of PDMS are: good transparency for wavelengths above 230 nm and low autofluorescence, enabling various optical detection methods [172]. Gas permeability reduces staying of air bubbles in the channels and it also enables growing and handling of living cells in the enclosed fluidic reservoirs without need of transporting oxygen [178]. Biocompatibility of PDMS has been reported to be good enabling many biochemical applications [171].

Drawback of PDMS is instability. For example many organic solvents swell PDMS easily [VII]. In analytical applications PDMS absorbs samples and easily releases chemicals from the material itself, disturbing analysis. This can be seen easily in mass spectrometry applications [174]. Release of chemicals is emphasized if microchip has to be heated during the analysis [10]. Also the coefficient of thermal expansion might limit applications at elevated temperatures. High CTE can cause deformation of structures. The CTE value for PDMS is in the range of 310 ppm / °C according to manufacturer. Replication techniques are generally fast, but microchip fabrication of PDMS is rather time consuming compared to other replication techniques. Curing of PDMS can be done

within an hour [68], but the stability of the material can be increased if curing is done for longer times (for 48 hours) [174]. Despite slow replication process advantage of the material is fast throughput time. First microchip from design to ready chip can be accomplished within one day [68]. Therefore ideas for new microfluidic chip designs can be tested immediately. An example of PDMS microchip used for ESI is shown in Figure 9.

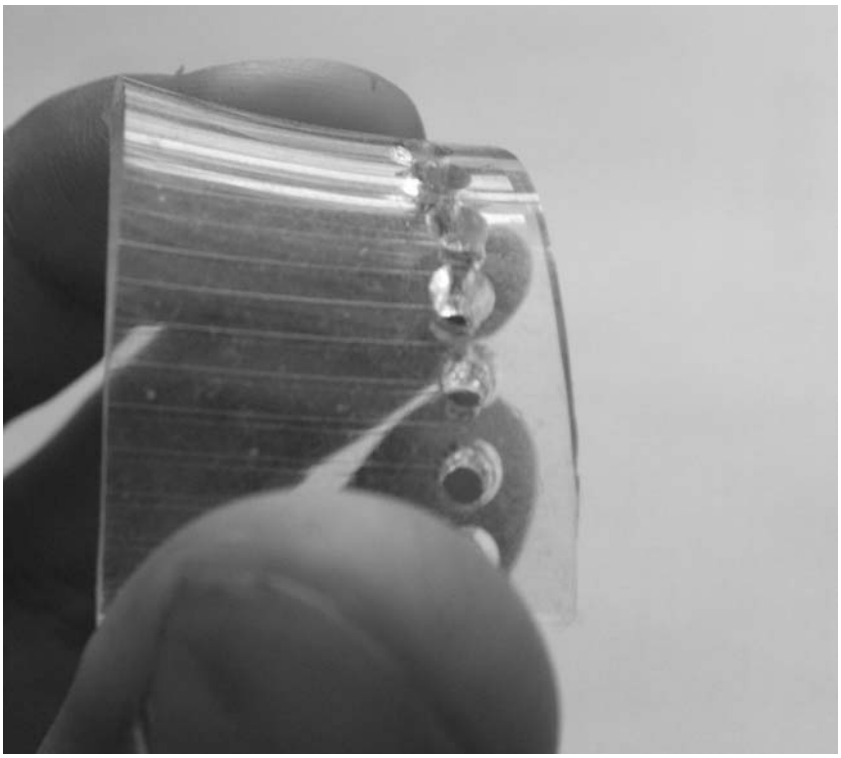


Figure 9 A PDMS microfluidic device bent between fingers. Elastic material enables bending without any damage to the device.

Besides PDMS, other polymer materials have been cast as well; for example epoxies [179], polyurethane [180] and polyester [181]. Epoxies are known to be thermally, mechanically and chemically relatively stable materials and therefore suitable for microfluidics. Electrophoresis microchips and PCR chamber have been fabricated by casting epoxy materials [179]. Casting of epoxy has been done with silicon masters. In contrast to PDMS, epoxy sticks eagerly to the master and therefore antisticking layers have to be used. For example parylene or Teflon-like polymer coatings have been used for silicon masters [179]. Due to easy fabrication process and non-sticking property of PDMS, it has been used also as a master material for casting of other polymers. Other benefits of PDMS master are transparency of the material and flexibility that makes visualization and handling of master during the casting process easier. However, softness of PDMS might cause deformation of the structures and high CTE of PDMS can also cause change in structure dimensions.

5.2 Hot Embossing

Hot embossing is a method that can be used for patterning of larger variety of polymers than casting. Principally all thermoplastic materials are suitable for embossing. However, printability of the microstructures is not as good with all polymers [64]. The most commonly used polymers are PS, PC and PMMA, but several others have been

used as well including even biodegradable polymers [182]. Hot embossing is also called imprinting. Especially if nanometer sized structures are replicated with this method it is called nanoimprinting lithography (NIL) [183, 184]. The main principle of hot embossing technique is shown in Figures 7 and 10. In hot embossing the master structure is pressed with force to softened polymer. Normally this is done by heating the polymer substrate above its glass transition temperature (T_g). Above T_g polymer chains can easily flow past each other and therefore material can be patterned by pressing the structured master to the polymer sheet. Force is applied until the sheet is cooled again under the T_g. After that polymer sheet preserves the embossed shape and the master can be released [185]. As an example of processing conditions for PMMA pressures of 6550 kPa and temperature of 155 °C have been used [64]. In another version of embossing, the master is applied to a monomer solution and curing is done either with heat or with UV-exposure [186].

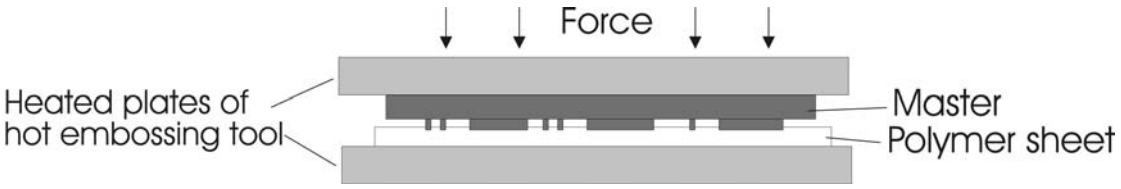


Figure 10 Principle of hot embossing. Master is pressed with force to heated polymer sheet. After full contact the plates holding the master and the polymer sheet are cooled and separated from each other.

Hot embossing, in comparison with casting, is suitable for replication of larger batches of similar microchips. Therefore method is more beneficial for commercial applications. Structures have been fabricated with simple laboratory-made tools, but if fast throughput of the method is required, commercial hot embossing tool has to be used. The main advantage of hot embossing in comparison with casting is shorter cycle time in the replication. Processing of one polymer plate can be done in a timescale of 10 minutes. However, the master fabrication for hot embossing is normally more expensive and time consuming as comparison to casting. Therefore the total throughput time is increased.

Lithographically fabricated SU-8 structures have been used for hot embossing of low T_g polymers [187] and cast PDMS structures have been used as masters for embossing microchannels to PMMA [188]. However, temperature and pressure requirements in hot embossing do not normally allow the use of polymeric masters. Thermal expansion induced changes and structure deformations with higher pressure are limiting especially the use of PDMS masters. Normally masters are done with mechanical machining of metal [189], silicon or glass etching [185, 190] or with electroplating [64, 189]. In the first generation of hot embossing the microchannels were defined with wires between PMMA and flat glass layer [185]. The method was cheap and simple, but alignment was random and it did not enable fabrication other geometries like standard channel crossings. In contrast to PDMS casting, sticking of polymer to the hot embossing master remains a problem. In some cases anti-sticking layers with low friction coefficient have been used to ease the releasing. These materials typically are fluorine containing polymers like Teflon [191] or fluorinated silanes [192] which provide hydrophobic surface and good thermal stability in temperature range required for hot embossing. An example of hot embossing master and hot embossed microchannels in PMMA are

shown in Figure 11. The master is four inch electroplated nickel master made by X-ray LIGA-technique.

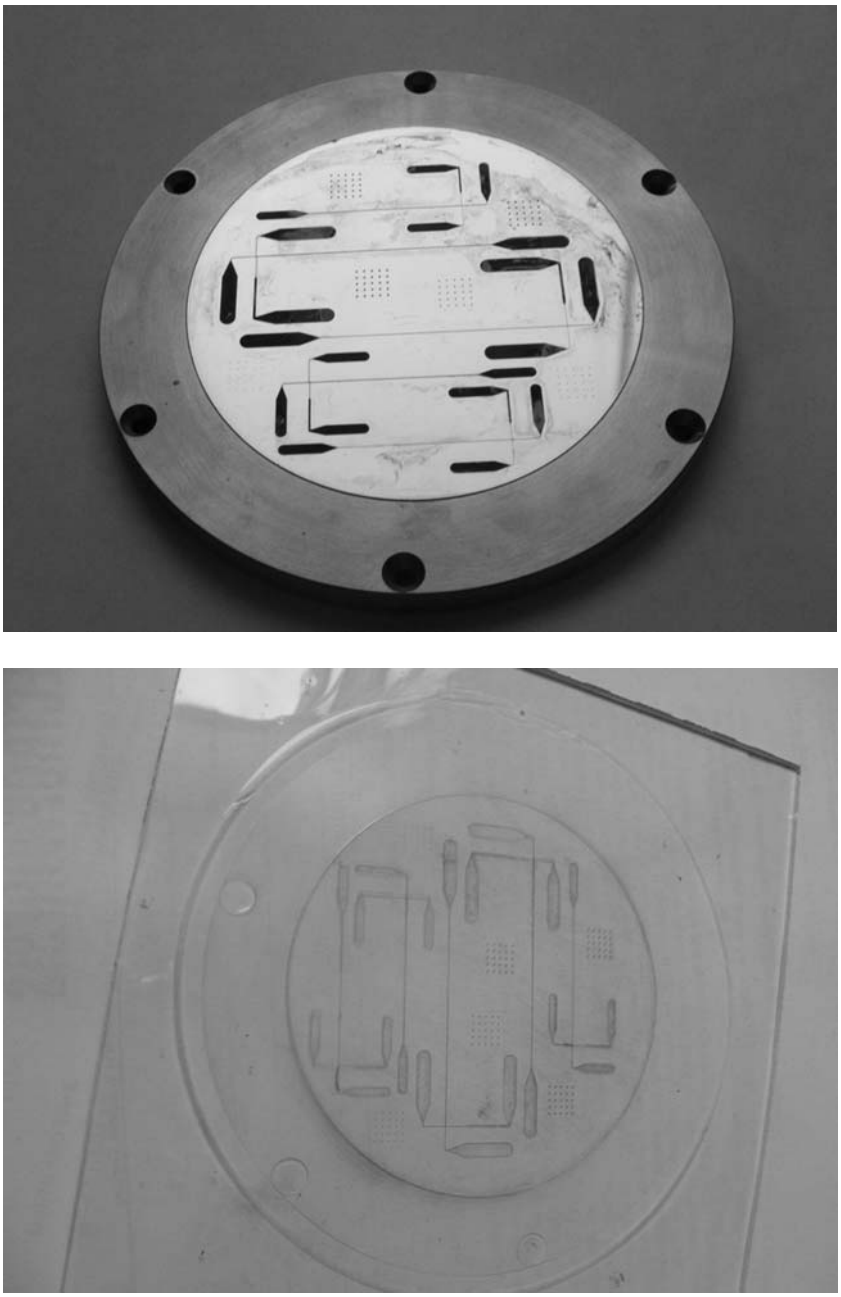


Figure 11 Hot embossing master made by X-ray LIGA technique of nickel and embossed channels in PMMA done with the same master.

Problems of hot embossing are related to the fact that only low aspect ratio structures can be patterned. The problem is related to the filling of narrow gaps. Air is easily trapped in the cavities of high aspect ratio structures in the hot embossing master rendering embossing difficult or impossible. Maximal aspect ratios are in the range of 10:1 for trenches, but individual pillars have lower aspect ratios [193]. Embossing through the polymer layer would lead to braking of the master or embossing tool and therefore it is avoided. Due to this fluidic inlet fabrication to embossed structures is difficult. Normally drilling of inlets is required. Geometries of the structures are limited by the master fabrication process, but mostly structures are with relatively simple

shapes compared with lithographic patterning. Multilayer structures are more difficult to fabricate and undercut structures are not possible. Also integration of other structures like electrodes is more difficult in hot embossed microchips.

5.3 Injection molding

In injection molding molten polymer is injected to an enclosed cavity containing the master. When hot polymer comes to contact with the room temperature mold, polymer cools down rapidly and hardens. After complete filling of the mold the hardened polymer structure can be demolded and new cycle can be started immediately. The main principle of the technique is shown in Figure 12. The method requires higher temperature than casting or hot embossing because the polymer needs to be melted. Temperatures are normally in the range of 200-300 °C. Pressure for injection is also high to ensure complete mold filling. Pressures are in the range of 1000 bar. Replication time of injection molding is very fast. In normal applications cycle time of one minute can be easily achieved, but simple chips can be produced in few seconds. Despite fast replication process micro-injection molding is still relatively rarely used method for producing microfluidic components [194-197]. One of the main reasons for this is that the throughput time from design to ready chip is the highest of the replication techniques. Also the price of injection molding tool is relatively high.

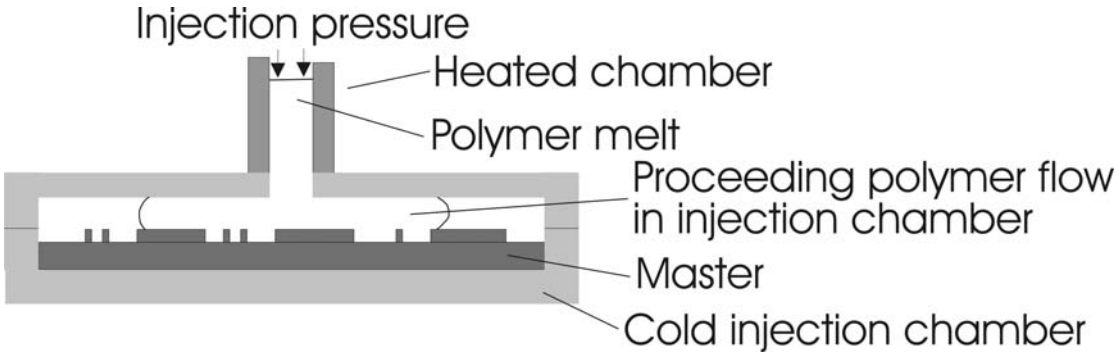


Figure 12 Principle of injection molding. Melted polymer is injected to a chamber containing the master. Polymer cools and hardens rapidly inside the cavity followed by chamber opening and removal of replicated polymer.

Injection molding of microstructures requires optimization of the structure design and the fabrication process. During injection molding the advancing polymer cools down very rapidly in the case of microstructures, because surface area to volume ratio is high and therefore heat transfer is efficient. Cooled polymer then restricts complete filling of the mold, leaving air gaps. Because of this aspect ratios in injection molding do not normally exceed 1:1. To avoid filling problems the mold can be heated up during the injection and cooled down before demolding. This enables polymer to flow more easily everywhere in the mold. Temperature ramping makes the method a mixture between hot embossing and injection molding and respectively increases the cycle time of replication.

High temperature differences during the injection molding cycle and high CTE values of polymers cause easily deformations to the molded structures. Polymer shrinkage has to be small to be able to reproduce structures accurately. Polymers that have been micro-injection molded are mostly PMMA, PS and PC. Master requirements are similar to hot embossing master, but both the master and the whole cavity have to be made of

material that tolerates high temperatures and pressures. Material selection for the master depends on the applied polymers and on the device for injection molding. However, good heat transfer coefficient is required to cool the master rapidly. Therefore mainly silicon or metals have been used as a master [194, 196]. Also temperature stable epoxies have been used as injection molding of PS and cyclo olefin copolymers (COC) [197]. For relatively simple low aspect ratio structures injection molding can provide the fastest fabrication time for large number of similar chips.

6 Summary of the channel fabrication methods

Existing polymer microfabrication techniques are numerous and the best fabrication method for each design has to be determined carefully. Polymer physical and chemical properties have to be determined as well for the application. Furthermore the number of chips required for the application will affect the selection. Direct fabrication is good for research purposes or for fabrication of relatively small number of the chips. Later, if a successful design is found and commercial application is contemplated, the process could possibly be transferred for some replication technique to minimize fabrication costs. Naturally the selection of the technique should be based on available processing tools and knowledge.

Direct fabrication methods can provide microstructures comparable to silicon or glass microfabrication. Range of possible geometries and structure widths and depths is wide. Processes are normally cheap and fast compared to silicon or glass fabrication producing similar structures. This holds true especially with lithographic patterning that requires only lithography process to produce such structures. Lithographic fabrication of one wafer for standard 50 μm channel depth can be accomplished within 2 hours, but several wafers can be processed simultaneously that is not possible with replication methods. Although processes can be used for fabrication of highly integrated devices, only few have been demonstrated with direct polymer microfabrication methods. Therefore fabrication of complex devices requires more process development than with silicon that has most processes already established. If direct fabrication techniques are compared with replication methods, possible structures and functionalities in the devices are more versatile with direct fabrication. First microfluidic chips can be accomplished also faster after the design than replicated chips because the master fabrication step can be eliminated from the process flow.

Replication techniques are good methods for producing large number of simple chips. After master fabrication similar chips can be realized in fast process. Methods are especially good when optimized geometry for fluidic device has been found and larger number of chips is required. Limitations in geometries and in level of integration have to be taken into account in design. However, more complex devices can be realized by combination of different techniques. In analytical applications the easily contaminated chips can be disposable replicated chip whereas the complex devices can be done in another platform that can be reused. Replication techniques can provide microchips with lower fabrication and material cost than direct polymer microfabrication. If compared with silicon fabrication the difference becomes even bigger. However, the advantage is achieved only if a larger number of chips are produced. This is due to master fabrication costs. Structure dimensions with different polymer microfabrication techniques are summarized in Table 2. In Table 3 are summarized some properties of most commonly used polymers in microfluidics. So far polymer properties for microfluidics have been reported incoherently for polymers with different fabrication techniques. Now the

studies concentrate mostly on one material or one technique [64, 170]. Therefore, special fluidic properties like chemical stability or biocompatibility for certain applications have to be studied case-specifically.

Table 2 Microstructure dimensions achievable with different microfabrication methods

Technique	Thickness	Min. structure widths	Aspect ratios max	Normal aspect ratios
Contact UV lithography	Up to mm	1 μm	100:1	10:1
X-ray lithography	Up to few mm	1 μm	1000:1	20:1
Electron beam lithography	Few μm	10 nm	2:1	1:1
Laser ablation	Up to ~100 μm	<1 μm	2:1	1:1
Polymer etching	Up to ~100 μm	few μm	20:1	1:1
Casting	Up to mm	10 nm	10:1	2:1
Hot embossing	Up to mm	10 nm	10:1	2:1
Injection molding	Up to mm	1 μm	2:1	1:1

7 Channel enclosure

Polymer microfabrication methods generally produce open microfluidic channels. Only in few special cases are readily sealed channels obtained [146, 198]. Open microchannels have been used in capillary filling systems [199], but applications using open channels are rather limited. Therefore channel enclosure must be done as the following process step after microchannel fabrication. Selection of channel enclosure methods for polymeric microfluidic chips is as wide as the selection for the fluidic structure fabrication. Furthermore, different methods for channel fabrication and enclosure can be combined. The optimal enclosure method for microchannels is such that all four walls of the microchannel would be of the same material. This ensures uniform flow profile which is especially important in electrophoretic applications as they rely on uniform channel wall charge and correspondingly uniform EOF. Chemically similar channel walls are also important for surface modifications after channel enclosure to ensure uniform binding of the coating material. This is especially crucial if surface coating is planned to be covalently bound. Polymeric channel enclosure methods are not generally producing hermetic sealing because polymers themselves are usually permeable to gases. However, hermetic sealing is not necessary in most fluidic applications as long as the sealing is impermeable to liquid. A comprehensive study of the polymer bonding techniques has been published recently [55].

7.1 Enclosure by bonding

The easiest method for channel enclosure is to press the channel chip against some flat substrate that has fluidic inlets drilled through it. This requires completely flat structural and sealing layers or one of the layers should be elastomeric material. The method results in low pressure tolerance and channels are often leaky. Therefore it is suitable only for first testing of new fluidic devices. A somewhat more sophisticated way to enclose any channels is to use PDMS. It forms a reversible bond to nearly any clean substrate. The bond does not tolerate high pressure and uneven surfaces or particles easily lead to delamination. Reversible enclosure makes channel fabrication easy and fast. It also enables easier cleaning and second use of the channels. However, repeated opening and closing rapidly leads to deteriorated bonding.

PDMS channels can be closed irreversibly with plasma activation of the surfaces. Plasma treatment also makes hydrophobic PDMS to hydrophilic. Water contact angle decreases from over 100° down to 30° with plasma treatment [68]. However, hydrophobicity of PDMS is destroyed in less than an hour in contact with air. In comparison with other polymers PDMS has an advantage of easy inlet fabrication. Inlets can be done during casting or they can be easily punched through the soft material after fabrication. Wide use of PDMS is mostly based on the simplicity. PDMS works well in certain applications but chemical compatibility of the material limits its application also as a sealing material.

Various adhesives have been used to irreversibly enclose microfluidic structures. Adhesives can be cured in room temperature or by thermal or UV assisted curing. Dispensing of the adhesive is problematic. Thick layers of adhesives cause easily microchannel blocking by the adhesive, while thin layers are difficult to dispense and require completely flat substrates for leak-free enclosure. The resulting channel has a ceiling covered by the adhesive. Therefore the method is comparable to the use of different materials for structures and for the enclosure that is not optimal. Furthermore adhesives can be toxic to biological samples limiting the application possibilities. Other properties like optical characteristics might change and some chemicals are easily dissolved from the adhesives disturbing sensitive chemical analyses [VI]. To improve bonding with adhesives technique additional structures have been used to hinder adhesive flow to the microchannels [200]. However, these techniques require complicated manual dispensing of the adhesive. Specially designed equipment has been used for dispensing thin layer of adhesive on top of structural layer without any adhesive in the channels [201]. This requires special equipment and extremely flat wafers for high bonding yield.

A basic bonding method for polymer substrates is to heat patterned and non-patterned polymer sheets or chips and apply high enough force to press those together. Softened polymers will be joined and during cooling they will stay bonded. The method is called thermal bonding or thermo-compression. The technique has been used to enclose mostly replicated microfluidic systems [64, 187], but also lithographically made SU-8 structures [99, 202] and parylene microchannels [153]. Polymer sheets are heated above the T_g (in the case of thermoplastics) or to temperature when polymer becomes reactive (in the case of cross-linked materials). Applied forces, temperatures and time used for the bonding have wide range depending on polymers and applications. For example PC chips have been bonded at 135 °C and with pressure of 0,4 MPa [203] whereas corresponding values for parylene bonding are 200 °C and 24 MPa [153]. Bonding can be done either with a commercial bonding system or by laboratory made tool. If alignment of the cover and structural layer is required, special equipment has to be used. Drawback with the method is structure deformation. When polymer sheets are heated above T_g microstructures can easily deform. This concerns mostly the microstructured wafer, but also the heated cover sheet can easily intrude inside the channels blocking them. Temperature, force and bonding time have to be optimized carefully to avoid this.

Solvent bonding is another method for producing microfluidic channels with all walls from the same material. This technique has been used as an enclosure method for hot embossed microchannels [204, 205], but also for lithographically made structures [70]. Solvent that dissolves the structural polymer is applied on top of the polymer sheets, dissolving the top layer of the polymer. Structural polymer sheet and cover sheet are

pressed together and sheets become bonded during evaporation of the solvent. The method has an advantage of low temperature and it does not require expensive equipment if alignment is not required. Solvent selection and time for the bonding has to be optimized for each polymer separately. Low temperature of this technique would theoretically enable deposition of sensitive materials like biological compounds to the channels, but normally the solvents are too strong for these molecules. As a drawback of the method solvent easily deforms structures, especially if smaller structures are combined with larger bonded areas. In the worst case the whole channel can be blocked by partially dissolved polymer. Short exposure times to the solvent have been used to reduce the problem [205]. In another method sacrificial filling of the channels during the bonding has been proposed. Channels are filled with paraffin wax that is removed after bonding via inlets [204]. This process requires multiple additional fabrication steps, but channel shape is retained better during the enclosure.

Adhesive bonding, as the term is used in this work, is a more sophisticated method compared to enclosure by adhesives. Adhesive bonding uses polymeric materials for the microfluidic channel enclosure that are identical to structural materials. In contrast to thermal bonding, the substrates are bonded by curing the adhesive layer while in thermal bonding the cover is already cured. Bonding occurs by cross-linking of adhesive layer to the structural layer. Therefore both pressure and temperature in adhesive bonding are generally lower. The technique has become an important wafer bonding method in MEMS and it has been used for enclosure of silicon and glass structures as well. Low temperature of adhesive bonding enables even entrapping of liquid inside enclosed cavities [206, 207]. Particles or uneven substrates are not fatal to bonding if the particle diameter is smaller than the adhesive polymer thickness. Unintentional structure blocking due to polymer flow is a drawback also in this method. To overcome channel blocking, lower bonding temperatures have been proposed [98]. This, however, increases non-bonded area and consequently channels leaking. Secondary structures together with optimized bonding temperature aid the bonding retaining the channel shape and still have low non-bonded area [II]. Other methods to decrease non-bonded area are to rely on loosely cross-linked bonding layers [100] or to cure thermally non-cross-linked polymers [102]. These techniques, however, do not allow patterning of the top layer that is possible with non-cured bonding layer [104, VI, VII]. Microstructure enclosed by adhesive bonding that has also the bonding layer accurately patterned is shown in Figure 6.

In adhesive bonding the curing of the adhesive layer can be done by temperature or by UV-illumination. In the case of UV-curing at least one of the substrates has to be transparent to UV-light. If temperature is used for the curing, the possibility of patterning the bonding layer is lost. Depending on the carrier substrate for the bonding layer, method can be called either adhesive bonding (rigid substrate) [97, 98, I-VI] or lamination (flexible substrate) [98, 104, VII]. Both methods can be done either with commercial equipment or manually without additional equipment. There are also some commercially available polymers designed for enclosure by lamination [85]. Adhesive bonding methods have been used mostly with direct fabrication techniques, but can be used also for replicated microchannels. For enclosing replicated microchannels these methods produce channels with cover material different from the walls. Microchannels with walls and cover of the same material have been closed by adhesive bonding techniques mostly to SU-8 [98-104, II-VII]. Examples of microchannels made fully of SU-8 are shown in Figure 13. For adhesive bonding of microchips with different

materials on the walls and on the cover the material selection is wide: epoxies, polyimides, fluoropolymers, esters, negative and positive photoresists and especially designed bonding adhesives have been applied [208-210].

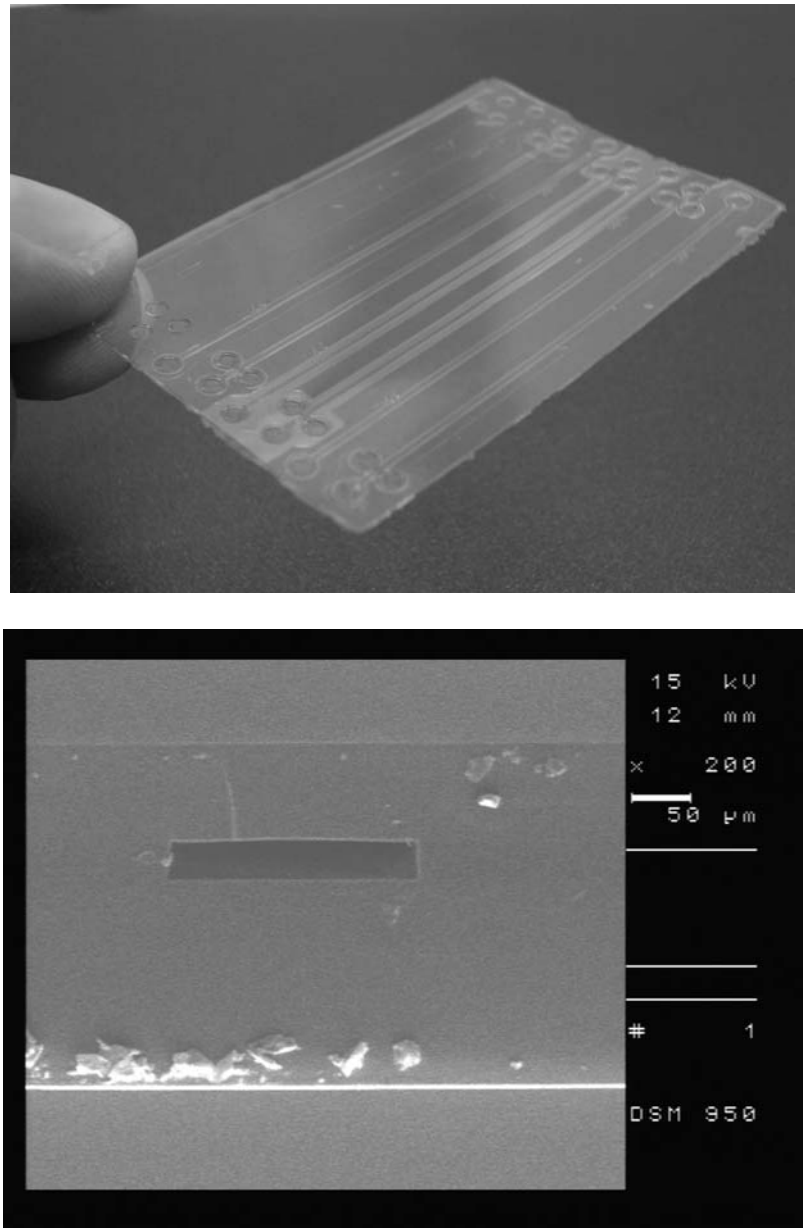


Figure 13 Fully SU-8 microchannels; (a) parallel enclosed microchannels made fully of SU-8 (b) cross-sectional picture of one channel made of SU-8. All three layers are SU-8.

More exotic methods for polymeric wafer bonding apply microwaves or laser energy. Microwave bonding requires polymer substrates to be transparent for the microwave energy and a thin absorbing layer at the bonding interface is needed. PMMA chips have been bonded using metal and polyaniline layers between the bonded substrates [166, 211]. Laser bonding (or laser welding) requires similarly cover to be transparent to laser light and some interface layer that absorbs light [212]. In both methods the energy absorbed at the interface is used to realize the bonding. Both methods require additional layers that may have an effect on the device performance. Furthermore, laser bonding is a slow method as the laser has to scan the edges of the channels. Both methods have been demonstrated in microfluidic fabrication, but have not been in wider use.

7.2 Enclosure with sacrificial methods

Channel enclosure with sacrificial techniques is also widely used method for fabrication of polymeric microfluidic structures. Sacrificial enclosure is mainly used with direct microfabrication methods. The idea is based on making the channel ceiling before opening the channels. The channels are then opened by removing the sacrificial material via fluidic inlets. The superior advantage of this method is that channel blocking by the sealing layer can be completely avoided. However, the method is very slow as sacrificial material removal from the microchannel is diffusion-limited. Removal rate is also highly dependent on the microchannel cross-section and material to be removed. Cross-sectionally smaller channels develop slower [84]. An important issue in this method is that sacrificially removed material has to be dissolved with a method that does not cause deformation to the structural polymer. Therefore material selections play a critical role in this method.

Microfluidic devices made of parylene are mostly fabricated with sacrificial methods [35, 151, 152]. Standard positive photoresist is used as the sacrificial layer prior to deposition of enclosure layer of parylene. Photoresist can be removed selectively underneath parylene with little deformation to structural layers. Removal times for the photoresists are long, of the order of 20 h for electrophoresis channels [35]. Despite long opening time the method has been used for successful fabrication of various fluidic structures from parylene.

For SU-8 microchannels various sacrificial removal methods have been proposed. They are mostly based on development of non-exposed SU-8 from the channels after enclosure [84, 97, 101, 213-215]. Exposure of the SU-8 inside the microchannels has been avoided by partial exposure techniques done with dose reduction. Only the roof SU-8 is exposed, but not the inside of the channels. Method has been realized with e-beam, UV and laser exposures [101, 213, 214]. Also metal deposition between the channel and cover layer of SU-8 has been used to cure only the ceiling layer [84, 215]. SU-8 development from centimeter long channels takes several days. Selectivity between exposed and non-exposed SU-8 is not as good as in the case of parylene and photoresist. This causes cracking and layer delamination in structural SU-8 as can be seen in Figure 14. This results in not well defined channels and therefore the method is usable only for very short microchannels [VI]. In alternative techniques more selectively dissolvable materials have been used in the SU-8 channels to improve channel quality after opening step. Channels have been filled with other materials prior enclosure [84]. Also HF-etching of manually assembled glass fibers has been proposed to produce round channels to SU-8 [216]. This limits channel positioning to manual accuracy and channel crossings are difficult to fabricate. PMMA lines in SU-8 have been exposed with X-rays in single exposure to produce channels. This works because PMMA is positive and SU-8 negative photoresist in X-ray exposures [217]. However, fabrication is complicated and expensive X-ray facility is required.

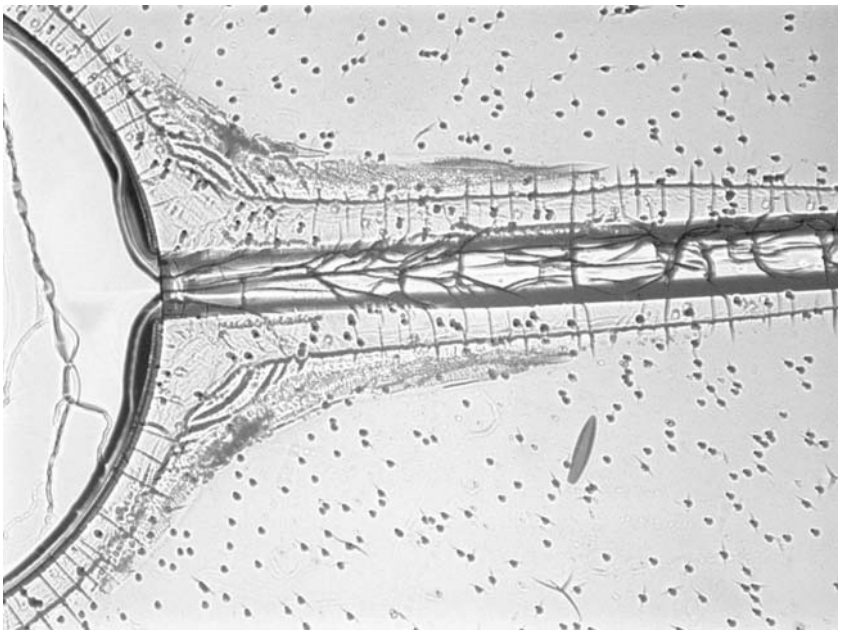


Figure 14 SU-8 channel done by development of non-exposed SU-8 in cured SU-8 channel via inlet. Cracking and delamination of cured SU-8 layers can be seen in the figure.

Besides wet chemical release of sacrificial material, thermal degradation / evaporation of sacrificial material, has also been used for fabrication of polymeric channels [218-221]. In this method thermally degradable polymer is used as a sacrificial material. Sacrificial polymer is coated with structural polymer. During final annealing the sacrificial polymer is decomposed and forms volatile compounds that can penetrate through the cover polymer. This reduces dramatically diffusion path length and therefore channel opening times are shorter. However, temperatures required for the decomposition are in the range of 200-400 ºC. These are extremely high temperatures for polymeric materials and therefore deformations easily appear to the structure shapes. These deformations can be reduced by optimization of the degradation process [219].

Thermally degradable sacrificial materials have mostly been different polycarbonates (polyethylene carbonate and polypropylene carbonate). Initially polycarbonate layers were patterned using metal hard mask and plasma etching [218]. Later photodefinable polycarbonates were developed to ease the process. Polymeric channels have been made with this method to PI, to BCB and to epoxies, [218-221]. The method enables fabrication of complicated multilayer structures, but only simple channels have been demonstrated. High temperatures reduce possibilities to integrate other functions to the microchips due to high CTE of polymers. The method has been rarely used in microfluidics.

7.3 Inlet fabrication to polymeric channels

Inlet fabrication to polymeric microchips is similar to glass chips in most cases. Drilling or etching the inlets to ready-made microfluidic chips is not an optimal method. The drilling depth is difficult to control. Stopping right after the cover sheet in micrometer scale channel requires accurate control. Dust and particles from the drilling easily intrude inside the channels causing blocking. Chips also easily break if the inlets are drilled close to each other. Enclosure after cover sheet drilling is possible for most polymer chips, but then the bonding requires dedicated equipment with alignment. Furthermore, after drilling the cover sheet can be slightly deformed making the bonding

more difficult. In an alterative method inlets can be brought from the sides of sawed chips, but then the height of the channels is limited by the outer diameter of the capillaries used for the connection [V].

The adhesive bonding method using UV-curable polymer utilizes the excellent alignment capability of a lithographic mask aligner. This reduces equipment cost and still makes accurate alignment of the inlets possible. Inlet fabrication with this method does not produce additional dust or particles and the thickness can be accurately controlled to cover layer only. Inlets can then be opened by releasing the polymer chip completely from the substrates [II, VI] or by releasing the substrate for the cover layer [104, VII]. UV-exposure of the inlets does not limit the spacing, the size or the shape of the inlets and all the fabrication steps can be done in a cleanroom to ensure microchips with no contaminating particles inside the channel. Fluidic inlets made lithographically to SU-8 microchannels are shown in Figure 15 (a). In Figure 15 (b) a fluidic connection done by bringing a capillary from the side of the chip is shown.

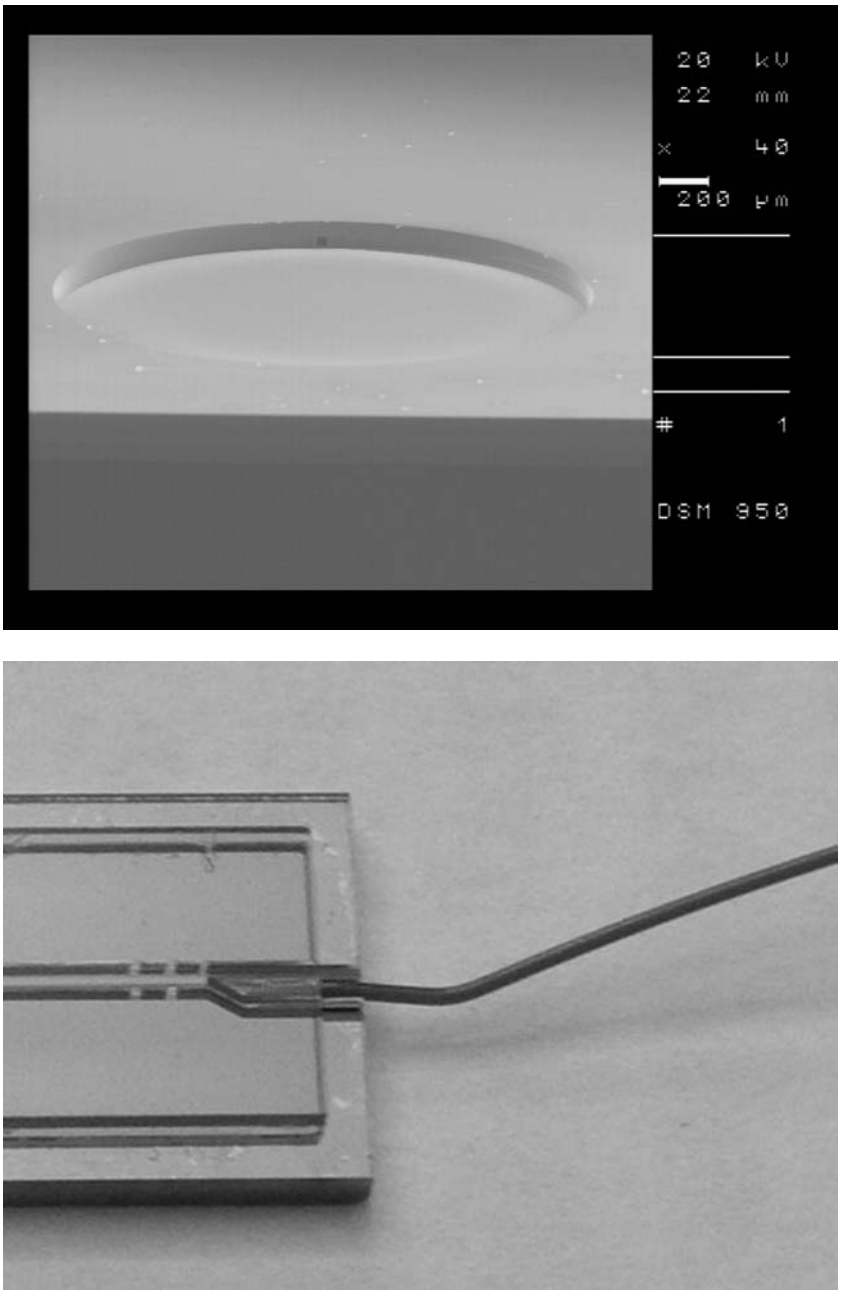


Figure 15 Two different inlet designs for a microfluidic chip. (a) An open reservoir where channel starts from the side of the reservoir. (b) Inserted capillary from the side of the sawed chip. Both chips are done of SU-8, but Figure (b) has silicon and glass wafers as supporting layers for the chip.

Table 3 Properties of most common polymers for microfluidic chips

	Thermal properties:				Optical properties:	
Material	**T_g [°C]**	**Melting/ degradation T [°C]**	**CTE [ppm/°C]**	**Thermal conductivity [W/mK]**	**UV-transparency**	**Visible light transparency**
PMMA	106	205	70	0,19	opaque	good
PC	148	230	65	0,19	>350 nm	good
PS	105	240	60-80	0,12	>300 nm	good
PDMS	-125	400	310	0.15	>230 nm	good
SU-8	240	>340	102	0,2	>350 nm	good
Polyimide	400	620	3	0,2	opaque	good
Parylene	150	290	35	0,084	>300 nm	good

	Chemical properties		Other		
Material	**Effect of organic solvents**	**Effect of acids/bases**	**EOF-mobility [cm^2/Vs]**	**Contact angle [°]**	**References**
PMMA	soluble to many solvents	acids affects	$2,07*10^{-4}$	73	[64], [222]
PC	soluble to many solvents	both dissolves	$2,22*10^{-4}$	80	[64], [222]
PS	soluble to many solvents	resistant	$1,54*10^{-4}$	94	[64], [222]
PDMS	swelling with many solvents	little swelling	$1\text{-}3*10^{-4}$	>100	[68],[223], [224], [IV]
SU-8	mostly no effect	very resistant	$4,5*10^{-4}$	85	[83], [84], [III], [IV]
Polyimide	mostly no effect	resistant	N.A.	50	[119]
Parylene	Very resistant	very resistant	$1*10^{-4}$	108	[155],[225], [226]

Polyimide values are for PI 2611

N.A. = not available

Summary of own papers

In this chapter the experimental results achieved in the articles of the thesis are shortly reviewed.

Paper I:

In this study the basic phenomena of wafer-level bonding with epoxy photoresist SU-8 were investigated. Bonding was done for two SU-8 layers on top of silicon and glass wafers. Bonding parameters were optimized to have complete sealing of the structures. Bonding of large areas and small pillars was investigated in wafer scale process. Bonding with SU-8 was found to have numerous excellent qualities. Bonding at temperature less than 100 °C enables application of various materials like metals or other polymers, enabling low cost MEMS structures with closed channels and cavities to be fabricated. Bonding process is straightforward and no special equipment is required. Mechanical strength of the bonds was good: breakage occurred at the SU-8 - silicon or at the SU-8 – glass interface, but not at the bond interface. As a drawback the microstructure blocking during bonding remained problem. Controlling of unintentional blocking was found to be difficult if low non-bonded area was as a target. Therefore the process yield was lower for structural layer thicknesses below 100 μm.

Bonding with non-bonded area less than 5 % was reproducibly demonstrated. Bonding of large, centimeter-scale, and small, micrometer-scale, SU-8 structures was successful on the same wafer. Pillars of 25 μm in diameter were successfully bonded with SU-8 as shown in Figure 16. Stability of high aspect ratio structure shape was found to be good if both bonded wafers were heated to the same temperature during bonding. This paper formed the basis of the future development for SU-8 microchannel fabrication.

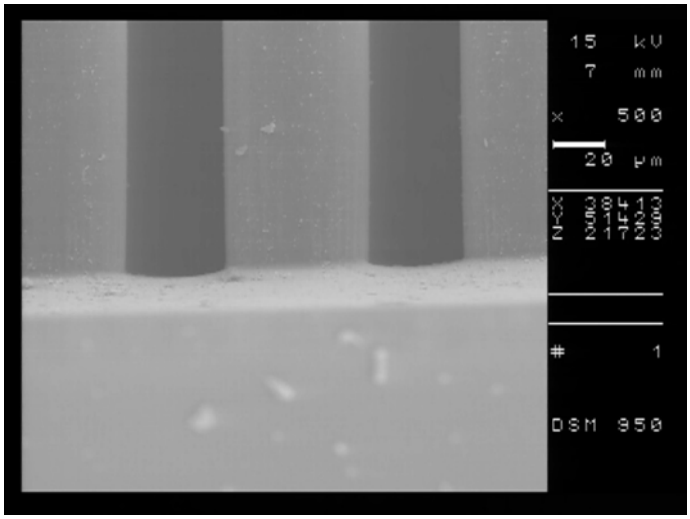


Figure 16 Bonding interface between small pillars and adhesive SU-8

Paper II:

In this paper the SU-8 bonding studies were continued with fabrication of fluidic channels fully of SU-8. The goal was to find parameters for SU-8 bonding that are suitable for all possible structural dimensions of the microchannels. We found out that stresses caused by the structural layer were the main reason for channel blocking noticed in [I]. Successful bonding for channel enclosure can be achieved by avoiding high stresses. This can be accomplished by selection of suitable bonding temperature slightly above SU-8 T_g and by controlled temperature ramp rates. Besides the optimal process parameters the process requires also auxiliary structures designed next to the main channels. Microchannels up to 6 cm long with heights between 10 – 500 μm were fabricated successfully. The bonding method described here is suitable for enclosing structures with various lateral dimensions on the same wafer with non-bonded area less than 5 % in wafer scale.

Furthermore, we found out that three-layer SU-8 chips can be removed from the substrates and those can be used as stand-alone microfluidic chips. The simplicity of fluidic inlet fabrication in fully SU-8 chips makes this fabrication scheme very attractive. By patterning the fluidic inlets to the first SU-8 layer and by releasing the structures from the substrate after bonding it is possible to fabricate fluidic inlets with lithographic accuracy. The combination of low unintentional channel blocking and low non-bonded area enables fabrication of long microfluidic chips required in many μ-TAS applications. By application of auxiliary structures described in this article, yield of 6 cm long microfluidic channels was 90 %; compared with 10 % yield without them, using identical bonding parameters. Initial fluidic experiments with capillary filling were made to test the usability of the channels. They showed promise of reproducible filling and reusability. Cross-section of 3-layer, fully SU-8 microchannel is shown in Figure 17 (a). Released fully SU-8 microfluidic channels are shown in Figure 17 (b)

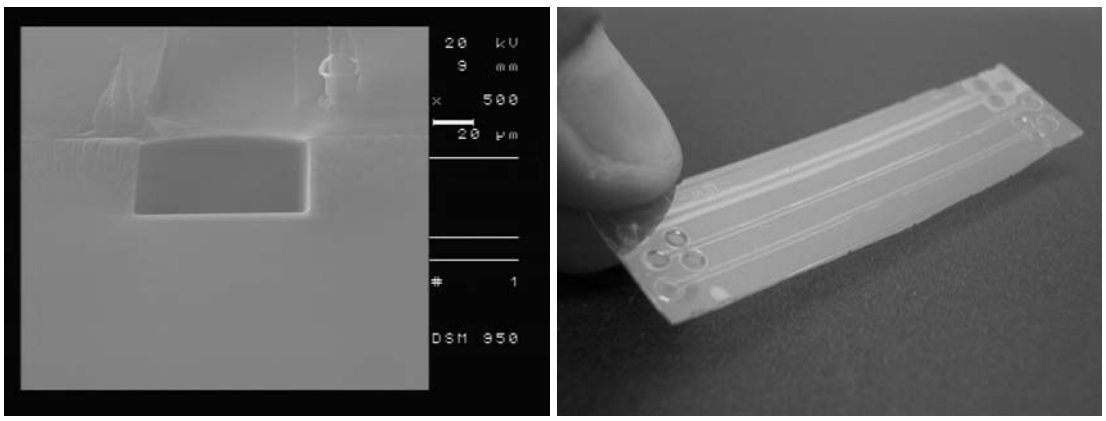


Figure 17 (a) Cross-section of microfluidic channel made fully of SU-8. (b) Released free-standing SU-8 microfluidic chip.

Paper III:

Since the first presentation of electrophoresis in a microchannel [25] the technique has become very popular separation method in the microchip world. Improved separation efficiency and reduced separation time in microscale have been driving the development of the technique. Polymeric electrophoresis chips have also been developed from various materials and with various techniques. In this paper fully SU-8 microchannels were used to determine the material suitability for electrophoresis. Although many earlier papers have proposed SU-8 channels for electrophoresis [101-103], this was the first paper measuring EOF in fully SU-8 microchannels. Measurements were compared with commercial glass microchips. SU-8 supports EOF mobility comparable to glass microchannels. This is very high value for polymeric microchannels and it promises fast separations in SU-8 microchannels. Clear pH dependence was noticed in SU-8 channels and furthermore the flow can be reversed in low pH values, which could enable new types of fluidic systems without need for additional coatings for the microchannels. The pH dependence of the EOF in SU-8 channel compared to glass channel is shown in Figure 18.

Reproducible results from EOF measurements in SU-8 channels show that channel fabrication method developed in [II] is suitable for fluidic applications. The EOF values were double checked with zeta-potential measurements with SU-8 particles in buffer solution. The particles were fabricated on silicon wafer with sacrificial aluminum layer. Releasing of the particles was based on our earlier work on SU-8 microparticle fabrication [227].

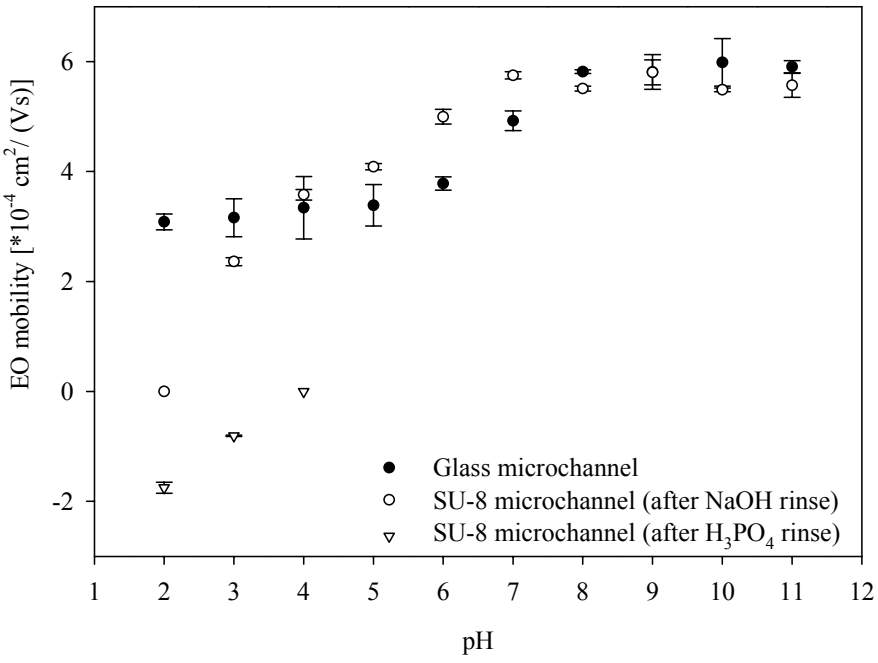


Figure 18 Results from EOF measurement in fully SU-8 microchannel at different pH

Paper IV:

In this paper the first electrophoretical separations using SU-8 microchannels were demonstrated. Separation of two fluorescent markers was performed in less than 30 s applying channels described in [II, III]. These first separation results promised successful application of SU-8 in electrophoretic applications. The channels showed fast separations and no interaction was noticed between SU-8 material and the analytical chemicals applied for the tests. An electropherogram of a separation done in SU-8 microchannel is shown in Figure 19.

The chemical stability of SU-8 was studied by immersing pieces of SU-8 to various chemicals up to 7 days. The results were compared to PDMS. SU-8 showed good stability for most chemicals. No interaction was noticed with the buffers applied for the separations. With some organic solvents changes occurred in long exposures to the chemicals, but in short exposures the SU-8 material was intact. Therefore the material stability was determined to be good for analytical applications.

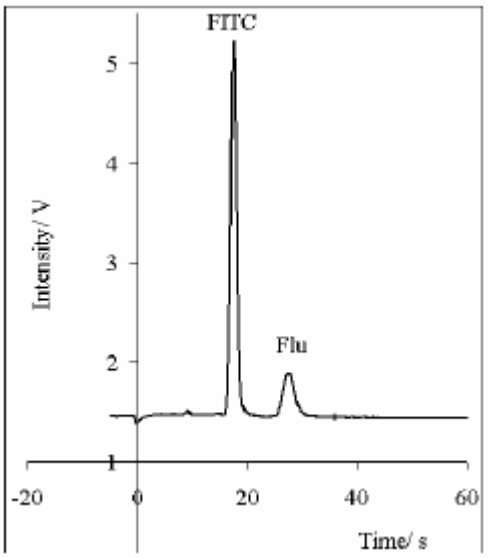


Figure 19 An electropherogram of separation of 50 μM fluorescein and its isothiocyanate derivative in SU-8 microchannel.

Paper V:

The knowledge gained from papers [I, II] was used to fabricate electrophoresis chip with an integrated SPE-reservoir. The microchip was designed, fabricated and tested. High aspect ratio SU-8 pillars were used as bead stopping filter to create SPE reservoir. Pressure driven flow was used to move liquid through the extraction channel and therefore the open inlet method described in [II] was not used. Fluidic capillaries were brought from the sides of the sawed chips. This determined the height of the SU-8 channel for extraction. Electrophoresis channel was made in the second (50 μm thick) SU-8 layer ensuring suitable channel size and all walls from the same material. Two layer patterning of SU-8 was developed so that alignment accuracy for high aspect ratio pillars could be achieved. All microstructures were made of SU-8 and silicon and glass were used as supporting wafers only. A finished microchip is shown in Figure 20 (a). In Figure 20 (b) is shown detailed view of the SPE area of the chip.

Fabrication process was optimized for high yield of the SU-8 chips. The combination of shallow electrophoresis channel and high aspect ratio pillars made the fabrication difficult. Yield of separation chips with 5 cm long electrophoresis channel and high aspect ratio pillars was 75 % after process optimization. Fluidic and electrical behavior of the chip was simulated. According to the simulations pillar-type bead filter shows uniform fluid flow which ensures that all the matrix in the SPE-reservoir is available for the extraction. From the simulations it can also be noticed that electric field is uniform in the reservoir and therefore the electrokinetic transportation of the analyte from the reservoir to the electrophoresis channel occurs as desired. Detection from the microchip was made with LIF through the cover glass and bonding layer of SU-8. Fluidic tests with fluorescein samples showed successful extraction, elution and detection of the analyte on the SPE-ZE chip.

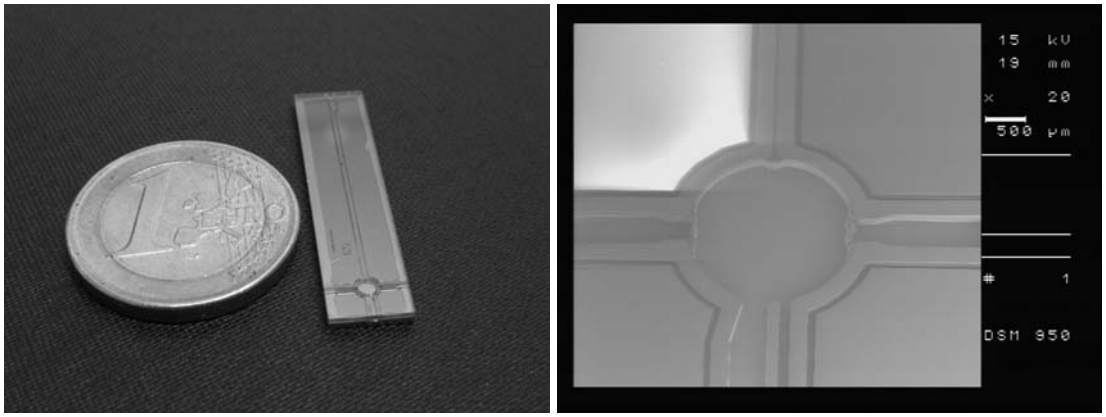


Figure 20 (a) A ready SPE-ZE chip fabricated of SU-8. (b) SEM picture of the SPE-part of the chip. Extraction channels are from right to left and electrophoresis channel goes upwards in the picture.

Paper VI:

In fabrication of ESI emitter microchips for MS the biggest problem has been in patterning of nozzles accurately. Widely applied technique based on manual assembly of ESI capillaries is a laborious approach and spray directly from glass chips is not successful due to hydrophilicity [45] as hydrophilic surface destroys the Taylor cone. Replication methods for polymeric ESI chips mostly require manual processing after microfabrication to realize the tip for the chip [196, 190]. In this paper we demonstrated the first enclosed SU-8 ESI tips. Earlier versions have been open to air being prone to sample evaporation and poorly suitable for pressure driven flows [106-108]. The process described in [I, II] was developed further to be able to pattern all three layers of SU-8. Masked exposure through the glass wafer and development via lanes enabled third layer patterning in a fast and simple process.

Tips with cross-sectional microchannel sizes between 10 μm *10 μm and 50 μm *200 μm were fabricated and tested successfully. MS tests were carried out with both pressure driven and electroosmotic flows, both showing stable performance. With electroosmotic sample transport stable spray is maintained in the timescale of tens of minutes, but with pressure driven flow stable functioning of the tip is extended to hours. Tips with microchannel cross-section of 50 μm * 80 μm and larger showed the most stable performance with RSD values repeatably less than 10 %. Due to accurately defined tip and hydrophobicity of the material Taylor cone and generated spray are well defined. SU-8 was shown to be a good material for analytical applications because no background was noticed from the material itself even at low m/z values. Fully released SU-8 microtips are shown in Figure 21.

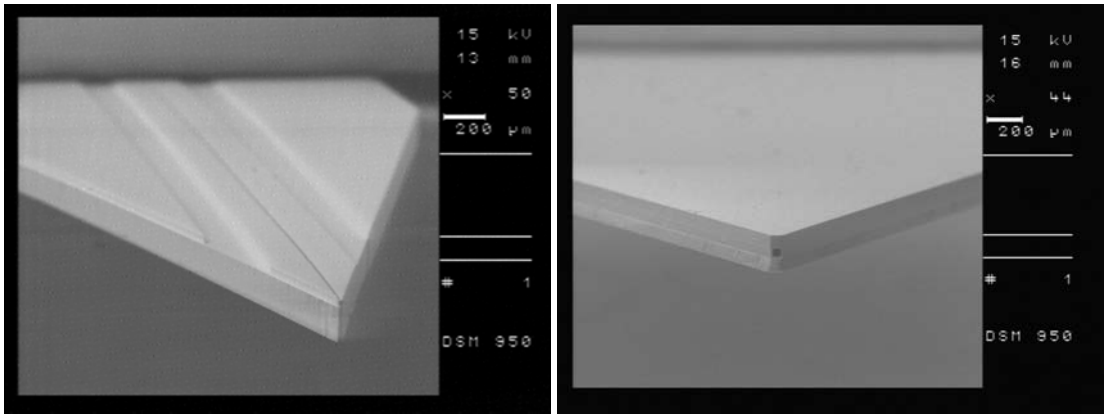


Figure 21 ESI tips made of SU-8; (a) Open channel before enclosure and (b) ready enclosed ESI tip.

Paper VII:

In this paper the fabrication process for the enclosed SU-8 ESI tips was improved. Channel bonding was done applying a polymer sheet instead of a rigid glass wafer. After exposure and curing of the bonding layer, the polymer sheet can be peeled off from the SU-8 structures enabling development of the third layer directly without lanes used in [VI]. The method reduces material costs because the sacrificial glass etching is eliminated. Application of thin polymer sheet as a substrate improves also the accuracy of third layer patterning because gap between photomask and exposed SU-8 is reduced from 500 μm to 100 μm. Integration of platinum electrodes inside the SU-8 structures was realized. Electrodes were patterned by lift-off after the first SU-8 layer patterning. After metal patterning the other two SU-8 layers were patterned similarly as in the basic process.

Different shapes of the tips were tested to optimize tip shape. Shape of the tip and size of the microchannel were explored according to results from mass spectrometric measurements. Stable electrospray from SU-8 tips was demonstrated reproducibly with both pressure driven and electroosmotic flows. Tips were characterized by signal-to-noise ratios and stability of total ion current (TIC). Also the reproducibility of the analysis was tested. In Figure 22 is shown the shapes of few novel designs of tested ESI-tips.

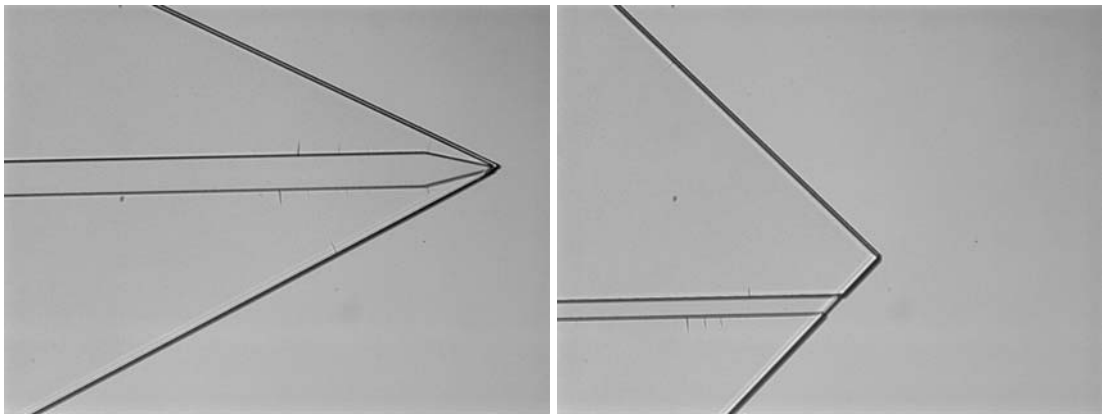


Figure 22 Two of the novel channel and tip geometries tested in MS measurements.

Conclusions and outlook

Microchannel fabrication of SU-8 is straightforward process to make cross-sectionally rectangular channels. Both depth and width of the channels can be varied in the range from micrometers to millimeters. High yield from the microchannel fabrication enables complex microfluidic networks or parallel fluidic systems. The process was demonstrated for three-layer SU-8 structures, but adhesive bonding process using removable bonding substrate enables fabrication of complex multilayer fluidic systems.

Fabrication process is relatively easy and therefore SU-8 microchannels offers good platform for studying new types of microfluidic systems. Besides simple channels with wide range of geometries, SU-8 enables integration of high aspect ratio pillars, electrodes and other additional functions to the microchips. Therefore fluidic systems with various functions can be realized.

First electrophoretic separations were made using SU-8 microchips. SU-8 showed good properties for electrophoresis. EOF-mobility was high in the SU-8 microchannels and therefore additional coatings which have been used with many other polymer electrophoresis chips are not required. More detailed separation performance studies with SU-8 microchannels are currently being done.

ESI from the microchips works well because accurate ESI tips can be fabricated of SU-8. Taylor cone is small in volume and the electrospray is well funneled. Stability of the electrospray is good both with and without pressure assistance for driving the flow. Furthermore, SU-8 as a material is stable and compatible with most analytical solutions. Good results from the ESI-MS detection enables further development for integrated separation systems with MS detection. Electrophoretic or chromatographic separations could be combined together with MS detection without major differences in the fabrication process. This integration is a topic of our future research.

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