Fabrication and characterization of micro/nano filter for isolation of waterborne pathogens

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**Fabrication and Characterization of
Micro/Nano Filter for Isolation of
Waterborne Pathogens**

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School of Mechanical & Aerospace Engineering

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Abstract

Water plays a central role in our lives, and access to safe and clean water is essential for each person. Water destined for human and industrial use is required to be quality tested for a wide variety of contaminants, including potentially harmful microorganisms. Life-threatening diseases caused by some waterborne pathogens such as *Cryptosporidium parvum* oocysts are largely associated with contaminated drinking water supply. The highly infectious nature of *C. parvum* oocysts and the lack of effective medication until now urge a reliable routine test to monitor *C. parvum* oocysts contamination in the drinking water supply system. However, the *C. parvum* oocysts contamination level of concern in drinking water is far below the sensitivity of most current detection technologies, such as flow cytometry, immunological methods or polymerase chain reaction assays (PCR). As a result, a rapid and effective method for concentrating *C. parvum* oocysts present in a large volume of drinking water is critical for accurate detection and quantification. Filtration-based concentration techniques have been widely used for isolation and recovery of *C. parvum* oocysts into small volumes for downstream analysis. Negative features of commercial filters such as rough surface, tortuous pore path and low pore density are the major factors which compromise their efficiency, lower their throughput and prolong processing time in microfiltration processes.

Micro-fabricated membranes that contain pores with the same size and shape can overcome these micro-structural defects. The fabrication process allows enough flexibility to control the porosity and pore geometry according to the desired application in order to

have a higher flow rate, lower clogging ratio, better recovery and sufficient reliability. In addition, a straight instead of a tortuous pore path helps preventing accumulation of particles inside the pores. Existing techniques for fabrication of screen-type microfilters suffer from major impediments such as membrane folding (curling), membrane failure and adhesion upon release from the mold.

The central topic of this thesis is the development of a novel microfabrication technique (i.e., *dissolving mold technique*) to produce a polymeric microfilter which has advantages over existing membrane fabrication methods. Firstly, it resolves completely the demoulding problem by dissolving the polymer pillar mold; secondly, folding (curling) of the membrane upon release from the mold is also solved with this method by bonding the membrane to a support mesh before dissolving of the mold. This process is low-cost and high-yield and can be used to make a molecular filter with a sub-micron pore diameter. The resulting microfilter has micro-structural features such as high pore density, uniform pore size, smooth surface, and straight pore path.

In addition to the dissolving mold technique, two different techniques, which take advantage of multi-level lithography and MEMS techniques were also proposed for fabrication of polymeric and metallic micro/nano filters. Surface treatment processes using an oxygen plasma and wet chemical etching were carried out on the micro-fabricated filters to improve their performance during microfiltration. The measured features of contact angle, surface roughness (AFM analysis) and chemical composition of the surface (XPS analysis), both prior and after the surface modifications, are highlighted. In this context, three important characteristics of micro-fabricated membranes (i.e., high throughput, high recovery ratio and low turbidity of eluent) were addressed and

appropriate experiments for proving these hypotheses performed. In addition, we demonstrated the benefit of micro-fabricated membranes with identical pore sizes as a promising tool for studying fouling phenomenon for different pore geometries. We have also shown the use of transparent polymeric micro-fabricated filters as a useful tool for direct observation through the membrane (DOTM) applications.

Lastly, design and fabrication of an automated filtration system for isolation and recovery of waterborne pathogens is presented. Results of some preliminary genetic tests using our fully integrated bio-chip is also depicted.

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Nomenclature

A	Area, [m ²]
A_m	Membrane area, [m ²]
C	Concentration, [g/l]
C_b	Bulk foulant concentration, [g/l]
CV	Coefficient of variation, [-]
C_1	Constant of integration, [-]
C_2	Constant of integration, [-]
D	Flexural rigidity of the plate, [N·m ²]
D_{eff}	Effective flexural rigidity of the plate, [N·m ²]
d	Pore diameter, [m]
E	Young's modulus, [Pa]
E_{eff}	Effective Young's modulus, [Pa]
$f(\epsilon)$	Porosity function, [-]
g	Acceleration due to earth gravity, [9.81 m/s ²]
h	Membrane thickness, [m]
J	Filtration flux, [l/m ² h]
$J_{critical}$	Critical flux, [l/m ² h]
K	Relative perforated area, [-]
L	Length, [m]
M	Mean pore diameter, [m]
$M_r \text{ and } M_t$	Bending moments, [N·s]
n	Filtration constant, [-]
n	Number of pores, [-]
$P = (J/\Delta p)$	Permeability, [l/m ² hbar]
(P_{max})	Maximum pressure, [Pa]
P_w	Maximum water permeability, [m/Pa.s] or [l/m ² hbar]

P_{latex} / P_w	Relative permeability, [-]
ΔP	Pressure drop, [Pa]
Q	Intensity of load (distributed load), [N]
Q_0	Initial volumetric flow rate at time t=0, [m ³ /s]
q	Volumetric flowrate, [m ³ /s]
R	Radius, [m]
R_m	Membrane resistance, [1/m]
R_p	Resistance of the foulant deposit, [1/m]
Re	Reynolds number, [-]
ρ	Density of the fluid, [kg / m ³]
t	Membrane thickness, [m]
t'	Filtration time, [s]
u, v	Velocity components in the x, y direction, respectively, [m/s]
V	Volume, [m ³]
w	Maximum deflection
x, y	Coordinates parallel and normal to the vertical plate, respectively, [m]

Greek Symbols

α	Pore blocking parameter, [m ² /kg]
η	Kinematic viscosity, [Pa.s]
σ	Standard deviation, [m]
σ_{yield}	Yield strength, [Pa]
ν	Poisson's Ratio
μ	Viscosity of liquid, [kg /m.s]

Subscripts

i	Node index in the x direction
j	Node index in the y direction
P	Constant pressure

List of publications

Patents:

- 1- **Majid E. Warkiani** and Hai-Qing Gong "A multi-layer polymeric micro-filter with slotted pores" US Patent (2011), filed, Application no: 201106927-5.
- 2- **Majid E. Warkiani** and Hai-Qing Gong "A microporous metallic micro/nano filter with integrated back-support" US Patent (2011), filed, Application no: 201106802-0.

Journals (published)

- 1- Longqing Chen, **Majid E. Warkiani**, Hao-Bing Liu, Chao-Ping Lou, and Hai-Qing Gong, "Polymeric micro-filter manufactured by dissolving mold technique", *J. of Micromechanics and Microengineering*, 20 (7), art. no. 075005, 2010.
- 2- **Majid E. Warkiani**, Longqing Chen, Hao-Bing Liu, Chao-Ping Lou, Zhang Rui, and Hai-Qing Gong, "Capturing and recovery of *Cryptosporidium* oocysts with polymeric micro-fabricated filter", *J. of Membrane Science* 369, pp.560-568, 2011.
- 3- **Majid E. Warkiani**, Chao-Ping Lou, and Hai-Qing Gong, "Fabrication and characterization of microporous polymeric micro-filter for isolation of *Cryptosporidium parvum* oocysts", *J. of Micromechanics and Microengineering*, 21(3), art. no. 035002, 2011.
- 4- **Majid E. Warkiani**, Chao-Ping Lou, and Hai-Qing Gong "Fabrication of multi-layer polymeric micro-sieve having narrow slot pores with conventional ultraviolet-lithography and micro-fabrication techniques", *J. of Biomicrofluidics* 5 (3), art. no. 036504, 2011.
- 5- **Majid E. Warkiani**, Hai-Qing Gong, A.G. Fane and F. Wicaksana "Effects of membrane pore morphology on fouling behaviour of polymeric microfabricated membrane during crossflow micro-filtration", *Technical Proceedings of the 2011 NSTI Nanotechnology Conference and Expo, NSTI-Nanotech 3*, pp. 569-572, 2011.
- 6- **Majid E. Warkiani**, Hai-Qing Gong, and A.G. Fane, "Surface modification of micro/nano-fabricated filters", *J. of Key Engineering Materials* 508, pp.87-98, 2012.
- 7- **Majid E. Warkiani**, Liu-Hoabing, Chao-Ping Lou, and Hai-Qing Gong "A high-flux isopore micro-fabricated membrane with integrated back-support for isolation of waterborne pathogens", *J. of Biomedical Microdevices*, pp.1-9, 2012.

Journals (in preparation)

- 8- **Majid E. Warkiani**, Khoo Bee Luan, Ali Asgar S. Bhagat, Hai-Qing Gong, A.G. Fane, C.T Lim and J. Han, "Isopore micro/nano engineered membranes and their applications: A review", *in preparation*.

9- Majid E. Warkiani, Chao-Ping Lou, Hai-Qing Gong, and A.G. Fane, "Isolation and recovery of *Cryptosporidium parvum* and *Giardia* (oo)cysts from drinking water samples using a micro-engineered membrane", to be submitted to the J. of Biotechnology and Bioengineering.

10- Majid E. Warkiani, Liu-Hoabing, Chao-Ping Lou, and Hai-Qing Gong "A fully automated machine for isolation and detection of *Cryptosporidium parvum* oocysts from tap-water", to be submitted to the J. of Sensors and Actuators B.

11- Majid E. Warkiani, Hai-Qing Gong, A.G. Fane and F. Wicaksana "Investigation of membrane fouling at pore scale using isopore micro-engineered membranes", submitted to the J. AIChE.

Conferences:

1- Majid E. Warkiani, Hai-Qing Gong and A.G. Fane "Surface modification of polymeric micro/nano-fabricated membranes", ASPT 2011, 1-2 December, Sendai, Japan.

2- Majid E. Warkiani, Hai-Qing Gong, A.G. Fane and F. Wicaksana " Direct observation of particle deposition on the surface of micro-fabricated membrane during crossflow microfiltration", ICOM 2011, 23-29 July, Amsterdam, The Netherlands.

3- Majid E. Warkiani, Hai-Qing Gong, A.G. Fane and F. Wicaksana " Effects of Membrane Pore Geometry on Fouling Behavior of High-flux Micro-fabricated Membranes", MST 2011, 24-26 August, Singapore.

4- Majid E. Warkiani, Longqing Chen, Hai-Qing Gong, "Fabrication of polymeric microfilter with dissolving mold technique*", AMN-APLOC 2011, 5-7 January, Singapore.

**This paper got the "best student paper award" from the conference organization.*

5- Majid E. Warkiani, Longqing Chen, Hai-Qing Gong, "A polymeric micro-sieve*", NanoMemCourse 2010, 7-16 April, Enschede, the Netherland.

**This paper got the "best student paper award" from the conference organization.*

Chapter 1

Introduction

A **membrane** is a permeable or semi-permeable material, which restricts the motion of certain species. In this chapter, a brief review of membranes and their applications is presented and the major **drawbacks** of commercial membranes are also addressed. Additionally, the motivation and the **objectives** of this research are expressed.

1.1 Membranes

A membrane is a permeable or semi-permeable, solid phase (polymer, inorganic or metal), which can be employed as a barrier for selective transport and separation of chemical/biological species [1,2] or can be utilized as a stencil for cell-patterning [3] and synthesis of nano-structures [4]. It can also serve as a platform for use in

controlled, long-term, protein-delivery devices [5]. Micro/nanoporous membranes with hydrophilic pore environments are extremely ideal for specific biological applications, such as antibody or enzyme immobilization [6]. Isopore membranes can be employed as a short-pass filter in optical devices [7] or can be integrated inside the lab-on-chip devices for blood fractionation [8]. Purification of water and clarification of beer are two current technologies that could realize significant improvements from the incorporation of micro/nano porous membranes [9]. These applications are summarized with a schematic diagram in Figure 1.1.

Figure 1.1: An illustration listing the different applications of a membrane. The types of applications can be classified into various categories (not exhaustive) such as micro/nano filtration, molecular sieving, drug delivery, photonics, tissue engineering, and microfluidics.

Performance of a membrane is normally defined in terms of flux and retention (and/or selectivity) [1,2,10]. Flux is the volumetric (mass or molar) flow rate of fluid passing through the membrane per unit area of membrane per unit time. Selectivity is a measure of the relative permeation rates of different components through the membrane. Retention normally defined as a fraction of solute in the feed retained by the membrane [10,11].

1.2 Membrane processes

The main mechanisms of separation in industry are summarized in Table 1.1. The success of using membranes is closely related to the intrinsic properties of the membrane. The interactions between fouling materials and membrane surface, nature of feed and solutes govern membrane performance to a great extent [12]. These interactions have a considerable impact on transport characteristics, selectivity, fouling tendency, and biocompatibility of the membrane [10].

Table 1.1: Main membrane separation processes arranged according to the mechanism of separation [10].

Separation mechanism	Membrane separation process
Size exclusion (filtration)	Nanofiltration (NF), ultrafiltration (UF), microfiltration (MF)
Solubility/Diffusivity	reverse osmosis (RO), gas separation, pervaporation, liquid membranes
Charge	electrodialysis

1.2.1 Filtration through size exclusion

The different separations achieved by membrane filtration are classified on the basis of their separation threshold. This is shown in the following illustration (Fig. 1.2).

Figure 1.2: Schematic of different filtration methods. Reverse osmosis offers the finest degree of separation, followed by nanofiltration, ultrafiltration, and microfiltration, which has the largest pore size [13].

1.2.1.1 Microfiltration

Microfiltration (MF) is the oldest process in membrane technology that has been employed for various applications such as bacteriological analysis of water, sterile fruit juices and wine, and aseptic pharmaceuticals [12,13]. In microfiltration (MF), some of the target solutes or particles to be retained comprise mainly microorganism, proteins, yeast cells, and also synthetic latex particles or colloidal silica. The approximate size of such components ranges from 0.05 to 10 μm , and they can be filtered at pressures ≤ 2 bar [10,14].

Two different operation modes of microfiltration are: dead-end and cross-flow. Both

use a pressure drop across the membrane as a driving force for permeation. The simplest design is the dead-end operation mode (see Fig. 1.3), where the feed flow is perpendicular to the membrane. The retained particles build up in time on the membrane, forming a cake layer, which increases the resistance to filtration and causes the permeate flux to decline [15].

Figure 1.3: Schematic of dead-end-filtration mechanism [2].

In 1907, a group of researchers found that in the filtration of suspensions and particles, a flow parallel to the membrane surface can enhance the filtrate flow and prevent the concentration polarization (i.e., gradual build-up of non-permeating or slowly permeating components near the surface of membrane during filtration) for longer periods of time [2,15]. Afterward, this method of filtration was called cross-flow (or tangential flow) and has been used in the past decades as a successful alternative to dead-end. The shear exerted by the feed flowing parallel to the membrane surface can sweep the deposited particles towards the retentate side so that the cake layer remains

relatively thin (see Fig. 1.4). In dead-end filtration, the cake grows constantly and causes a severe flux decline. In the case of cross-flow microfiltration, however a cake layer is built up slowly. Eventually, a steady state condition is reached because the transport of particles to the cake layer is in equilibrium with the back transport of particles into the feed stream [2,12,15].

Figure 1.4: Schematic of cross-flow filtration mechanism [2].

1.3 Membrane preparation methods

In the filtration industry, membranes are usually classified as either porous (or microporous), as applied in MF and UF, and non-porous (solution-diffusion membrane) as applied in gas separations and also Reverse osmosis (RO) [2,16]. In the membrane industry, the most important methods which are normally used for fabrication of membranes are: (1) stretching, (2) phase inversion, (3) sintering, (4) track-etching, (5) template leaching, and (6) coating [10,15]. Membranes made with these methods normally have either a symmetric or asymmetric structure. Each of them has its advantages and disadvantages in terms of pore-size distribution, surface

properties, homogeneity, fabrication cost, etc. Figure 1.5 shows SEM photos of commercial membranes made with abovementioned techniques.

Figure 1.5: SEM photos of (a) Envirochek standard, (b) Envirochek HV, (c) Cellulose, (d) Glass fiber type, (e) Teflon filter, and (f) Track-etched polycarbonate membrane filters [17].

The best homogeneity in terms of pore-size distribution and pore shape in commercial membranes is possessed by the track-etched membranes (normally made from polycarbonate) by cylindrical pores, but the irregular array of pores on the surface, low porosity and also their angle with the surface limit the strength, flow rate and reliability of them on the process-scale [10].

1.4 Applications

Microfiltration is currently used in a very wide range of applications in food (e.g., protein and bacteria filtration), pharmaceuticals (e.g., antibiotic production), biomedical (e.g., blood filtration), biological (e.g., drug delivery), microelectronics and chemical industries. Membrane processes are also used extensively in the treatment of wastewaters and effluent streams, both industrial and municipal. Some of the existing commercial MF processes are summarized in Table 1.2 using the basic classification scheme [14].

Table 1.2: Application of microfiltration in different industries [14].

Concentration
<ul style="list-style-type: none"> • Proteins (enzymes, milk protein, egg white), mammalian cell harvesting • Polymer lattices
Recovery
<ul style="list-style-type: none"> • Electro-deposition paint from wash/rinse bathes • Textile sizing agents
Clarification
<ul style="list-style-type: none"> • Fruit juices and wine • Beer (yeast and bacteria removal) • Sugar refining (removal of colloidal impurities, proteins)
Effluent treatment
<ul style="list-style-type: none"> • Oily wastewaters • Bleach effluents in pulp and paper industry
Purification
<ul style="list-style-type: none"> • Blood fractionation • Separation of antibiotics or vaccines from fermentations broths

1.4.1 Hygiene and safety of drinking water

Water safety has been a critical issue in many parts of the world, which causes more attention paid on the cleanliness of drinkable water [14]. Water destined for human consumption and industrial use is required to be quality tested for a wide variety of contaminants, including potentially harmful microorganisms [18]. Life-threatening diseases caused by waterborne pathogens like *C. parvum* oocysts are largely associated with a contaminated drinking water supply. The highly infectious nature of *C. parvum* oocysts (and other pathogens) and the lack of effective medication until now urge a reliable routine test to monitor their contamination in the drinking water supply system [17]. However, the *C. parvum* oocyst contamination level of concern in drinking water is far below the sensitivity of the most current detection technologies, such as flow cytometry, immunological methods or polymerase chain reaction assays (PCR). Consequently, a rapid and effective method to concentrate *C. parvum* oocysts present in a large volume of drinking water is critical to their accurate detection and quantification in drinking water. Filtration-based concentration techniques have been widely used to recover *C. parvum* oocysts into small volume for downstream analysis [17].

1.5 Major drawbacks in commercial MF membranes

Based on the literature review and SEM analysis, it was realized that commercial membranes, which are usually used for microfiltration processes suffer from major micro-structural defects. These deficiencies in their structure, which are mainly caused by their fabrication process, lead to some major drawbacks such as low water flow rate and poor cell recovery rate. The micro-structural defects in commercial membranes

include: (1) a tortuous pore path; (2) rough surface; (3) low pore density and large pore-size variation [10,17].

1.6 Micro-fabricated membranes

Micro-fabricated membranes contain pores with the same size and shape and therefore, are short of the micro-structural defects of the conventional membranes. The micro-fabrication process allows enough flexibility to control the porosity and the pore size and shape according to desired application (e.g., bacteria isolation and recovery) in order to have higher flow rate, lower clogging ratio, better recovery and enough reliability. In addition, a straight instead of a tortuous pore path helps prevent accumulation of particles inside the pores. Figure 1.6 shows a schematic comparison between pore-size distribution of commercial and micro-fabricated membranes (Note: It may not apply to the track-etched membranes).

Figure 1.6: Schematic of pore-size distribution in commercial and micro-fabricated filters [17].

1.7 Objective

The main objective of this work is to develop a new method for fabrication of isopore micro/nano filters of high-flux using micro-fabrication techniques. The micro-fabrication process allows enough flexibility to control the porosity, pore size and pore shape of the filter according to desired application in order to reach higher flow rate, lower clogging ratio, better recovery and sufficient reliability. The novel method aims at low-cost and high yield and can solve the existing obstacles in current micro-fabrication techniques. The micro-fabricated filters will be used for isolation and recovery of waterborne pathogens like *Cryptosporidium parvum* oocysts or other parasites.

1.8 Scope and goals

- To make a novel polymeric/metallic microfilter with microfabrication methods (i.e., *dissolving mold technique, multi level lithography and electroplating techniques*) in order to achieve a high flux, high recovery ratio and low turbidity level of eluent during microfiltration.
- To solve the obstacles in existing microfabrication techniques such as membrane folding (curling), membrane failure and adhesion upon release from the mold.
- To fabricate a strong microfilter that endures high pressures during microfiltration using appropriate materials and fabrication techniques.
- To characterize and evaluate their performance with standard characterization and testing methods.

- To use the micro-fabricated filters for isolation and recovery of *Cryptosporidium parvum* oocysts from large volumes of water samples in a short period of time (e.g., less than 1 hour) and perform genetic analysis using molecular approaches.
- To improve the performance and productivity of the micro-fabricated filters using well-known surface modification techniques.
- To use the micro-fabricated filters as a new tool for investigation into membrane fouling at the pore scale with appropriate model particles like latex.
- To use the micro-fabricated filters as a new tool for direct observation through the membrane (DOTM) applications and investigation into the concept of critical flux.

1.9 Report overview

This thesis is organized in the following manner:

Chapter 1 introduces the microfiltration (MF) membranes in details and points out the major drawbacks in their use, followed by the scopes and goals of the present study. In the first part of Chapter 2, the advantages and disadvantages of the existing methods for fabrication of screen type microfilters are discussed and major impediments in their use for mass production are indicated. In the second part, fouling phenomena, which is the most important obstacle in the membrane industry for liquid feed streams is reviewed briefly, and the available methods for mitigation of fouling are discussed.

A novel method (i.e., *dissolving mold technique*) for fabrication of polymeric micro/nano filters, which could thoroughly solve the existing problems in current fabrication methods is described in detail in Chapter 3.

In the Chapter 4 another approach for fabrication of polymeric micro/nano filters with integrated back-support is investigated. This method takes the benefit of conventional

UV-lithography and MEMS techniques, which are widely used in the semiconductor industry.

Chapter 5 illustrates another low cost method for fabrication of metallic micro/nano filters using multilevel lithography and nickel plating techniques. These filters have a long life time and can be used for harsh conditions where chemical cleaning is required. An integrated back-support mesh helps the membrane to withstand high pressures during microfiltration. Analytical and experimental investigations on deflection and maximum load of these membranes are also carried out. An appropriate correlation based on modified Young's module theory for the metallic micro-fabricated filters is derived and FEM analysis for validation is carried out.

Chapter 6 summarizes the details of surface treatment processes carried out on the micro-fabricated filters to improve their performance during microfiltration. The measurement details of contact angle, surface roughness and chemical composition of the surface, both prior and after the surface modification, are highlighted.

In Chapter 7 three important characteristics of the micro-fabricated filters (i.e., flow rate, recovery ratio and turbidity of eluent) are investigated in detail. Numerous tests are performed to evaluate the performance of the micro-fabricated filters in terms of flux, recovery rate and turbidity of eluent. The results are compared quantitatively with available commercial microfilters for the same purpose.

In the first section of Chapter 8 we demonstrate the benefit of micro-fabricated membranes with identical pore sizes as a promising tool for studying fouling phenomena for different pore geometries. In the second part we demonstrate the use of polymeric micro-fabricated filters as a useful tool for direct observation through the membrane (DOTM) applications. Design and fabrication of an automated filtration system for isolation and recovery of waterborne pathogens are also presented in

Chapter 9. In addition, we also describe the fabrication process of a novel bio-chip and an integrated genetic analyzer for extraction of DNA from *Cryptosporidium* oocysts and subsequent PCR process.

Lastly, Chapter 10 presents a summary of the findings and experimental work conducted during this Ph.D research. Future research work that might be undertaken is also described.

2

Literature review

Micro-fabricated filters, mainly screen filters, are different from other microfiltration membranes. Their unique properties open the way to new separation possibilities. In the first part of this chapter an effort is made to explain what makes them so special and what are pros and cons of their fabrication methods. In the second part fouling phenomena, which is the most important obstacle in the membrane industry, is reviewed briefly, and available methods for mitigation of fouling are discussed.

Part 1

2.1 Microfabrication technologies

Two types of membranes are distinguished in microfiltration: screen filters and depth filters [1,15]. Screen-type filters have straight-through pores and the separation process happens at the membrane surface. All particles with sizes bigger than the pore diameter are retained while particles smaller than the pore size can pass through the pores. Track-etched polycarbonate membranes and Anodisc filters are two examples of screen filters [2,10]. Conversely, depth filters have a random, tortuous pore structure and retain particles by trapping within their microstructures. Schematics of both membrane morphologies are depicted in Figure 2.1.

Figure 2.1: Schematic comparison of (a) a screen filter, and (b) a depth filter.

Immense efforts are nowadays being deployed in developing new membrane materials and designs to replace conventional polymeric and ceramic membranes used in traditional MF applications. Recent developments in micro/nanotechnology have provided novel techniques for controlling the detailed microstructure of membrane materials, allowing the fabrication of membranes with precise pores. Therefore, membranes with identical pores (quasi two-dimensional) and a completely controlled geometry can be achieved [10].

In the following sections we review the current methods for fabrication of screen-type isopore microfilters in details. The advantages and disadvantages of each fabrication process will be discussed accordingly.

2.1.1 Fabrication of inorganic isopore membranes with silicon micromachining techniques

The first silicon nitride (Si_3N_4) membrane with precise pore dimensions was developed by Ogura et al. [19] in 1991 to investigate the deformability of individual red blood cells (RBCs) by simulating the passage of RBCs from capillaries. Afterward, Kuiper and co-workers [20,21] at University of Twente devoted comprehensive investigations for development of silicon nitride membranes (so called microsieves), employing them for various industrial applications. Figure 2.2 schematically shows the fabrication process for the realization of thin silicon nitride membranes. In the first step, silicon nitride (1 μm thick) was deposited on a thick polished silicon wafer by means of low-pressure chemical vapor deposition (LPCVD). Then, a photoresist layer was formed on the nitride layer by spin coating. This layer was patterned with small holes ($> 1 \mu\text{m}$) by UV lithography through a glass mask and followed by appropriate development. The pattern in the photoresist layer (array of circular holes) was transferred to silicon nitride layer by RIE (reactive ion etching) with a CHF_3/O_2 -plasma, followed by a backside etching with KOH-solution to make the support bars beneath the nitride layer [20]. Figure 2.3 shows an SEM photo of a membrane obtained by this method [22]. It can be seen that the membrane surface is very smooth and the pores are perfectly ordered. Due to the resolution limit of conventional mask lithography, projection of small features ($< 1\mu\text{m}$) in the photoresist is technically impossible [21]. To solve this problem, Kuiper et al. [23] used a laser interference lithography technique to transfer the desired pattern into the silicon nitride thin layer. Figure 2.4 gives a schematic representation of a typical holographic set-up for interference lithography [24].

Figure 2.2: Fabrication process of a microsieve with pores larger than 1 μm [20].

Figure 2.3: SEM images of a silicon nitride microsieve surface. (a) Sieve bed, (b) pore grid, (c) close-up the pores, and (d) cross-section of the porous layer [22].

Figure 2.4: Interface lithography set-up used to produce submicron grating [24].

Part of an incoming plane wave will be reflected by the mirror and interferes with the undisturbed part of the wave to form an interference pattern (grating) on the substrate surface. After the beam splitting, each beam travels the same optical path before interfering in the region where the sample is placed. After exposure with laser and transferring the pattern to the photoresist layer, this pattern transferred into holes by evaporating a thin layer of chromium (15-20 nm) onto the substrate and removing the dots in the acetone bath (lift-off process). Similar to the previous section, the obtained pattern of the lift-off process transferred to the silicon nitride layer by an RIE process [25]. An impediment in this method is bubble formation during the anisotropic etching with KOH solution [20]. This problem caused by hydrogen molecules may damage the thin nitride layer by their explosion. To solve this problem, silicon was first etched through the pores (with SF_6/O_2 plasma) to form the bars and then, similar to the previous section, back-side etching was performed by KOH (25%, 75°C) solution. Figure 2.5 (a) shows an SEM photo of the resulting membrane supported by silicon

bars. Figure 2.5 (b) illustrates the microsieve breakdown because of bubble formation during the fabrication process.

Figure 2.5: (a) SEM photo of a microsieve after etching with an SF_6/O_2 -plasma through the pores, (b) membrane failure during the fabrication process caused by hydrogen bubble explosion [20,21].

Inorganic micro-fabricated filters have been used for various applications such as particle filtration [26,27], emulsification [28] and protein filtration [29]. Identical and uniform pore-size of the resulting membranes with this technique allows them to have low transmembrane pressures (TMP) and large fluxes (10 times higher than commercial membranes), but restrictions in the membrane material (limited to nitride) and the small thickness of the silicon nitride film ($< 1 \mu\text{m}$), which allows only low working pressure ($< 2 \text{ bar}$), limit the application of this type of filtration membrane. In addition, these microsieves have to be made in a clean-room environment (i.e. the entire process) with expensive equipments; hence, most of its applications will be in small scale systems and in the filtration of precious liquids [21].

2.1.2 Fabrication of polymeric isopore membranes with phase separation micromolding

The principle of phase-separation micromolding (PS μ M) was first introduced by Vogelaar [30] as a new generic approach for microstructuring various materials [31]. This method can be used to structure a broad range of polymers without the need for clean-room environments. This method basically relies on phase separation of a polymer solution which is cast on a mold. Intrinsic shrinkage, which happens during the phase-separation process, helps in release of the replica from the mold [30]. A schematic representation of the PS μ M process is illustrated in Figure 2.6 [30]. In this process a layer of polymer solution is applied to a mold which possesses micrometer-sized relief profile on the surface.

Figure 2.6: Schematic representation of the phase-separation micromolding (PS μ M) process [30].

The polymer solution is solidified by either thermally induced (change of temperature of the solution) or liquid-induced phase separation (immersion in a non-solvent). During the solidification stage, the polymer assimilates the profile of the master mold and the polymer microstructure is easily peeled off from the master mold. Phase-separation micromolding (PS μ M) can also be used for polymeric microsieve fabrication [32]. For this purpose, the master mold contains pillars that perforate the polymer film. The master mold features such as the pillar shape, diameter and support dimensions are critical for the final microsieve morphology. In this process phase separation occurs in two stages: vapor-induced phase separation (VIPS) and liquid-induced phase separation (LIPS) [32]. During solidification, shrinkage of the polymer happens in three directions: lateral (in-plane) and thickness-wise. Thickness shrinkage takes place to a larger extent than lateral shrinkage, and it allows perforation of the film. A volatile co-solvent is usually added to boost shrinking during the first-stage of polymer solidification. After the solution is applied on the mold, the volatile additive evaporates causing a reduction in the polymer film thickness [22,32]. The contraction of the film towards the mold structures (thickness shrinkage) will result in perforation and a completely open microstructure will be obtained. Lateral shrinkage facilitates the loosening and easy release of the replica. Figure 2.7 shows SEM photos depicting the fabrication process.

Figure 2.7: SEM photos of polymeric microsieve fabrication [22].

Figure 2.8: SEM images of a membrane with broken pillars in its structure [22].

By employing this method a variety of polymers can be used to fabricate polymeric microsieves with different pores sizes, but this technique is not viable for mass production due to some technical limitations. The major difficulty is related to the

adhesion of polymer to the master mold upon release, which can damage the membrane in this stage. In addition, failure of the pillars in the silicon master mold is another obstacle, which occurs due to the sticking of the polymeric membrane to them [22]. Figure 2.8 shows a released membrane with broken pillars inside the pores. Pore enlargement and membrane curling are the other impediments, which are associated with this technique.

2.1.3 Fabrication of polymeric isopore membranes using aperture-array lithography

In another attempt for fabrication of polymeric isopore microfilters, ion beam aperture-array lithography was employed to produce through-hole membranes with ideally ordered cylindrical pores [33]. In this method a stencil mask having an array of circular pores is irradiated by helium ions (30keV) that pass through the mask pattern to expose a photoresist on a substrate placed near the mask. The schematic representation of the aperture-array lithography system is illustrated in Figure 2.9. This set-up consists of a duo-plasmatron ion source, which can generate He^+ ions with 30 keV acceleration, for purpose of exposure. The total exposure dose is measured by a picoameter and a Faraday cup that are both connected to a software for online monitoring [33]. The membrane material used in this study was polyimide, which was spin-coated on a silicon wafer followed by sputtering of a thin silicon oxide layer (0.07 μm) on top. Then, a thin PMMA layer was spin-coated on the top of the SiO_2 layer that is used as a mask for RIE etching (CHF_3) after patterning by ion-beam lithography in the next step. Following removal of the oxide layer, another RIE etching (O_2) was performed to etch through the polyimide layer to make a through-hole membrane [33].

Figure 2.9: Schematic of the aperture-array lithography system [33].

After releasing the membrane, a coarse filter paper was used to provide additional strength to the thin micro-fabricated membranes. This coarse filter paper should not significantly affect the total hydraulic resistance. Figure 2.10 shows the resulting through-hole membranes with varied porosities from this method. By using this process, submicron sieves with pore size down to $0.2\ \mu\text{m}$ can be achieved.

The resulting membranes have good uniformity and a low coefficient of variation ($CV < 7\%$), but like the previous methods this technique cannot be used as a reliable process for mass production. One reason is that the ion-beam lithography is a costly process and needs expensive equipments. In addition, exposure of a small area (e.g., $10 \times 10\ \text{mm}^2$) takes few hours to complete. Furthermore, membranes manufactured with this method are too thin to be used for normal microfiltration processes.

Figure 2.10: SEM photos of polymeric nano-sieves with different porosities manufactured by aperture-array lithography [33].

2.1.4 Fabrication of polymeric isopore membranes with imprinting technology

The feasibility of producing polymeric microsieves with imprinting technology was also investigated by some researchers at the University of Twente [21]. Figure 2.11 schematically shows the imprint process for fabrication of microsieves. In this process a silicon mold is first made using silicon micromachining technology.

Figure 2.11: Schematic illustration of the imprint process for the fabrication of microsieves [21].

A thick sheet of polycarbonate is then placed in-between the mold and a flat disc. The whole is placed in a vacuum oven and heated to a temperature of 250°C, which is 100°C above the glass transition temperature of polycarbonate. A continuous pressure is applied by placing a weight on the disc. 1 kg weight on the 0.5×0.5 cm² mould area gives a pressure of 4 bar [10,21]. Part of the viscous polymer is pushed into the pattern and the excess escapes to the sides. After 1 hour the oven is cooled down and the

polycarbonate is released from the disc and the mould. In the places where the mold touched the disc, a thin layer of polymer (residue layer) normally remains, which can be removed with an O₂ plasma. Figure 2.12 shows SEM photos of the micro-fabricated mold and the imprinted membrane. It can be seen that the imprinted sieve is very good replica of the mold, but the membrane appears to have been stretched and damaged at several places.

Figure 2.12: (a) SEM micrograph of the mold used for making microsieves with imprinting technique, and (b) released polycarbonate microsieve with slotted holes [21].

With this method, different thermoplastic polymers (e.g., PMMA, PC) can be employed to fabricate through-hole membranes. Although it is useful for fabricating polymeric microsieves with a wide range of perforation sizes (i.e., down to 50 nm), due to its intrinsic limitations the process cannot be used for large-scale production. First, fabrication of the mold with silicon micromachining is a high-cost process, and the mold service life is short. Second, difficulty in demolding and membrane rupture upon release are the major impediments to the application of the method. Moreover, removal of the residual layer by an O₂ plasma adds additional cost to the technique.

2.1.5 Fabrication of polymeric isopore membranes with anodic porous alumina templates

In recent years, anodic aluminum oxide (AAO) membranes with honeycomb-like pore structure have attracted significant attention due to their utilization as a nanoporous template for development of various functional nanostructures, such as nanowires [34], nanotubes [35], and nanomaterial synthesis [36].

The application of porous alumina templates for fabrication of isopore membranes was introduced by Masuda et al. [37] in 1995. They proposed a novel approach to precisely control the growth of the channel array in anodic porous alumina, which could be used to fabricate the long-range-ordered channel-array architecture on the millimeter scale. Afterward, the same group proposed a method for fabrication of polymeric nanoporous membranes from metal molds, which were prepared by a replication process using an anodic porous alumina template [38]. The process was based on the nanoimprinting concept consists of two main steps: (i) preparation of a metal mold with an ideally ordered array of pillars, (ii) fabrication of polymeric through-hole membranes by nanoimprinting as schematically shown in Figure 2.13. In this process an alumina template was firstly produced by anodization of a pre-textured Al (~99.99% purity) foil inside an acidic solution such as sulfuric acid (H_2SO_4) and a direct current (DC) bias. The initial pattern on the Al foil was imprinted using a mechanical mold (i.e., SiC) made by Electron Beam (EB) lithography [39,40]. In order to replicate Ni molds from the Al template, a thin layer of platinum (Pt) was coated inside the Al hole by means of sputtering. In the next step Ni electroplating was carried out and finally a Ni mold was obtained by dissolving the Al template. Then a polymeric solution was spread on the metal mold and pressed by a quartz-glass substrate coated with a PMMA layer [38].

Figure 2.13: Schematic of fabrication of polymer through-hole membrane using Ni molds: (a) Anodization of aluminum, (b) Pt layer coating, (c) Electrodeposition of Ni, (d) Removal of porous alumina templates, (e) Filling of monomer, (f) Imprinting and UV irradiation, (g) Release of mold from polymer, and (h) Dissolution of PMMA layer [38].

Figure 2.14: SEM images of the polymer through-hole membrane after dissolving the PMMA layer in acetone. (a) Top-surface, (b) back-surface, and (c) cross-section view [38].

After performing UV exposure and polymerization, the mold was detached, and a polymeric microfilter was obtained by dissolving the PMMA layer in acetone. Figure 2.14 shows the SEM images of a through-hole membrane obtained by this method. Following the same procedure, various resins can be used to make a through-hole membrane with the hole-diameter down to 100 nm. One of the main problems of the method is the difficulty in demoulding of the cured membrane from the master mold because peeling off of the membrane from the mold often results in membrane damage. In addition, by increasing the size and thickness of the membrane, the friction and adhesion forces between the membrane and pillar mold would be too high to peel off the through-hole membrane [38]. Likewise, the choices of pore size and pore density are restricted and limited due to the use of alumina templates.

2.1.6 Fabrication of polymeric isopore membranes using an excimer laser

Excimer laser micromachining is a versatile technology that offers several applications in the fabrication of precise three-dimensional and high aspect ratio microstructures [41]. Early attempts for fabrication of polymeric microfilters using a laser were done by Atkin et al. [42].

The main problem in this method is the pore size variation in the entrance and exit of the membrane, which is caused by laser machining (normally giving ablated wall angles of approximately 10-15 degrees). Figure 2.15 shows a polymeric membrane fabricated by an excimer laser on a 12 μm Polyethylene terephthalate (PET) film.

Figure 2.15: Excimer-ablated filter membrane in 12 μm PET film. (a) Entrance holes 8 μm , (b) Exit holes 1 μm [42].

Recently, Saxena et al. [43] tried to optimize this method for membrane fabrication. By using a KrF-based excimer laser (248 nm), which generates a pulsed laser beam with a 25 ns pulse duration, passing through a beam-delivery system. The beam delivery system comprises a series of optical lenses to homogenize the incoming beams. The beam then passes through a mask for the desired shape on the substrate surface and then passes through a demagnifying lens to reduce the beam size by eight

times. In their study a polyimide sheet with 40 μm thickness was used as a substrate for laser ablation. The schematic representation of the process is shown in Figure 2.16.

Figure 2.16: Schematic representation of the process for fabrication of microsieves with a laser excimer (a) Beam-delivery system: (1) attenuator, (2) 45° mirror, (3) telescopic lens, (4) spherical lens, (5) homogenizer, (6) condenser lens, (7) field lens, and (8) mask. (b) Mask and the demagnification system [43].

By controlling the process parameters such as beam energy, repetition and number of pulses, various through-hole membranes were produced with 25 μm , 18.75 μm and 12.5 μm pore sizes from the masks (fabricated by micro-drilling on thin copper sheets) with hole sizes 200 μm , 150 μm and 100 μm , respectively [43]. Figure 2.17 shows some images of the polyimide membranes fabricated by the excimer-laser micromachining process.

Figure 2.17: Microfilters fabricated using an excimer laser (a) 25μm, (c) 12.5 μm [43].

The resulting membranes have a good uniformity and present sufficient mechanical strength in filtration tests, but they also suffer from pore size variation in the front and back of the membrane (sloped walls) as well as debris due to laser ablation. Moreover, this process is time consuming and needs expensive equipment. In addition, the high variation from the target at smaller pore size compromises the reliability of the process.

2.1.7 Polymeric isopore membranes manufactured by inkjet technology

Recently, Jahn et al. [44] introduced a new methodology for microsieve fabrication by means of inkjet printing technology. Inkjet printing technology is a versatile and feasible process for positioning small volumes of a liquid precisely and quickly onto a substrate and has become a common tool for many applications.

In their work, inkjet printing was used to deposit sessile drops of a water-based liquid (mixture of water and ethylene glycol) onto a hydrophobic substrate (aluminum foil), which was covered with a thin layer of a polymer solution (liquid PMMA). After evaporation of the solvent and solidification of the liquid polymeric layer, the sessile drops imprint their shape into the film. In fact, tiny drops act as a template to create the

pores inside the polymeric film [44]. The process is schematically illustrated in Figure 2.18.

The resulting membranes from this process show a good uniformity in thickness and size, which enables this technique to be used as a quick method in laboratories for membrane production. Figure 2.19 shows optical and SEM images of a membrane fabricated by this process. Despite some advantages of the method, it suffers from several problems limiting its application as a reliable method for mass production. Firstly, it has again the problem of membrane folding (curling) upon release from the substrate. Secondly, it requires a precise device for printing of the sessile drops, which would be expensive. In addition, fast evaporation of droplets during the fabrication process causes some non-uniformity in pore size.

Figure 2.18: Schematic of Inkjet fabrication method [44].

Figure 2.19: Microsieve manufactured by inkjet printing technology [44].

2.1.8 Isopore micromachined planar filters for microfluidics

Microfluidics is an interdisciplinary field at the interface of chemistry, engineering, and biology [45], and have been used extensively for various applications such as DNA and proteins synthesis [46] and cell/molecular separation [47] due to advantages associated with miniaturization, integration, improved sterility and faster sample processing times. Size and deformability based cell sorting that use arrays of micro-scale constrictions or pores to selectively isolate/purify biological samples (such as cancer cells, DNA, etc.) have been used in various microfluidics devices due to ease of integration as part of the chip fabrication process, despite low specificity and also clogging issues [8,47].

Wilding and co-workers [48] were the first group to introduce the use of silicon micromachined filters for blood separation and other biological assays. They designed a weir-type filter with a 3.5 μm gap between the silicon structure and the Pyrex top cover (i.e., attached together using anodic bonding) for effective isolation of WBCs from RBCs. In a detailed study, Ji et al. [49] investigated the isolation efficiency of four different types (i.e., weir, pillar, cross-flow and membrane filter type) of planar

microfilter for separation of white blood cells (WBCs) from red blood cells (RBCs) inside a silicon biochip (see Fig. 2.20). In their proposed method, silicon wafer was patterned by deep reactive ion etching (RIE) on the front side to create sieving structures as well as reservoirs. Back-side etching (with KOH) was performed for all methods to create the openings and fluidic channels. The critical dimension of all microfilters was fixed to $3.5\ \mu\text{m}$. Based on their experimental results, they found that cross-flow design has superior performance in terms of throughput (i.e., whole blood handling capacity) and also isolation efficiency [49].

Figure 2.20: Schematics of the microfilter designs, (a) weir filter (side view), (b) pillar filter (top view), (c) cross-flow filter (top view), and (d) membrane filter (side view) [49].

In another study, Peh and his colleagues [50] used the silicon micropillars to make a filter-based biochip for isolation and detection of *C. parvum* oocysts and *Giardia lamblia* (see Fig. 2.21). Their design included a bypass region ($2\text{--}4\ \mu\text{m}$ pitch), acting as an alternative fluidic flow routes to relieve pressure changes, and a fine region ($1\ \mu\text{m}$ pitch) for trapping the protozoa cells.

Figure 2.21: (a) Schematic of silicon pillars (b) SEM photo of trapped bacteria between micropillars [50].

Isopore planar membranes with comparable molecular dimensions have been also employed by researchers as an alternative to conventional approaches (e.g., polymeric gels and electrophoresis) for separation and concentration of complex biomolecules such as DNA, RNA, proteins etc. For instance, Austin and coworkers [51] used a biochip consisting of micrometer-sized pillars of silicon for electrophoresis of DNA. Further on, Han and his group [52,53] developed a nanofluidic chip with entropic traps for the separation of long DNA molecules (see Fig. 2.22(a)). *In situ* fabrication of polymeric isopore membranes inside the microfluidic channels was also realized using advances in holographic and inclined lithographic techniques [54,55]. Using this method, a triple sieving system with multiple inlet and outlet streams and three different mesh sizes of 57.3 μm, 27.3 μm, and 10 μm had been fabricated by Yoon et al. [54] for filtration of microparticles, as shown in Figure 2.22(b).

Figure 2.22: (a) SEM image of a planar nanosieve consists of a deep region and a shallow region for DNA separation [53], (b) SEM photo of a triple sieving system inside the microfluidic channels for particle separation [54].

Fabrication of isopore planar filters for lab-on-chip devices is a straightforward process because it can be a part of the chip fabrication process. However, the specificity and also rapid clogging are the major issues for their large-scale use [47]. These kinds of filters normally produced for single use applications such as point-of-care devices; therefore, they must be manufactured in a large-scale with relatively low cost.

2.1.9 Polymeric isopore membranes produced by self-assembly techniques

Block copolymers which are macromolecules with long, covalently connected blocks of two or more distinct repeating units of the same monomer have received great attention over that past decade for fabrication of complex structures with small feature sizes [9,56]. Depending on the volume fraction of the components, they can perfectly self-assemble into highly-ordered arrays of nanoscopic domains with various morphologies such as spheres, lamellae and cylinders [56].

The combination of new synthetic methods with an understanding of phase behavior enabled researchers to fabricate high porosity isopore membranes with cylindrical

nano-channels for various advanced technologies, such as nanolithography [57], water purification [58], and drug delivery [59]. The most general strategy involves selective dissolution of the minority component (e.g. PMMA), which is normally embedded in the majority components (e.g. PS) in a thin film of a block copolymer (e.g. PS-PMMA), having cylindrical microdomains on the plane of a thin-film [9,60], a process shown schematically in Figure 2.23. This thin film is then transferred onto a high porosity microporous membrane with relatively large pore size in order to support the membrane during filtration. Using this approach, Yang and coworkers [61] produced an isopore polymeric membrane using a polystyrene-*block*-poly(methyl methacrylate) copolymer (PS-*b*-PMMA) with 15 nm pore size. Well-oriented cylindrical microchannels were obtained in a thin film of about 80 nm thick by removal of PMMA homopolymer inside an acetic acid solution. This asymmetric nanoporous membrane (see Fig. 2.24(a)) showed impressive selectivity while still maintaining a high flux for the separation of human rhinovirus type 14 (HRV14) from an aqueous dispersion. The same nanoporous membrane with tailored pore size (i.e., using gold sputtering) has been used recently for drug delivery applications by other researchers [62]. They have successfully demonstrated the application of isopore membranes made with self-assembly of block copolymers for long-term controlled release of therapeutic proteins such as bovine serum albumin (BSA) and hGH using a single file diffusion (SFD) mechanism.

Figure 2.23: Schematic of the procedure for the fabrication of isopore nanoporous membranes using block-copolymers [61].

Particle assisted wetting (or float-casting) is also another attractive approach which has been developed by Xu et al. [63,64] for fabrication of ultra-thin freestanding porous membranes. In this technique, a mixture of nanosized spherical particles coated with a hydrophobic polymer and a non-water soluble, polymerizable liquid monomer is applied to a water surface. Eventually, this mixture forms a monolayer of colloids on the water surface, which are embedded in a layer of the liquid monomer. After cross-linking of monomer using photo-polymerization and removal of particles, a freestanding membrane containing pores with similar diameter to the colloids can be formed [63,64]. Structures with very narrow pore size distribution and small pore diameters (50-100 nm) can be obtained with this technique. Figure 2.24(b) shows an SEM image of nanoporous membrane with a square-shape back support. The inset image shows the structure of pores.

Figure 2.24: (a) SEM image of a nanoporous membrane prepared from PLA-PDMA-PS triblock copolymer precursor [61], (b) SEM image of a thin free-standing porous membranes that cover a gold grid with 100-μm wide openings. The inset image shows the structure of pores from bottom view [64].

Isopore membranes produced with self-assembly techniques are extremely uniform and have a smooth surface, but the major hurdle in their use is the large-scale production because achieving a large defect-free surface area is difficult in these approaches. Furthermore, the pore sizes and shapes and their distribution cannot be controlled perfectly similar to MEMS techniques.

2.2 Summary (Part 1)

One of the advantages of microsieves, regardless of their inorganic or polymeric nature, is that the fabrication process allows enough flexibility to control the pore geometry and porosity precisely according to the desired application [22]. Micro-fabricated filters also have high porosity, a smooth surface, high throughput, and very thin sieving (or selective) layers compared to the commercial depth-filters. The main advantage of the thin selective layer is that the membrane resistance during filtration is very low.

Based on this comprehensive literature review and comparison of different methods for fabrication of microfilter, we realized that all the existing methods suffer from major problems and impediments such as:

- Membrane fouling (curling) upon release from the master mold.
- Membrane failure and rupture upon release from the master mold.
- Adhesion of the membrane to the mold and difficulty in demoulding.
- Limitation in material in use.
- Low strength of fabricated membrane.
- High-cost and low-yield process.

As described earlier, the great aim of this project is to develop a robust and reliable method for fabrication of microfilters that are able to isolate waterborne pathogens and recover them easily from large volume of water samples. In addition, the proposed method should overcome completely all problems in existing microfabrication techniques and must be productive and cost-effective.

In the next Chapter, we will demonstrate a novel microfabrication technique for fabrication of polymeric microfilters based on the soft-lithography and UV embossing techniques.

Part 2

2.3 Fouling of the membranes

Membrane fouling refers to the deposition or adsorption of material (e.g., colloidal, algae, microorganisms, etc.) on the surface of the membrane or within the pores [1,2,12]. Through the microfiltration process, the permeate flow through the membrane creates the convective transport of particles to the membrane surface [11]. During a membrane separation process, the particles retained on the feed side (retentate) start to accumulate near the membrane surface and consequently, the concentration of particles becomes higher near the membrane than in the bulk retentate. This phenomena is called concentration polarization, which is a major obstacle in the widespread application of membrane microfiltration [14]. When the feed contains small particles, the concentrated layer forms a dense “cake” layer. In case of solutions containing microorganisms (e.g., bacteria and algae), the retentate can form a biofilm layer on the membrane surface. Furthermore, with a large number of particles near the membrane surface, there remains a possibility of particle intrusion into the pores of the membrane which may result in pore clogging and adsorption in the pore [65,66].

2.3.1 Concentration boundary layer on membrane surfaces

In cross-flow filtration, the growth of the concentrated layer is restricted by the high shear exerted by the tangential flow of the feed during filtration. Concentration polarization of particles near the membrane surface in cross-flow filtration is schematically shown in Figure 2.25 [67]. During the filtration process, the concentration of retained particles on the surface of the membrane (c_m) is much higher than in the bulk (c_b).

Figure 2.25: Concentration polarization of particles on the membrane surface during cross-flow filtration [67].

The distance from the membrane surface over which the concentration reduces to bulk concentration ($c_m \rightarrow c_b$) is defined as concentration boundary layer (δ_c). The concentration boundary layer is assumed to be thin in comparison to the viscous boundary layer under the hydrodynamic flow conditions in channels [12,66,67].

2.4 Fouling mechanism

The analysis of the flux decline due to particle deposition is necessary since it can provide some insight into the fouling phenomena that take place during microfiltration. Depending on the solute and the process conditions, different blocking mechanisms that explain the flux decline during membrane filtration have been identified [12,66]:

- Complete blocking (pore blocking)
- Standard blocking (pore narrowing)
- Intermediate blocking (long term deposition)
- Cake formation (gel/cake layer)

These mechanisms are schematically shown in Figure 2.26.

Figure 2.26: Mechanism of particle deposition. (a) Complete pore blocking, (b) intermediate pore blocking, (c) pore narrowing and (d) cake formation [14, 66].

2.4.1 General filtration models

Filtration flux (J) is defined as the volume flowing through the membrane per unit area and time. If the membrane is clean, the clean water flux can be determined by Darcy's law [2,66,68]:

$$J = \frac{dV}{Adt} = \frac{\Delta P}{\eta_p R_m} \quad (2.1)$$

where J is the flux ($\text{m}^3/\text{m}^2\text{s}$), V is filtrate volume (m^3), A is filter surface area (m^2), t is filtration time (s), ΔP is the differential pressure applied across the membrane (Pa), η_p is viscosity of the permeate (Pa.s) and R_m is the membrane resistance (m^{-1}). When membrane fouling occurs, in addition to the clean membrane resistance (R_m), the blocking resistance (R_b) and cake resistance (R_c) also need to be taken into account when calculating the total filtration resistance [66-68]. This can be modelled via the simple well known multi-resistance law:

$$Flux(J) = \frac{\Delta P}{\eta_P (R_m + R_b + R_c)} \quad (2.2)$$

2.4.2 Single pore blocking model

A number of fouling models exist to describe each fouling mechanism encountered during the filtration process. For constant pressure filtration, Hermia [69] presented a unified power-law model (Eq. 2.3) to incorporate the four different mechanisms using parameters k and n .

$$\frac{d^2t}{dV^2} = k \left(\frac{dt}{dV} \right)^n \quad (2.3)$$

where t is the filtration time, V is filtered volume, k is rate constant depending on filtration mechanism and n is filtration constant characterizing the filtration mechanism. Complete blocking has the highest fouling rate ($n=2$) and cake filtration has the lowest fouling rate ($n=0$). The summary of the model parameters is listed in Table 2.1.

Table 2.1: Summary of parameter k and n for constant pressure blocking filtration law [69].

Model	k	n
Complete pore blockage	$\sigma_d \frac{Q_0}{A}$	2
Standard pore blockage	$\frac{2\Phi_d}{LA} Q_0^{1/2}$	1.5
Intermediate pore blockage	$\frac{\sigma_d}{A}$	1
Cake filtration	$\frac{\alpha \rho_p C_p \eta_p}{A^2 \Delta P (1 - m C_b)}$	0

2.4.3 Combined pore-blocking and cake-filtration model

During a filtration process a complex mechanism is occurring that cannot be predicted by models that consider only a single mechanism. Some researchers divided filtration into a few stages and model each stage separately using a single mechanism [70]. However, it is arbitrary to divide the stages since the transition between the stages is not smooth. More recently, Ho and Zydney [71] developed a combined pore-blocking and cake-filtration model for protein fouling during microfiltration. A simplified analytical form is given in Eq. (2.4):

$$Q = Q_0 \left[\exp\left(-\frac{\alpha \Delta P C_b}{\eta_p R_m} t\right) + \frac{R_m}{R_m + R_p} (1 - \exp\left(-\frac{\alpha \Delta P C_b}{\eta_p R_m} t\right)) \right] \quad (2.4)$$

where Q is the volumetric flow rate at time t (m^3/s), Q_0 is the initial volumetric flow rate at time $t=0$ (m^3/s), α is the pore-blocking parameter (m^2/kg), C_b is the bulk foulant concentration (g/l), R_m is the membrane resistance (m^{-1}) and R_p is the resistance of the foulant deposit (m^{-1}), which is a function of filtration time. The first term in the bracket is equivalent to the classical pore-blocking model and gives a simple

exponential decay in the volumetric flow rate. For long time runs, the volumetric flow rate is dominated by the second term (classical cake-filtration model) [72]. This model provides a smooth transition from pore-blocking to cake-filtration behaviour during filtration, eliminating the use of separate mathematical descriptions in these fouling regimes [72].

2.5 Interactions between the foulant and membrane surface

The affinity of the foulant to the membrane can considerably influence the membrane fouling and permeate quality. The interaction between the foulant and membrane is more pronounced for colloidal and macromolecular organic matter rather than particulates because they have smaller sizes [72]. There are many factors, which can influence this interaction, e.g., charge, pH, ionic strength, multivalent ions (Ca^{2+} and Mg^{2+}), hydrophobicity, and membrane morphology [66].

2.5.1 Charge

If the colloids and the membrane surface have the same charge, the colloids will be repelled by the membrane due to electrostatic interactions between them. Consequently, the adsorption of colloids is relatively low [66,72]. Many colloids and macroorganics are negatively charged at neutral pH conditions [66]; therefore, the microfiltration membranes in water and wastewater filtration processes are often manufactured or modified to be negatively charged. However, it should be noted that the charge of the membrane can be changed by the adsorption and deposition of colloids [66].

2.5.2 pH

The pH can influence the charge of colloids and macroorganics during microfiltration. The majority of colloids and microorganisms are more negatively charged at high pH conditions due to the deficiency of protons, which promotes the dissociation of protons from the colloids into the solution [73,74]. However, the filtration of water and wastewater is normally performed at neutral pH conditions; hence, it is not feasible to adjust the pH only for the purpose of controlling fouling.

2.5.3 Hydrophobicity

Particles that foul in aqueous media tend to be hydrophobic such as metal colloids, proteins, and oily particulates [2,12]. Hydrophobic particles tend to cluster or group together to form colloidal aggregates because this lowers their interfacial free energy due to decreased surface area. Hydrophobic particles in an aqueous environment have a tendency to attach to any material less hydrophilic than water because of less exposure of their surface to water can be achieved; therefore, by increasing the hydrophilicity of the membrane, foulants prefer the water over other materials and less fouling will occur [65]. It should be noted that the hydrophobicity of the membrane can be changed by the adsorption and deposition of colloids during filtration; eventually, the membrane tends to have similar hydrophobicity to the deposited colloids [66,72].

2.5.4 Ionic strength

Filtration with low ionic strength feed water may reduce the adsorption of colloids and microorganisms and therefore, retarding the fouling. The impact of ionic strength is indirect. In the filtration of proteins, screening of the charges of the proteins is reduced at low ionic strength. Therefore, protein molecules strongly repel each other,

especially at the membrane surface, where the concentration of proteins is high [66,72,75].

2.5.5 Membrane morphology

Membrane morphology, (e.g., pore opening, pore-size distribution and surface roughness) can affect membrane fouling. Recent studies demonstrated that the membrane pore morphology could have a significant effect on the rate of flux decline (i.e., initial stages of filtration) where the dominant fouling mechanism was pore blockage [76]. For instance, membranes with slotted openings and a high porosity would be less likely to get blocked during filtration of typical cell or particle suspensions [77,78]. Such membranes can provide adequate selectivity needed for sterile filtration and microorganism removal, while minimizing the rate of flux decline.

2.6 Fouling prevention techniques

Cross-flow filtration is much more effective than dead-end filtration in preventing particle deposition on a membrane surface, but fouling still exists. In most membrane operations, the feed is subjected to pretreatment that includes pH adjustment, chlorination, addition of complexing agents, etc. to reduce the extent of concentration polarization during filtration [79,80]. Studies have shown that membrane pretreatment results in improvement of the product quality and significant flux and pressure recoveries during the pilot tests [80,81]. However, fouling prevention techniques are necessary to sustain high flux through the MF and UF membranes in the pretreatment module as well as in standalone applications of MF/UF. Over the past decades, most of the work in membrane research has been to determine effective techniques to

prevent and control the build-up of the cake layer on the membrane surfaces [67,80]. The currently available methods can be categorized in three classes as shown in Table 2.2.

A majority of the fouling control techniques is directed toward creating disturbances in the flow field near the membrane (hydrodynamic control), leading to removal of the retained particles from the membrane surface. A turbulent flow condition near the membrane surface enhances the mass transfer from the cake layer [82-83].

Table 2.2: Classification of available methods for fouling prevention in the membrane industry [67].

Different methods for generating disturbances in the hydrodynamic fields to decrease membrane fouling include use of turbulence promoting spacers, use of flow geometries to promote near-membrane mixing [84-86], back-pulsing [87], and back-washing [88]. Creating a vibration-induced shear field near the membrane surface (i.e., VSEP system) has been found effective in preventing concentration polarization [89]. Another method known as gas/air sparging was reported to be effective in reducing the impact of fouling by introducing a two-phase cross-flow (gas-liquid) in the feed chamber [90]. Air sparging in combination with cross-flow filtration in flat-sheet membranes is very useful to enhance the flux and retard the fouling phenomenon. Several studies have also reported significant improvement in flux enhancement when the membrane surface is irradiated with ultrasonic waves. Results indicate that

ultrasound reduces the thickness of the cake layer by increasing turbulence in the concentration polarization layer [91]. Membranes subjected to plasma treatment have shown improved antifouling characteristics [92]. Over the past decades, some attempts to reduce fouling by means of an electric field are also reported (i.e., electro-filtration) [93-95]. The effect of an external electric field on the charged interface of the solute and the solution has been thoroughly studied in electro-kinetics [67,96].

2.7 Summary (Part 2)

In the second part of this Chapter, the basic mechanism of particle accumulation and consequent loss of permeate flux was explained to indicate the major problem encountered in almost every membrane filtration process. Based on the information gathered from the available literature, some effective solutions have been recognized to diminish the fouling related issues. This thesis deals with a novel generation of high-flux screen microfilters: polymeric/metallic micro-fabricated membranes and their potential use as microfiltration membranes for isolation of waterborne pathogens. Relatively few literature is available concerning their filtration performance, which could be very interesting and promising for future industrial applications. For this purpose, one part of this thesis is dedicated to study the fouling phenomena during filtration process and also investigate some strategies to retard/suppress flux decline and prevent cake formation during microfiltration. To do this, a comprehensive study of fouling phenomena with a model particle (i.e., latex) and also clay or bentonite will be carried out. By understanding the mechanism of fouling in this type of microfilters appropriate techniques such as back-flushing and surface modification will be employed to retard the particle accumulation on the surface of micro-fabricated filters.

A scanning electron micrograph (SEM) showing a dense array of vertical, cylindrical microstructures. The structures are arranged in a regular grid pattern. In the foreground, some structures appear to be broken or tilted, revealing a porous, fibrous internal structure. The background shows the full array of intact structures.

Chapter 3

3

Design and fabrication of polymeric micro/nano filters with dissolving mold technique

Over the past decades, microfabrication has developed into a rapid expanding field for miniaturization of various devices and structures, of micrometer sizes and smaller. Traditionally, photolithography has been the desired method, but recently the micromolding method is finding increasing applications. Based on this, we review first the micromolding history and then present a novel “dissolving mold technique” for fabrication of polymeric micro/nano filters.

Parts of this chapter have been published previously in the Journal of Micromechanics and Microengineering and also the Journal of Membrane Science [17,97].

3.1 What is micromolding?

3.1.1 Introduction

Microfabrication is the set of technologies for fabrication of miniature structures with micrometer size or smaller. Over the past decades, microfabrication has been the workhorse behind the explosive growth in many industries such as microelectronics, pharmaceuticals and the automotive [30,98]. Microfabrication has also supported many new technologies such as micro/nano fluidics, optical MEMS, Bio-MEMS, photonics, and biosensors where miniaturization and large-scale integration are required for a reduction in time, costs, reagents, sample size, or power consumption as well as portability and reliability [30]. In the areas where microfabrication is applied, methods to realize structures on such small length scales can be divided in direct writing methods and replication methods [98].

3.1.2 Direct writing methods

Directs writing methods in the semiconductor industry refer to those techniques can be combined directly to CAD/CAM software to generate micrometer patterns for the manufacturing of microelectronic devices and other micro-engineered components such as MEMS and micro-fluidic devices [98,99]. For example, writing with a focused laser beam is normally used to fabricate photomasks that contain the structure necessary for conventional photolithography. Other examples of direct writing methods are writing by dip-pen micro/nanolithography (i.e., using atomic force microscopy (AFM)), droplet-based direct writing, filament-based direct writing (FBDW), electron-beam (e-beam) lithography, focused ion-beam (FIB) lithography, laser-based direct writing and inkjet printing. In some cases high resolution features (i.e., down to 10 nm) can be created using the aforementioned techniques [100,101].

Even though direct-write processes have demonstrated their potential impact on future microelectronic manufacturing, there are still some significant technical limitations to be overcome before any further breakthrough can be achieved in terms of widespread industrial application. The main barrier in direct-writing methods is their serial character that makes the process too slow and expensive for large scale production of micro-devices [30].

3.1.3 Photolithography

Photolithography is a process used widely in microfabrication to transfer the desired pattern from a photo-mask to a film of photoresist on the substrate [98,102]. The patterns generated by lithography are then transferred into the underlying material by etching (i.e., wet or dry etching), or transferred into other layers by deposition. The substrate materials that normally are used in the semiconductor industry are mainly silicon, glass, quartz, metals, and some polymers such as PMMA, PC, etc. [30,103]. The limited transparency of these materials, and the high costs are the major drawbacks for the use of photolithography in integrated optics. In addition, the inability to pattern non-planar surfaces as well as limitations in geometrical design and surface chemistry caused researchers to look for alternative approaches, where micromolding methods emerged [104]. Micromolding relies on replication of a master mold having a micro/nano structured relief profile on its surface, and can be divided to following categories: (i) Soft-lithography and (ii) Micromolding against a rigid mold.

3.1.4 Soft-lithography

In technology, soft-lithography refers to a family of techniques for fabricating or replicating structures using “elastomeric stamps, molds, and conformable photomasks”

[105]. The master mold mostly is prepared from a soft material (i.e., polydimethylsiloxane (PDMS)), by curing on a micro-fabricated mold (see Fig. 3.1). Soft-lithography has some advantages over others such as lower cost in mass production, being ideal for biotechnology applications and the ability to the transfer pattern on non-planar surfaces [106].

Figure 3.1: Schematic of the fabrication of PDMS stamp: (a) and (b) photoresist is spincoated on a silicon wafer, (c) a mask is placed in contact with the layer of photoresist, (d) the photoresist is illuminated with ultraviolet (UV) light through the mask. An organic solvent dissolves and removes photoresist that is not crosslinked (e) PDMS is poured on the master, cured thermally and peeled away, and (f) the resulting layer of PDMS has microstructures embossed in its surface [106].

Soft-lithography techniques, which mainly developed within the group of G.M. Whitesides at Harvard University [107-109], can be classified into six main groups as illustrated in Figure 3.2. Microcontact printing (μ CP) and replica molding (REM) are the most important categories of soft-lithography that have found the most practical applications until now. In μ CP the elastomeric microstructure is used as a stamp to transfer the patterns of self-assembled monolayers (SAMs) of ink on the surface of a substrate through conformal contact [106,110-112]. The transferred ink to the substrate can be used in subsequent fabrication steps (See Fig. 3.3a).

Figure 3.2: Classification of the soft-lithographic techniques: (1) replica molding, (2) microcontact printing, (3) micro transfer molding, (4) solvent assisted micro molding, (5) capillary force lithography, and (6) micromolding in capillaries [106,112].

In replica molding (REM), a topographically patterned layer of PDMS is used to transfer the pattern to the surface of another polymer (See Fig. 3.3b). A prepolymer is

deposited on the PDMS master mold by casting or spin-coating, and then separated from the master by peeling them apart [106]. The advantage of the use of an elastomeric over a rigid mold is the low surface tension and elasticity of the mold, which facilitate the release [30,106].

Figure 3.3: The schematic representation of the two major soft-lithography techniques: (a) microcontact printing, and (b) replica moulding [106].

3.2 Dissolving mold technique

The methodology to be employed in this study for fabrication of polymeric micro/nano filter is based on replica molding (REM), which was described earlier. In order to fabricate a through-hole polymeric membrane, a silicon master pillar-mold is first fabricated by silicon micromachining technology. Then an interim blind-hole mold is

replicated with PDMS from the silicon mold, and finally a dissolvable polymer pillar mold is obtained by replication from the PDMS mold. The fabrication process will be described in details in the following sections.

3.2.1 Fabrication of dissolvable polymer pillar mold

Figure 3.4 schematically illustrates the process for fabrication of dissolvable polymer pillar mold [97]. Firstly, a silicon wafer was cleaned in piranha solution (98% H_2SO_4 + 30% H_2O_2 in the ratio of 3:1) for 20 minutes at 120°C to remove any organic contaminations and then submerged in buffered oxide etchant (BOE) for 3 minutes. Afterward, a 3 μm thick positive photoresist layer (AZ7220, Clariant Corporation) was spin-coated on the wafer surface (Fig. 3.4a). Photolithography is carried out on a mask aligner (Karl-Suss MA6), and a resist dot array with a diameter of 2 μm and space of 2.5 μm was patterned on the silicon wafer (Fig. 3.4b). The patterned resist was used as an etching mask and silicon etching is then conducted on an STS deep reactive ion-etching (DRIE) system to form high aspect ratio micro-pillars with about 2 μm diameter and 20 μm height (Fig. 3.4c). If molecular filters with sub-micron or nano hole-diameter are required, the diameter of the pillars can be further reduced by thermal oxidation followed by HF etching method (see section 3.2.3). After stripping off the resist and processing a passivation treatment, the silicon pillar mold can be used as a master mold for fabrication of an interim blind-hole mold by means of soft lithography. Figure 3.5 shows a scanning electron microscopy (SEM) image of the silicon micro-pillar mold. It can be seen that a perfectly ordered array of uniform-sized silicon pillars are formed after the process.

In order to fabricate the dissolvable polymer pillar mold, a PDMS (polydimethylsiloxane) interim blind-hole mold was firstly replicated from the silicon

master pillar mold. For this purpose, PDMS (Sylgard[®]184, Dow Corning) was prepared according to the manufacturer's instructions (mixing 10:1 elastomer to curing agent by weight) and then cast on the silicon mold (Fig. 3.4d). After degassing in a vacuum chamber, the PDMS prepolymer is kept inside an oven for 3 hours on 85°C for curing, and subsequently the PDMS mold is obtained by peeling it off from the silicon master mold (Fig. 3.4e). The cross-section of obtained PDMS interim blind-hole mold is depicted in Figure 3.6, which shows that high aspect ratio cylindrical holes are created inside PDMS mold.

Figure 3.4: Schematic representation of the fabrication process for the dissolvable polymer pillar mold, (a) spin coating of photoresist, (b) photolithography, (c) Si micro-pillar mold, (d) dispensed PDMS on the Si pillar mold, (e) PDMS interim blind-hole mold, and (f) dissolvable polymer pillar mold.

To obtain the dissolvable polymer pillar mold, PVA (Polyvinyl alcohol, Sigma-Aldrich[®]) solution was prepared (mixing 9:1 water to PVA granule by weight) and

then dispensed on the PDMS interim blind-hole mold. After degassing in a vacuum chamber for 2 hours, it was left in the room temperature for 24 hours and finally the dissolvable polymer pillar mold was obtained by peeling it off from the PDMS mold (Fig. 3.4f).

The material for making the dissolvable polymer pillar mold should be curable (e.g., UV curable, hot curable, etc.) and also dissolvable in a proper solvent such as water or acetone. PVA, Polyimide (PI), Polymethyl methacrylate (PMMA) and photoresist can be used to fabricate the dissolvable polymer pillar mold. In the present work, PVA was used because of its good mechanical property and dissolvability in water.

Figure 3.5: SEM photo of a silicon micro-pillar mold.

Figure 3.6: SEM photo of PDMS interim blind-hole mold.

Figure 3.7 shows a SEM image of a dissolvable polymer pillar mold made of PVA. Similar to the silicon master pillar mold, a perfectly ordered array of PVA pillars is obtained after the replication process.

Figure 3.7: SEM image of a dissolvable polymer pillar mold.

3.2.2 Fabrication of polymeric through-hole microfilter

In order to obtain the through-hole polymeric membrane, a UV curable resin such as polyurethane (PU), SU-8 or other epoxies can be employed to cast on the solvable polymer pillar mold. In this study we used polyurethane (Polyurethane, Sigma-Aldrich®) because it is a versatile polymer material with a wide variety of physical and chemical properties [113]. UV curable polyurethane is typically composed of three basic components [96,114]: (i) a resin (i.e., an oligomer or a prepolymer), (ii) a reactive diluent and (iii) a photoinitiator capable of absorbing UV radiation. The polyurethane solution was firstly prepared (mixing oligomer, reactive diluents and photoinitiator by weight in ratio 70:20:10) and then dispensed onto the PVA mold. Afterward, a glass plate was pressed onto the uncured resin to spread it between the pillars, and finally the polyurethane was exposed under UV light (I line) for 90 seconds (Fig. 3.8a). A passivated transparent plastic sheet was placed between the glass plate and the UV curable resin to prevent their adhesion. After exposure and detaching the passivated sheet and the glass plate from the cured resin, oxygen plasma dry-etching was carried out to remove the residual resin layer remaining on the top of the pillars. To strengthen the polyurethane membrane and to avoid curling of the membrane during subsequent processes and handling, a piece of stainless steel mesh was bonded to polyurethane membrane by UV curable glue (UV Cure, IllumaBond™) (Fig. 3.8b).

Figure 3.8: Schematic representation of the fabrication process of the filter membrane with micro-pores and support meshes, (a) dispense UV curable resin on the polymer pillar mold, press the resin with glass plate, and expose the assembly with UV, (b) bond a support mesh to the cured polyurethane on the dissolvable pillar mold, (c) dissolve the polymer pillar mold to obtain the through-hole membrane bonded with the support mesh, and (d) the final microfilter with support meshes on both sides.

Subsequently, the entire membrane was immersed into DI water (60°C over a night) to dissolve the polymer pillar mold to obtain the through-hole membrane (Fig. 3.8c). Lastly, another piece of steel mesh is bonded to the backside of the through-hole membrane to complete the process of making a filter with micro-pores supported with two steel meshes (Fig. 3.8d).

Figure 3.9 shows an SEM image of a polymeric microfabricated membrane is obtained by this method. This image confirms that the uniform-sized holes are arranged in a uniform interval. Figure 3.10 depicts the cross-section of the resulting membrane to confirm that perfectly cylindrical pores are formed inside the membrane. An SEM image of the membrane bonded to the steel meshes is illustrated in Figure 3.11.

Figure 3.9: SEM image of a polymer through-hole membrane.

Figure 3.10: Cross-section view of a polymeric membrane with 2 μm pore size.

Figure 3.11: SEM image of a micro-fabricated filter membrane bonded to a support mesh.

Steel meshes with a variety of aperture sizes can be used as a support layer, but the apertures should be 50 to 100-fold larger than the membrane pore size so that the mesh does not affect the hydraulic resistance [33].

3.2.3 Fabrication of silicon nano-pillar mold using KOH etching and laser-interference lithography

In conventional UV lithography it is technically impossible to project a clear image of a small feature ($< 1 \mu\text{m}$) inside a photoresist layer due to the limitation in wavelength of the light and also reduction lens system [10]. In this study we used two different strategies to fabricate a silicon nano-pillar mold to be used for fabrication of a nano-filter using our novel dissolving mold technique. In the first strategy we employ wet etching of the silicon micro-pillar mold (i.e., using KOH at room temperature) that is

initially coated with a thin layer of silicon oxide (SiO_2) to obtain uniform nano-pillar mold for fabrication of polymeric nano filters. Figure 3.12 (b) shows an SEM image of a silicon nano-pillar mold ($0.45\ \mu\text{m}$) that obtained by wet-etching of an initial micro-pillar mold ($2\ \mu\text{m}$). The initial micro pillar mold is also depicted in Figure 3.12 (a). It can be seen that the cross-section of pillars changed from square to circular after wet etching using KOH. We employ this nano-pillar mold in later steps to fabricate nano-filters with pore sizes down to $0.45\ \mu\text{m}$.

Figure 3.12: (a) SEM photo of a silicon micro-pillar mold and (b) SEM photo of a silicon nano-pillar ($0.45\ \mu\text{m}$) obtained from KOH etching of a silicon micro-pillar mold.

The second strategy that we used for fabrication of sub-micron size filters was laser-interference lithography [23,24]. Figure 3.13 shows a schematic of the exposure set-up used to transfer the desired pattern onto the photoresist. In this technique part of an incoming laser beam (i.e., $351\ \text{nm}$ line of a Kr ion laser) is reflected by the mirror and forms an interference pattern on the Si substrate with the part that reaches the substrate undisturbed [23]. The period (Λ) of the generated interference pattern is constant and given by $\Lambda = \lambda/2 \sin \theta$, where λ is the laser wavelength and 2θ is the angle formed

between the interfering beams. This angle can be changed by rotating the mirror or substrate [24].

Figure 3.13: Schematic of experimental set-up used for the laser-interference lithography [23].

In our experiments we use a 500 nm thick AZ 7220 photoresist on top of a silicon wafer. Using our laser interference set-up with $\theta = 23^\circ$, a post pattern with period $\Lambda = 500$ nm is achieved. A thin layer of aluminum is then sputtered between the photoresist patterns. After aluminum lift-off, the resulting pattern (i.e. ≈ 450 nm square-shape dots) is used as a mask for dry etching of the silicon wafer using DRIE machine. Figure 3.14(a) shows an SEM image of a silicon nano-pillar mold after dry etching using DRIE machine. The resulting through-hole nano-filter is also shown in Figure 3.14(b). It can be seen that an array of well-defined pores is formed for this case as well.

(a)

(b)

Figure 3.14: (a) SEM photo of a 0.45 μm silicon nano-pillar mold, and (b) SEM photo of a micro-fabricated nano-filter with 0.45 μm pore size obtained with dissolving mold technique.

3.3 Results and discussion

3.3.1 Membrane morphology

In present work, we fabricated over 80 membrane filters with 25 mm² and 47 mm² filtration area. The pore density of the fabricated membranes varied between 2.5×10^7 pores/cm² to 0.21×10^7 pores/cm². Figure 3.15 shows the SEMs of 2 μ m filters, which are designed and fabricated for capturing and collection of *C. parvum* oocysts: (a) 2.5×10^7 pores/cm² (b) 0.81×10^7 pores/cm² (c) 0.4×10^7 pores/cm² (d) 0.21×10^7 pores/cm². This figure indicates that the micro-fabricated membrane comprised narrow and uniform pore-size distributions and also a smooth surface, which is independent of pore density.

Figure 3.15: SEM photos of 2 μ m HAR micro-fabricated filter pores with four different densities: (a) 2.5×10^7 pores/cm² (b) 0.81×10^7 pores/cm² (c) 0.4×10^7 pores/cm², and (d) 0.21×10^7 pores/cm².

3.3.2 Pore-size distribution of the micro-fabricated membranes

The UV lithographic technique is a precise process and almost any shape, size and distribution can be obtained by this technique. In the fabricated polymeric membrane measurement of the pore-size was realized using digitalized photographs from HITACHI S3500 scanning electron microscope (SEM), which is equipped with the 'in-built dimension measurement' module and image analysis program (SEMICAPS 2200, Semicaps Pte Ltd) from random areas of the samples. The mean pore diameter (M) and standard deviation (σ) of the polymeric filter are $2\ \mu\text{m}$ and $65\ \text{nm}$, respectively. The corresponding coefficient of variation ($CV = \sigma/M$) for this membrane is 3.25%, which is much lower than commercial membranes such as track-etched membranes with CV around 20% [10,115]. The histogram of the analyzed samples is illustrated schematically in Figure 3.16.

Figure 3.16: Pore size distribution of a $2\ \mu\text{m}$ micro-fabricated membrane filter.

3.3.3 Pillars failure and membrane occlusion

In fabrication of the silicon master micro/nano pillar mold, increasing the height of the pillars excessively will cause their breakdown upon stripping the PDMS interim blind-hole mold from the silicon master mold. Therefore, finding the optimum aspect ratio for the silicon pillar mold according to the desired size of the pores is an important issue during the fabrication process. Furthermore, salinization of the silicon master pillar mold before casting PDMS on it could help to release PDMS mold without difficulty. Figure 3.17 shows the breakdown of pillars in a silicon master mold after peeling off the PDMS interim blind-hole mold.

Figure 3.17: SEM photo of pillar failure in silicon master mold upon PDMS peeling-off stage.

In addition to above-mentioned problem, finding the optimum pressure that was required to reach the lowest residual thickness in stage seven of the fabrication process (see Fig. 3.8 (a)) is a critical issue. We are currently working on a new method (i.e.,

interferometric measurement of displacement) to control the thickness of residual layer precisely. By employing this method, we can apply precision weights on the top of the UV-curable resin to decrease the thickness of the film to hundreds of nanometers and measure it concurrently. Then without using the RIE technique, we can simply flush away the residual layer (~100 nm thickness) by the pressure of the filtration. Figure 3.18 illustrates the failure of the PVA pillars due to excessive pressure, which is applied to the glass plate.

Figure 3.18: SEM photo of pillar failure in the PVA pillar mold upon applying pressure on the uncured resin.

Another important issue in fabrication of the polymeric micro/nano filters was finding the required time for dissolving the polymer pillar mold inside the proper solvent. In this stage if the membranes are not kept for an adequate time inside the solvent (e.g., water in this work), some pores may remain closed because of incomplete dissolving

of the polymer pillar mold. Figure 3.19 depicts the surface of one membrane for which the polymer pillar mold was not dissolved completely.

Figure 3.19: SEM photo of a membrane with some closed pores.

3.3.4 Reusability

Commercial filters are not designed to be reusable, and they can hardly retain their initial status after filtration and the back-flushing process. On the contrary, microfabricated filters can easily retain their original condition with a simple back-flushing or shaking process. Therefore, the long lifetime and easy cleanability make this filter a good option for large scale applications for which conventional filters must be replaced frequently such as in the water purification industry or in the breweries.

3.3.5 Applications

Unique properties of the polymeric microfabricated filters such as uniform pore size, high porosity (i.e., up to 50-60% depending on the pore size and shape), a smooth

surface and diversity in material in use (i.e., different UV curable resins) make them appropriate for different applications. For instance, in the water industry they can be used for fast detection, capturing and recovering of waterborne pathogens such as *C. parvum* oocysts and *Giardia* in very dilute suspensions. For this purpose we need to isolate a small amount of the oocysts from a large volume of water with high flux. In our tests (i.e., the details are presented in Chapter 6) for this purpose, the polymeric microfabricated filter shows a recovery ratio of around 90% to 95%, which is much higher than commercial filters where recovery ratio is around 50% [17]. Separation of milk fat globules from raw milk, filtration of white blood cells (leukocytes) from blood-cell concentration, cytology and cell cultures, detection of microorganisms and air monitoring can be other applications of polymeric microfabricated filters [10].

3.4 Summary

Although photolithography is still the driving force for fabrication of many micro-devices, the versatile spectrum of micromolding methods can provide a useful alternative for low-cost and high-yield production. Micromolding methods offer a very broad range of materials that are inexpensive, which make them advantageous, in particular for use in integrated optics, microfluidics and biomedics. Micromolding also gives more freedom to place intricate features in products to enhance the ability to create more innovative products. In addition, micromolding methods do not have a fundamental limitation for patterning in the nanometer range [30].

In this chapter we presented a novel “dissolving mold technique” for fabrication of high aspect ratio (HAR) polymeric micro/nano filters. This fabrication method for membranes has some advantages over existing methods. Firstly, it resolves thoroughly the demoulding problem in existing membrane fabrication techniques by dissolving

the pillar mold. Secondly, folding (curling) of the membrane upon release from the mold is solved by bonding the membrane to a support grid before dissolving the pillar mold. This process is capable of low-cost and high-yield and can be used to make a molecular filter with a sub-micron hole-diameter. The resulting membranes have good mechanical properties, high porosity, a smooth surface and uniform pore-size distribution.

Chapter 4

4

Fabrication of polymeric micro/nano filters with multilevel lithography technique

The application of SU-8 as negative based photoresist to develop thick microstructures for MEMS has attracted great interest. A promising approach for fabrication of complex features is formation of **multilevel** microstructures by performing multiple photolithographic steps of exposure and a single development. In this chapter we present the results of our investigations on fabrication of single layer and multi-layer polymeric micro/nano filters using this technique.

Parts of this chapter have been published previously in the Journal of Micromechanics and Microengineering and also the Journal of Biomicrofluidics [116,117].

4.1 Introduction

The SU-8 is a high contrast, negative, epoxy-type photoresist based on EPON SU-8 epoxy resin (from Shell Chemical) that was originally developed and patented by IBM in 1989 [118]. This photoresist can be as thick as 2 mm and micro-structures with an aspect ratio > 20 and even higher have been made with standard contact lithography equipment [119]. Over the past decades, SU-8 has been used extensively for fabrication of complex features through the combination of micro-stereolithography and UV lithography techniques [120]. The increased interest in SU-8 photoresist due to its great chemical and mechanical properties has resulted in a number of innovative processing methods as reported by various research groups [120-122]. In order to advance the capabilities of MEMS fabrication technology to construct complex microstructures with increasing thickness while maintaining fine minimum feature size, this chapter reports on the development of a novel process to fabricate polymeric micro/nano filters with precise pore size using several layers of SU-8 photoresist through multiple exposure and single development.

4.2 Fabrication of single layer polymeric membranes with integrated back-support

4.2.1 Fabrication of perforated polymeric membrane

The schematic representation of the entire fabrication process is illustrated in Figure 4.1. First, a silicon substrate, $\langle 100 \rangle$, p-type, and 100 mm in diameter, is cleaned in piranha solution (96% H_2SO_4 and 30% H_2O_2) for 25 minutes at 120°C to remove any organic contaminations on the wafer surface. Then, the substrate was submerged in the buffered oxide etchant (BOE) for 2 minutes to clean the native oxide layer.

Figure 4.1: Schematic representation of the fabrication process for fabricating the polymeric micro-filter with an integrated support mesh, (a) spin-coating of a thin sacrificial layer, (b) spin-coating of a thin SU-8 layer, (c) first UV-exposure through a quartz mask, (d) development to obtain a perforated thin filter layer, (e) spin-coating of a thick SU-8 layer, (f) second UV-exposure through a plastic mask, (g) formation of a support layer made of a thick SU-8 film after second development, and (h) the final micro-filter with a support mesh after releasing from the wafer substrate.

After rinsing with DI water and drying with N₂ gas, the dehydration bake step was performed in a Suss machine (Delta 150 VPO) for 2 minutes. To facilitate the release of the membrane from the substrate, a thin sacrificial layer of polystyrene film (Sigma-Aldrich) 2 μm thick is spin-coated on the silicon wafer and cured on a hot plate at 90°C for 10 minutes. After curing the sacrificial layer (i.e., polystyrene film) and cooling down the wafer to the room temperature, a 4 μm thin layer of SU-8 photoresist (SU-8 2005, MicroChem corp.) is spin-coated on the top of the cured sacrificial film. After soft baking the SU-8 on the hot plate, a chrome coated quartz mask with circular, square and rectangular features (1.5 and 2.5 μm openings) is used to transfer the patterns to the SU-8 photoresist layer.

UV-Lithography was then carried out using a Karl Suss MA6 mask aligner (Karl Suss) in a vacuum contact mode between the silicon wafer and the mask at 365 nm wavelength. Then the SU-8 is kept again on the hot plate for 5 minutes for the purpose of post-exposure at 95°C and cooled down to the room temperature gradually. In this step, the cationic photo-polymerization of the epoxy occurs in SU-8 resist. Finally, the exposed resist layer is developed by immersion of the whole wafer inside the SU-8 developer (MicroChem Corp.) with manual agitation for 2 minutes. After development, the sample is rinsed with isopropyl alcohol (IPA) and subsequently dried with N₂ gas. The resulting structure is an array of circular and rectangular holes with different sizes registered across the SU-8 film. Figure 4.2 shows the SEM images of a thorough-hole membrane, in which arrays of rectangular and circular pores are formed in the SU-8 film. It should be noted that this membrane has a very small thickness and therefore the transmembrane pressure (TMP) during filtration will be low.

(a)

(b)

Figure 4.2: (a) SEM image of a polymeric micro-filter with an array of rectangular pores, (b) SEM image of a polymeric micro-filter with array of circular pores (3 μm pore diameter) made by the direct lithography technique.

Fabrication of high aspect ratio (HAR) pores with small dimensions ($< 2 \mu\text{m}$) inside a thick SU-8 film is practically impossible due to the tapering effect which normally happens during UV lithography [10]. To solve this problem, we proposed a novel multilayer technique that will be explained in greater detail later.

4.2.2 Fabrication of support structure

The perforated SU-8 film is too thin to be used directly for water filtration applications, and it also folds easily upon release from the wafer substrate; therefore, we constructed a backside support with larger openings using a thick layer of SU-8. For this purpose, a second layer of SU-8 (SU-8 2015, MicroChem corp.) film with thickness of $20 \mu\text{m}$ was spin-coated on top of the first layer. After soft baking on the hotplate for around 45 minutes, a second exposure through a plastic mask was carried out to form the support layer with $600 \mu\text{m}$ apertures in the backside of the membrane. After this second exposure, the substrate was held on the hotplate for 40 minutes for post-exposure and also good adhesion of the second layer to the first layer. Subsequently, it was cooled down to room temperature and was developed in SU-8 developer for 5 minutes with manual agitation. The resulting structure is shown in Figure 4.3.

Figure 4.3: SEM image of a membrane with an integrated support mesh made by multi-level lithography technique.

4.2.3 Release of polymeric membrane

The most important impediment in fabrication of polymeric micro-filters with this method is the release of the membrane from the substrate without damaging it. Backside support [20, 33] helps the membrane stay flat upon release from the substrate, but the large thickness of the support structure may cause the entire structure to collapse and adhere to the substrate while the sacrificial layer dissolves in the appropriate solvent. Therefore, finding a suitable material to be used as a sacrificial layer for the releasing step is an important issue. In the literature some methods have been proposed for this purpose, including sputtering (or electroplating) copper [120] or chromium [123] as a sacrificial layer beneath the SU-8 film and etching the metal film in the final step. Using metals such as copper or chromium may require extra steps such as sputtering, and also use of a toxic material as an etchant for sacrificial layer

removal. Alternatively, we can employ appropriate polymers and solvents for the purpose of releasing. Table 4.1 shows a list of materials that were used as a sacrificial layer in this study. All the solvents have no effect on the cured SU-8 film. We found that AZ 9260 and polystyrene [124] presented better results in the releasing step with respect to the film quality and complete dissolution in the solvent.

Depending on the thickness of backside support layer and the sacrificial layer in use, it typically takes between 10-20 minutes to release the membrane from the substrate in the particular solvent. Ultrasonic agitation (low frequency ≈ 20 kHz) is required for releasing the membrane from the substrate because it expedites the releasing process prevents the adhesion of the SU-8 film to the substrate when the sacrificial layer dissolves in the solvent. It is noted that the openings of the support mesh must be large enough to avoid causing any significant effect on the total hydraulic resistance to flow through the membranes [33].

Table 4.1: Materials and process conditions used for release of the microfilter from the substrate.

Material	Solvent	Curing process	Release step
AZ 9260	Acetone	Thickness 2 μm @ 110°C for 20 min.	Immersion in acetone bath for 15 min. (with ultrasonic agitation)
PMMA	Chloroform	Thickness 2 μm @ 95°C for 25 min.	Immersion in chloroform bath for 5 min. (without ultrasonic agitation)
Polystyrene	Toluene	Thickness 2 μm @ 90°C for 10 min.	Immersion in toluene bath for 15 min. (with manual agitation)
Polyurethanes	Dimethylformamide (DMF)	Thickness 2 μm @ 80°C for 30 min.	Immersion in DMF bath for 15 min. (with ultrasonic agitation)

4.3 Fabrication of multi-layer polymeric membranes with integrated back-support

4.3.1 Fabrication of perforated membrane

In conventional UV lithography, fabrication of small micro-holes (i.e., 0.5–5 μm) inside a thick SU-8 film is impossible due to the tapering effect [108,109], which normally happen during UV exposure (i.e., usually the top layer is overexposed and tends to be wider than the bottom layer, which is relatively underexposed, resulting in a variation in the lateral dimensions). To overcome this problem, we employed a multi-level lithography technique to fabricate polymeric membranes using multiple coating and exposure steps and a single developing process. With this process we can have great control of pore size in manufacturing robust micro/nano-sieves.

For this purpose SU-8 2010 and SU-8 2015 (MicroChem corp.) were used for the development of multi-layer polymeric micro-filters. A schematic representation of the entire fabrication process is illustrated in Figure 4.4. First, a standard silicon wafer is cleaned carefully in piranha solution and dehydrated under vacuum in a Suss machine (Delta 150 VPO) for 2 minutes. After spin-coating a sacrificial layer (i.e., similar to previous section) and curing in the appropriate time, a 10 μm thin layer of SU-8 photoresist (SU-8 2010, MicroChem corp.) was spin-coated on the top of the cured sacrificial film. In order to avoid bubbles, the photoresist is poured onto the substrate directly from a bottle with a large aperture. A soft baking process is performed on a carefully leveled hot plate at 65°C for 1 minute and 95°C for 5 minutes, followed by a 10-minute relaxation at 25°C (Fig. 4.4b). Baking steps are really crucial in order to reduce the internal stress in the final film. After soft baking the SU-8 on the hot plate, a chrome coated glass mask with rectangular features (12×9 μm) is used to transfer the patterns into the SU-8 photoresist. The pitch size (i.e., distance between the mask

features in the x and y -directions) was $4\text{ }\mu\text{m}$ and $2\text{ }\mu\text{m}$ in x and y -direction, respectively (see Fig. 4.5a). UV-Lithography was processed by a Karl Suss MA6 mask aligner (Karl Suss Inc.) in the vacuum contact mode between the silicon wafer and the mask with a 350W mercury lamp with a high pass UV filter in order to cut off undesired short wavelengths (i.e., exposed for 14 seconds corresponding to a dose of 120 mJ/cm^2). Then SU-8 is kept again on the hot plate around 5 minutes for post-exposure at 95°C and cooled down to the room temperature for the purpose of relaxation for 15 minutes. Those parts that received UV light cross-link to each other, which render permanent (i.e., dark brown areas in Fig. 4.4c) and the areas that not exposed were removed by solvent during the development process. A micro-fabricated membrane must be robust enough to endure the pressure of filtration. A key parameter that has a direct effect on the membrane strength is the thickness of sieving layer. As discussed earlier, due to the non-uniform UV exposure dose in a thick SU-8 film, usually the top layer is overexposed and tends to be wider than the bottom layer that is relatively underexposed, resulting in pore closure.

Figure 4.4: Fabrication process of a multi-layer polymeric micro-sieve, (a) spin-coating of a sacrificial layer on the Si substrate, (b) spin-coating of first layer of SU-8, (c) exposure through the first quartz mask (i.e., dark brown areas are exposed areas), (d) spin-coating of second layer of SU-8, (e) exposure through the second quartz mask (i.e., dark brown areas are exposed areas), (f) spin-coating of third layer of SU-8, (g) exposure through the foil mask (i.e., dark brown areas are exposed areas), and (h) simultaneous development of all three layers and release of the membrane from the Si substrate by dissolving the sacrificial layer. *Note: this schematic is just for purpose of illustration and do not represent the exact geometric dimensions.*

To solve this problem, we proposed a novel solution to make large holes in the first layer and then reduce the pore size by laying parallel strips in the middle of the pores. For this purpose a second layer of SU-8 2010 with a 10 μm thickness was spin-coated on top of the first layer (Fig. 4.4d). After soft baking the second layer on the hot plate (i.e., at 65°C for 1 minute and 95 °C for 4 minutes, 10 minutes relaxation at 25°C), another quartz/chrome mask with array of strips features (3 μm width) is used to transfer the pattern precisely in the middle of previous features (Fig. 4.4e). Alignment of the second mask with the patterns on the first layer was carried out using the precise microscopes of the Karl Suss MA6 mask aligner, and exposure was performed for 12 seconds with a UV dosage of 120 mJ/cm^2 . To avoid excessive stress, adhesion failure, and pattern dimensional change, the exposure dose must be adjusted according to the substrate or under layer reflectivity [118]. For this case finding the optimum dose for the second layer was a serious challenge, because an extra or insufficient dosage can lead to the pore occlusion and pattern failure. After exposing the second layer, the wafer is kept again on the hotplate at 95°C for the post exposure and cooled down to the room temperature for the purpose of relaxation for 10 minutes. Similar to the previous step, those areas that exposed cross-linked to each other, which rendered permanent (i.e., dark brown areas in Fig. 4.4e) and the areas that not exposed are removed during development step by developer.

Figure 4.5 shows a schematic and optical image of a membrane obtained with this technique. An array of parallel strips (3 μm width) that perfectly placed at the center of rectangular (12×9 μm) pores can be seen. The final pores have high aspect ratio slits with 12×3 μm dimensions that can be used for different applications such as *C. parvum* oocysts isolation, blood filtration and protein purification.

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4.3.2 Fabrication of support structure

Similar to the previous section, to improve the strength of the micro-fabricated micro-sieves, we used a thick layer of SU-8 (SU-8 2015, MicroChem corp.) to make a back side support with large openings. For this purpose, a third layer of SU-8 film with thickness of 20 μm was spin-coated on top of the second layer (Fig. 4.4f). After soft baking on the hotplate for around 40 minutes, a third exposure through a plastic mask with square shape openings ($600 \times 600 \mu\text{m}$) was carried out to form the support layer with 600 μm apertures in the backside of the double layer membrane (Fig. 4.4g). Following third exposure, the substrate was held on the hotplate for around 25 minutes for post-exposure and also good adhesion to the second layer. After cooling down to the room temperature with slow ramping, all the SU-8 levels were developed simultaneously at room temperature in Propylene-glycol-Methyl-Ether-Acetate (PGMEA) (Fig. 4.4h). This single developing step process provides important advantages over a process for which each coated layer is developed prior to coating of subsequent layers. For example, simultaneous development of multiple SU-8 layers significantly reduces the processing time. In addition, coating uniformity is increased compared to a process that coats over the topography of previously patterned layers. With this method membranes with different thicknesses and pore sizes can be produced. In order to release the membrane from the substrate, we put the wafer inside an acetone bath for around 5 minutes with ultrasonic agitation. Ultrasonic agitation is necessary for releasing the membrane from the substrate because, firstly, it expedites the releasing process and secondly, it prevents the adhesion of SU-8 film to the substrate when the sacrificial layer is dissolving in the solvent. Figure 4.6 shows an SEM image of a multi-layer micro-sieve with integrated back support.

Figure 4.6: SEM image of a polymeric multi-layer micro-sieve with integrated back-support. Close-up view shows the perforated micro-sieve.

Figure 4.7: Back-side view of a polymeric multi-layer micro-sieve with slit-shape openings ($3 \times 12 \mu\text{m}$) and integrated support mesh.

The inset image shows how the array of stripes perfectly placed between the rectangular slits on the first layer. Figure 4.7 depicts the back-side view of a multi-layer micro-sieve. This micrograph reveals that the resulting membrane has a smooth surface and high porosity, which make it ideal for microfiltration of biological samples such as blood cells or isolation of microorganisms such as *C. parvum* oocysts.

4.4 Fabrication of polymeric membrane (single layer or multi layer) with integrated back-support made from silicon

In another approach for fabrication of polymeric micro/nano filters, we employed MEMS techniques to fabricate microfilters to be used for filtration of large volumes of water. For this purpose we employed 100 mm silicon wafers to construct our microfilters on top and to back-side-etch the wafers to open windows for filtration. Figure 4.8 shows schematically the entire fabrication process. Steps 4.8a–4.8d are similar to that of the previous sections, but fabrication of the integrated support in this design is quite different. In order to fabricate the back-support using a Si wafer, a thin layer of AZ 9260 photoresist was spin-coated on the back-side of the silicon wafer and pre-baked at 110 °C for 4 minutes (Fig. 4.8d). Then, photolithography was carried out with a plastic mask with rectangular shape openings (800×400μm) to transfer the pattern on the silicon wafer (Fig. 4.8e). Then, a carrier wafer was attached to the other side to protect the wafer during the dry-etching process (Fig. 4.8f). Dry etching was employed to open vertical trenches (i.e., through the wafer) inside the silicon substrate by using an STS deep reactive ion-etching (DRIE) machine (Fig. 4.8g). Finally, a micro-fabricated membrane with integrated back-support is achieved by detaching the carrier wafer in an acetone bath with ultrasonic agitation (Fig. 4.8h).

Figure 4.8: Schematic illustration of the fabrication process: (a) Spin-coating the SU-8 on a Si substrate, (b) first photolithography to transfer the first pattern to the SU-8 film (i.e., dark brown areas are exposed regions), (c) development of the exposed film, (d) spin-coating of AZ 9260 photo-resist on the backside of the Si substrate, (e) second photolithography to transfer the second pattern, (f) attachment of a dummy wafer for backside etching, (g) DRIE etching through the wafer, and (h) detachment of the dummy wafer and removal of the AZ photo-resist. Note: this schematic is just for purpose of illustration and do not represent the exact geometric dimensions.

Figure 4.9: Optical image a micro-fabricated polymeric membrane with integrated back-support. (a) Top view that shows the sieving layer, and (b) back view that shows the integrated back support.

Figure 4.10: SEM images of the polymeric membranes with different pore size and shape. (a) slit-shape pores with ($4 \times 10 \mu\text{m}$), (b) slit-shape ($2 \times 20 \mu\text{m}$), (c) square-shape membrane ($6 \times 6 \mu\text{m}$), and (d) multi-layer membrane ($12 \times 2 \mu\text{m}$).

Figure 4.9 shows the optical image of the micro-fabricated polymeric membrane with integrated back-support after release from the carrier wafer. It can be seen that a smooth layer of SU-8 film is covering the entire wafer, and rectangular windows are perfectly formed on the back-side of the wafer. With this method, we could successfully make polymeric membranes with different pore size/shape (see Fig. 4.10) by employing appropriate masks for a variety of applications such as pathogen isolation, absolute particle filtration, yeast harvesting, etc.

4.5 Membranes properties

By employing a conventional lithography technique, we demonstrated a simple and rapid process for fabrication of micro-filters. In this study we employed SU-8 resist for making a filter due to its wide use as a structural layer and its biocompatibility [10,117], and its good thermal and chemical stability. Similar to the Chapter 3, the measurement of the pore-size distribution of the SU-8 filter membranes was also performed using digitalized photographs from a HITACHI S3500 scanning electron microscope, which is equipped with a 'in-built dimension measurement' module and image-analysis program SEMICAPS 2200 (Semicaps Pte Ltd) from random regions of the samples. For example, for a sample membrane with a circular pore shape (i.e., 3 μm), the mean pore diameter was 3 μm for different regions and the standard deviation was around 100 nm. Therefore, the corresponding coefficient of variation ($CV = \sigma/M$) was 3.33%. The histogram of the analyzed samples is shown in Figure 4.11.

Figure 4.11: Pore-size distribution of a polymeric microfilter with 3 μm circular perforations.

The overall coefficient of variation (CV) for all the micro-fabricated filters (i.e., both with slotted and circular pores) was less than 5%, which is much lower than available commercial microfilters.

The strength of the polymeric membranes is an important issue that has not been investigated. The mechanical strength of a membrane depends on the thickness, the Young's modulus, the intrinsic tensile stress, the shape and distribution of the pores and the distance between the bars of the support structure [21]. Kuiper [21] proposed the following correlation for determining maximum load of a perforated membrane:

$$P_{\max} = 0.58 \frac{h\sigma_{\text{yeff}}^{1.5}}{lE_{\text{eff}}^{0.5}}$$

where P_{max} is the maximum load, h is the membrane thickness, l is the distance between the support bars, E_{eff} and σ_{yeff} are the effective Young's modulus and yield strength, respectively. The porosity in this model is accounted for by $(1-K)$ for calculation of E_{eff} and σ_{yeff} , where K is the porosity. By choosing $h = 4 \mu\text{m}$, $l = 600 \mu\text{m}$, $E = 2 \text{ GPa}$ [20], $\sigma_{yeff} = 60 \text{ MPa}$ [107], $K = 25\%$ and $E_{eff} = (1-0.25) \times E$ (Young's modulus) for the parameters of micro-fabricated membrane, the burst pressure would be 53 KPa (0.53 bar), which is enough for most microfiltration processes. Finite element simulation was also used in this work to investigate the stress distribution and the maximum load in the polymeric micro-filter with different frame sizes (backside support). Figure 3 shows that the largest stress is located at the middle of the edge because the total tensile stress at this location is the addition of the constant tensile stress due to stretching and the bending stress near the middle of the edge [125]. At the center the membrane also experiences the highest bending stress. Those holes which are located near the edges are also experiencing maximum stress. For a specified pressure the deflection of the perforated membrane is around 7% larger than that of non-perforated membranes. The perforation distribution has no significant influence on the mechanical stability of the membrane, but the aperture size of the backside support does. In addition, a membrane with a higher porosity deflects more than a membrane with lower porosity. The FEM results also confirm that the maximum stress is approximately proportional to the applied pressure.

Figure 4.12: FEM simulation of the stress distributions of perforated membranes, in which only the membrane within one support mesh element ($600 \times 600 \mu\text{m}$), is shown. The membrane is under vertical pressure (i.e., perpendicular to the surface to simulate pressure of filtration) and all 4 edges are clamped. Hence, it just can deflect in vertical direction.

In addition to the square-shape support mesh, we also employed hexagonal openings to take advantage of the membrane surface. Their dimensions depend on the thickness of the micro-fabricated membrane and on the pressure of the filtration process. For higher pressures and thinner membranes, the dimensions of the hexagons should be smaller [125]. Figure 4.13 shows an SEM photo of a membrane that was broken during the filtration. This photo also confirms that the rupture occurs in the center and middle of the membrane edges, where the stress is maximal.

Figure 4.13: SEM photograph of a broken membrane which shows the rupture occurs at the points of maximum stress.

4.6 Summary

In this chapter two methods for the fabrication of polymeric micro/nano filters with integrated back-support were investigated. Both methods used the advantages of conventional UV-lithography and MEMS techniques. With the first technique, we have successfully made polymeric microfilters with identical pore size and shape on a single layer of SU-8 resist. Due to the limitation of contact mask lithography, it is impossible to project small features ($< 2 \mu\text{m}$) inside a thick SU-8 film. To overcome this obstacle, we developed a novel multi-layer technique to fabricate slotted pore polymeric filters with micron and potentially sub-micron size using SU-8. The resulting membranes have smooth surfaces, narrow pore-size distribution, high porosity, and good mechanical strength. Concerning scaling-up, these methods are

promising because the photolithographic techniques are well-known; therefore, not much further research is needed.

Chapter 5

5

Fabrication of metallic micro/nano filters using conventional lithography and electroplating techniques

Two innovative methods for fabrication of polymeric micro/nano filters have been introduced in the previous chapters. These two methods are fairly easy to scale up since the technologies that have been used are fully developed. For applications where harsh conditions are dominant, we need to have membranes with high durability, chemical inertness and thermal resistance. In this chapter we employed the advantages of high-precision lithography and electroplating techniques to fabricate robust metallic micro/nano filters with integrated back-support.

Parts of this chapter have been published previously in the Journal of Biomedical Microdevices [126].

5.1 Introduction

A key component in all of today's membranes is a microporous structure with identical pore sizes and arbitrary shape as well as high porosity [127]. These structures can be used without further modification as microfiltration or nanofiltration membranes or as supports for composite membranes used in reverse osmosis, pervaporation, and gas separations. Micro/nano filters can be fabricated by conventional UV lithography followed by corrosion processing of the metal [128] or even by direct metal deposition on a patterned substrate (i.e., Photo Electro Forming) [129,130]. Photo Electro Forming (PEF) is an additive process that grows parts, molecule by molecule, via the electro-deposition of metal onto a pre-defined pattern, which is normally made using photolithography. This process is similar to the LIGA process, which has been widely used in industry for precision manufacturing of high-aspect-ratio micro-components such as micro-fluidic chips and optical fiber connectors [131]. In this chapter a new membrane fabrication technique is described that is capable of producing microporous structures of extremely high porosity and narrow pore size distribution from various metals (i.e., Nickel, Iron, Aluminum, Titanium, etc.) with pore sizes in the submicrometer range. An integrated support layer with large openings was also attached to the membranes to protect the membrane from tearing and failure during handling and filtration.

5.2 Design and fabrication

5.2.1 *Fabrication of the microfilter*

Figure 5.1 shows schematically the fabrication process of a metallic micro-fabricated membrane with integrated back-support.

Figure 5.1: Schematic of the fabrication process, (a) deposition of seed layer (Cr/Cu) on a Si substrate, (b) spin-coating of a thick layer of AZ9260 on the Si wafer, (c) UV exposure through a quartz mask with rectangular-shape features, (d) development of the exposed film inside AZ developer, (e) electroplating of Ni between photo-resist pillars, (f) spin-coating of another thick layer of AZ9260, (g) UV exposure through a plastic mask with square-shape features, (h) development of the exposed film inside AZ developer, (i) second electroplating of Ni between photo-resist features, (j) releasing the through-hole membrane with integrated back-support by dissolving the photo-resist and seed layer in acetone and Cu etchant, respectively. Note: this schematic is just for purpose of illustration only and do not represent the exact geometric dimensions.

First, a thick standard 100 mm diameter, (100)-oriented silicon wafer, was cleaned carefully in piranha solution (96% H_2SO_4 and 30% H_2O_2) for 20 minutes at 120°C to remove any organic contaminations on the wafer surface. After rinsing with DI water and drying with N_2 gas, the substrate was submerged in the buffered oxide etchant (BOE) for 5 minutes to clean the native oxide layer. This step has a significant impact on adhesion of seed layer to the substrate. Then electrical contact was provided through a 300 nm thick Cr/Cu seed layer that was deposited on the silicon wafer by sputtering process, using a magnetron sputtering machine (Fig. 5.1a). To enhance the adhesion of photo-resist to the seed layer, the dehydration bake-step was performed under vacuum in a Suss machine (Delta 150 VPO) for 2 minutes. Afterward, a 10 μm thick AZ9260 (Microchemicals GmbH) photo-resist was spin-coated on the wafer at a spin speed of 2000 rpm (Fig. 5.1b). In order to avoid bubbles, the photo-resist was poured onto the substrate directly from a bottle with a large aperture. A soft baking process was performed on a carefully leveled hot plate at 110°C for 4 min and followed by a 2 min relaxation at 25°C . After soft baking the AZ film on the hot plate, a chrome-coated glass mask with rectangular features ($3\times 8\mu\text{m}$) was used to transfer the patterns into the AZ photoresist. UV-Lithography was processed by Karl Suss MA6 mask aligner (Karl Suss Inc.) in the hard contact mode between the silicon wafer and the mask with a 350W mercury lamp with a high pass UV filter in order to cut off undesired short wavelengths (Fig. 5.1c). Finally, the exposed film was developed at room temperature in AZ 400k (Microchemicals GmbH) developer that was diluted with DI water (1:2) for 2 minutes with manual agitation (Fig. 5.1d). The width of the pillars can be controlled precisely using the UV exposure and development time. After rinsing with DI water and drying with N_2 gas, the wafer was mounted onto a holder that provides a homogeneous electric contact with the conducting layer coated on the

substrate. Then the holder was immersed in the electroplating bath to electroform the nickel between the photo-resist pillars (Fig. 5.1e). The bath solution has a volume of about 5 liters and operates at 50 °C. The bath composition is shown in Table 5.1. Figure 5.2 shows the photo of our fabricated electroplating bath for nickel electroplating.

Table 5.1: Chemical composition of the Ni electrolyte bath.

Chemical name	Formula	Bath concentration
Boric acid	H_3BO_3	30–50 (g/l)
Nickel chloride	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	5–20 (g/l)
Nickel sulphamate	$\text{Ni}(\text{NH}_2\text{SO}_3)_2$	320–400 (g/l)
Additives	N/A	5 – 20 ml/l

Figure 5.2: Optical image of our fabricated electroplating bath for nickel plating.

Nickel sulphamate is the main source of nickel ions for electroforming, which will be deposited on the cathode; while nickel chloride promotes nickel anode dissolution and prevents anode passivation (i.e., causing pH increase and sulfamate hydrolysis that leads to increase internal stress) [132,133]. The boric acid acts as a pH buffer that prevents basic nickel compound formation at the cathode and thereby minimizing the internal stress of the nickel deposits. Appropriate additives are added to the bath solution in order to shift the stress from tensile to compressive, preventing pitting formation on the cathode surface and adjusting the pH value during operation [133].

5.2.2 Fabrication of back-support

5.2.2.1 Integrated back-support made from nickel

The micro-fabricated membrane prepared by aforementioned procedure is too thin and may easily fold or break during filtration or handling. Hence, it is required to strengthen the membrane with a back-support with large apertures. For this purpose a thick layer of AZ 9260 was again spin-coated on the membrane surface (Fig. 5.1f) and lithography was performed using a plastic mask with square shape features (600×600 μm) to transfer the pattern to the photo-resist (Fig. 5.1g). Then, the exposed film was developed at room temperature in AZ 400k developer for 3 minutes followed by rinsing with DI water and N₂ gas drying (Fig. 5.1h). The second electroplating was performed in the same bath for around 15 minutes to form the support structure between the photo-resist gaps (Fig. 5.1i). Afterwards, photo-resists which remained from the previous steps, was dissolved in an acetone bath. After washing the wafer with isopropanol and also DI water, the seed layer was removed using Cu etchant (Sigma Aldrich) in order to release the micro-fabricated membrane from the substrate

(Fig. 5.1j). Ultrasonic agitation can be useful in this step because it expedites the releasing process and also prevents adhesion of the membrane to the substrate.

5.2.2.2 Integrated back-support made from silicon

In an alternative embodiment for fabrication of the back-support structure, the membrane support can be made by anisotropic/isotropic etching of the Si wafer using KOH or DRIE similar to the process that have been explained in Chapter 4, section 4.4 [10]. For this purpose we have used a double-sided polished silicon wafer, one side for the electroplated sieving layer, the other side for back-side etching of the Si wafer using KOH (or DRIE). In this case it is not required to release the membrane from the Si substrate. With this technique we can make a metallic micro/nano filter on top of a Si wafer with different thickness (i.e. 100 -1000 μm). A detailed explanation of the back-side etching can be found in the literature [10].

5.2.3 Fabrication of nanofilters using electroless plating

In conventional UV lithography it is technically impossible to project a clear image of a small feature ($< 1 \mu\text{m}$) inside a thick photoresist layer due to the limitation in wavelength of the light and also the reduction lens system [10]. In this study we employed an electroless nickel plating technique to reduce the pore size of the micro-fabricated microfilter evenly in order to achieve a high aspect ratio and robust nanofilter. The pore size of the final nanofilter can be controlled via the amount of Ni deposited [134]. It should be noted that electroless nickel plating is an extremely conformal process that offers distinct advantages such as uniformity of the deposits (i.e., even on complex shapes) when plating irregularly shaped objects and holes and almost zero stress in the deposited film [134]. In addition to conformal metal

deposition, other methods such as atomic layer deposition (ALD) as well as polymer deposition (i.e., Parylene C, Teflon, Heparin, etc.) can be employed for the purpose of pore reduction. The concept and schematic of conformal pore reduction are illustrated in Figure 5.3 [135,136].

Figure 5.3: Schematic of pore-size reduction using conformal deposition of metals or polymers.

5.3 Results and discussion

By employing conventional UV-lithography and electroplating techniques, we present a low cost method for fabrication of high-flux micro/nano-filters with perfectly ordered pores and integrated back-support. These techniques have been used widely in the semiconductor industry; therefore, this method can be scaled-up for mass production of micro-fabricated membranes.

5.3.1 Membrane morphology

Figures 5.4(a) and 5.4(b) show SEM photographs of the photo-resist pillars with the nickel thin film deposited between the gaps, respectively. The nickel film cannot be

thicker than about 85-90% of the photo-resist structure height; otherwise the nickel film will fill the microfilter pores. The thickness of electroplated nickel coating can be calculated from Faraday's law [133]. The resulting metallic membrane with slotted pores is also shown in Figures 5.4(c) and 5.4(d). It can be seen that an array of rectangular pores ($2.5 \times 8 \mu\text{m}$) with uniform intervals perfectly formed.

Figure 5.4: (a) SEM image of the photo-resist pattern (micro-pillars) used to electroplate the microfilter, (b) SEM image of a thin Ni layer electroplated between photo-resist pillars, (c) SEM image of a through-hole microfilter with slotted pores, and (d) cross-section view of the obtained microfilter.

The width of the photo-resist pillars was reduced from 3 to $2.5 \mu\text{m}$ precisely by controlling the exposure and development time during lithography. It should be noted

that a slotted pore design provides a significantly lower pressure drop than a circular pore membrane because the membrane resistance is much smaller [21]. In addition, it has been shown by other researchers that a slotted pore membrane is less vulnerable to the particle bridging (i.e., less fouling) than the one with circular pores [137]. Figure 5.5 also shows an optical microscopic image of a 100 mm diameter metallic membrane on a silicon substrate. The close-up view shows the SEM image of the Ni integrated back-support formed on top of the membrane. As discussed before, an integrated back-support safeguards the membrane from tearing and failure during micro-filtration, besides keeping it flat. Figure 5.6 also depicts a nanofilter (i.e., ≈ 400 nm pore size) obtained by conformal deposition of nickel onto the pore walls of a microfilter with an initial pore size of $2\ \mu\text{m}$.

Figure 5.5: Optical image of a metallic membrane with an integrated back-support before release from the silicon substrate. The close-up SEM images of the integrated back-support and through-hole membrane are also shown.

Figure 5.6: SEM image of a high aspect ratio nanofilter made by conformal electroless deposition of Ni onto the pore walls of a microfilter.

Figure 5.7 also shows SEM images of a micro-fabricated membrane with a silicon-integrated support. It can be seen that a smooth film of perforated nickel is perfectly deposited on the silicon wafer (Fig.5.7(a)); back-side windows also created successfully using DRIE machine (Fig.5.7(b)).

Figure 5.7: Optical image a micro-fabricated polymeric membrane with integrated back-support. (a) Top view which shows the sieving layer, and (b) back view which shows the integrated back support.

Figure 5.8 depicts a 100 mm micro-fabricated metallic filter attached to various PMMA supports (i.e., PMMA rings or PMMA with honey-comb openings), which were cut using a CO₂ laser machine. These membranes can be easily tailored using a scissors and can be used for various microfiltration purposes.

Figure 5.8: Optical image a micro-fabricated metallic membrane attached to PMMA supports.

5.3.2 Membrane strength

Experimental and numerical investigations were carried out to calculate the mechanical properties of the metallic micro-fabricated membranes. Based on the following correlation that was introduced by van Rijm et al [10,125] and explained in the previous Chapter, the mechanical strength of a perforated membrane depends on the membrane thickness and Young's modulus, the intrinsic tensile stress, and the distance between the bars of the integrated back-support.

$$P_{\max} = 0.58 \frac{h \sigma_{yeff}^{3/2}}{l E_{eff}^{1/2}}$$

In this model, the changes of mechanical properties due to the sieve-like structure of the membrane is considered by a global correcting factor $(1-K)$, where K is the relative perforated area. Based on this theory, a perforated membrane can be modeled as a non-perforated membrane with an adjusted Young's modulus and yield strength. For comparing the results from the analytical correlation with experiment, a small test device was made, by which the burst pressure of micro-fabricated membranes with different thicknesses could be measured using a pressure sensor (see Fig. 5.9 and 5.10).

Figure 5.9: Schematic of test setup for determining membrane strength [125].

Figure 5.10: Optical image of our test setup showing a membrane during the test. The pressure sensor is connected to the O₂ gas vessel.

Figure 5.11 shows the reciprocal values of the burst pressures for the metallic micro-fabricated membranes with a square-shaped integrated back-support ($600 \times 600 \mu\text{m}$ openings) in comparison to the reciprocal values of theoretical burst pressures calculated using Rijn's model [125]. The following typical values were used for the calculations:

$h = 2, 4, 6, 8$ and $10 \mu\text{m}$, $l = 600 \mu\text{m}$, $E = 210 \text{ GPa}$ [138], $\sigma_{\text{yield}} = 400 \text{ MPa}$ [138] and $K = 36\%$.

Figure 5.11: Reciprocal value of rupture pressure for the metallic micro-fabricated membranes with different thicknesses in comparison to the theoretical values calculated using Rijn's model. In all the analytical calculations using Rijn's model, the membrane assumed to be under vertical pressure (i.e., similar to the experimental condition) with the clamped edges. Hence, it just can deflect in vertical direction.

It can be seen that the measured rupture pressure (i.e., mechanical stability) of the micro-fabricated membranes with different thickness is larger than that calculated from Rijn's model. This under-estimation from this theory is attributed to the fact that

for ductile metals such as Ni, Stainless Steel, and Ti, there is a linear relation between the strain and the applied stress up to the yield stress (σ_{yield}). After this point, the membrane may not break and the stress in the middle of the edge (i.e., where the maximum stress is occurs) may increase up to the ultimate stress (σ_{ultimate}). Between the σ_{yield} and σ_{ultimate} , the membrane strain can increase significantly, and E cannot be a constant value [125,139]. Therefore, only an under-estimate of the maximum load can be given with the above correlation for the ductile materials.

Figure 5.12 gives an indication of the stress distribution using finite element simulation over the surface of a micro-fabricated membrane with slotted pores. It can be seen that the largest stress is located at the middle of the edges because the total tensile stress at the edge is the summation of the constant tensile stress due to stretching and the bending stress near the middle of the edge [125,138,140].

Figure 5.12: FEM simulation of the stress distributions over the surface of a perforated membrane with slotted pores, in which only membrane within one support mesh element ($600 \times 600 \mu\text{m}$) is shown. The membrane is under vertical pressure (i.e., perpendicular to the surface to simulate pressure of filtration) and all 4 edges are clamped. Hence, it just can deflect in vertical direction.

The highest bending stress is also located at the center. From the FEM analyses, it is also found that the deflection of perforated membrane is around 7-9% larger than that of non-perforated membranes for a specified pressure. The aperture size of back-side support has also a large influence on the membrane strength. The FEM results also confirm that the maximum stress is approximately proportional to the applied load and the deflection of membrane at the center is independent of the membrane pore size and thus the distribution of the pores [139,140]. It should be noted that for all the numerical calculations, a large displacement analysis with total Lagrange description has been chosen.

5.4 Summary

For applications where harsh conditions are dominant, a membrane must have high durability, chemical inertness and thermal resistance. In this Chapter, we employed the advantages of high-precision lithography and electroplating techniques to fabricate robust metallic micro/nano filters with an integrated back-support made from nickel, silicon or PMMA. Metallic membranes are very robust and have a smooth surface, which makes them ideal for many applications. By using an electroless plating technique, we successfully reduced the pore size of the microfilters to hundreds of nanometers evenly. The mechanical stability of these filters was also investigated both theoretically and experimentally. The results were also validated by FEM analysis.

A black and white photograph showing two micro-fabricated filter components. On the left is a dark, elongated, oval-shaped structure. On the right is a circular structure with a bright, glowing center, possibly a light source or a specific feature of the filter.

Chapter 6

6

Surface modification of micro-fabricated filters

Polymeric and metallic micro-fabricated membranes have excellent sieving properties. Their identical properties such as high surface porosity, perfectly patterned pore structure and mechanical strength make them ideal for many applications such as microorganism removal, blood filtration and protein purification. To improve the functionality and **performance** of the micro-fabricated filters, two different strategies for surface modification were employed, which will be discussed in detail in this chapter.

Parts of this chapter have been published previously in the Journal of Key Engineering Materials [141].

6.1 Introduction

Membranes are receiving increased attention for water treatment and other applications. However, a major barrier to further incorporation of membrane systems in industrial operations is flux decline resulting from fouling [1,12]. Membrane fouling refers to deposition of retained particles, colloids, etc., at the membrane surface or on the membrane pore wall, which normally reduces the membrane's performance [80]. The fouling generally happens due to the interaction between the membrane surface and the foulants including inorganic, organic, and biological substances in many different forms [80,142].

The separation process by a membrane is essentially a surface phenomenon. In particular, the sieving layer plays the vital role. Therefore, it is better to modify the membrane surface for reducing the fouling [2,142]. It is generally accepted that an increase in hydrophilicity offers better fouling resistance because:

i) Particles that exist in aqueous media tend to be hydrophobic, such as metal colloids, proteins, clays (e.g., silicates and alumina) and oily particles (e.g., paraffin, oils and surfactants) [2,12]. Hydrophobic particles tend to cluster or group together to form colloidal particles so that the interfacial area and therefore, the system-free energy can be reduced.

In addition, hydrophobic particles have a tendency to attach to hydrophobic or less hydrophilic surfaces to reduce their exposure to water (i.e., water has rather high surface energy by nature; it is a polar molecule and forms hydrogen bonds). Therefore, by increasing the hydrophilicity of the membrane, foulants prefer binding to water over other materials and less fouling will occur [2,84].

ii) Hydrophilic surfaces have a contact angle (θ) close to 0° , while more hydrophobic materials exhibit a contact angle close to or above 90° . The consequence

of the contact angle for capillary (or pore) intrusion behavior, which is important for membrane filtration, is shown schematically in Figure 6.1.

It can be seen that hydrophilic surfaces help in wicking of water into pores, while in hydrophobic pores; pressure is required to intrude water into pores [14].

Figure 6.1: The effect of contact angle on the pore-intrusion phenomenon, (a) hydrophilic surface ($\theta < 90^\circ$) (b) hydrophobic surface ($\theta > 90^\circ$) (c) water intrudes spontaneously (wicks) into pores if $\theta < 90^\circ$ and (d) no intrusion occurs (in the absence of pressure) if $\theta > 90^\circ$ [14].

For this purpose, some well-known strategies for surface modification of polymeric/metallic micro-fabricated filters were employed, including plasma treatment and wet chemical modification with subsequent coating [142,143].

6.2 Surface modification of polymeric micro-fabricated filters

In the previous chapters we described two different strategies for fabrication of polymeric micro/nano filters using MEMS techniques. SU-8, a biocompatible material

with extremely robust mechanical and chemical properties, was used as a structural component for fabrication of micro/nano filters [144]. It has been employed as a structural material in numerous bio-analytical microdevices, sensors, bioassays, and drug-delivery vehicles [145]. However, the hydrophobicity of SU-8 presents some impediments to many applications, including difficulty in surface wetting, biofouling and limited cell attachment [146,147]. Surface modification of SU-8 using plasma treatment [146,148], graft polymerization [147] and adsorption of bio-molecules [147] have been used to circumvent many of these problems. In this study, we employed two different strategies to make our polymeric micro-fabricated filters hydrophilic in order to improve their performance during microfiltration tests.

6.2.1 Plasma treatment

Plasma treatment is widely used to tune the surface properties of many polymers (e.g., PDMS, PMMA, PC, SU-8, etc.) to promote adhesion or to enhance wetting properties [149]. In this process plasma in the presence of oxygen is believed to promote peroxides on the surface of the membrane. The peroxides then decompose to form oxygen-containing radical groups such as hydroxyls, carbonyls, or carboxyls [136,149,150]. Plasma processes also can introduce specific binding sites or modify the polymer surface by cross-linking, chain scission or incorporation of functionalities [150]. The stability of the hydrophilization depends on the intensity of the plasma treatment.

For oxygen plasma treatment of our samples, we used a Harrick plasma cleaner (model: PDC-002) with an oxygen pressure of 0.4 mbar at a power of 30 W. The treatment was carried out for different times (from 1 to 8 min) in order to investigate the effect of treatment duration on the membrane's hydrophilicity. Figure 6.2 displays

the static contact angle (Ultrapure water, 18.2 M Ω cm) directly after surface treatment of the SU-8 samples, which was measured using a goniometer (Krüss GmbH, Germany). The sessile drop method was used to determine the static contact angle and the results were averaged over three measurements on each sample. It was found that the plasma treatment can significantly enhance the wettability of polymeric micro-fabricated filters. The decrease in the contact angle indicates the chemical changes (i.e., a significant increase in the surface-free energy) took place at the membrane surface after the plasma treatment. It should be noted that the treated surfaces remained hydrophilic for several days with only a moderate hydrophobic recovery.

Figure 6.2: Contact angles of samples directly after treatment with O₂ plasma.

Surface roughness of modified and unmodified samples was measured by atomic force microscopy (AFM) in a tapping mode (Dimension 3000 SPM, Veeco, Santa Barbara, CA). Silicon cantilevers with a nominal resonance frequency of 300 kHz were used in

all the measurements. Images were processed using SPIP 4.5 software (Image Metrology A/S, Lyngby, Denmark). It should be noted that roughness measurements by AFM can be affected by the choice of scan range and tip geometry [146,151]. To eliminate effects due to varying scan size, all images were taken at a scan size of $0.5\ \mu\text{m} \times 0.5\ \mu\text{m}$ with $512\ \text{pixels} \times 512\ \text{pixels}$. Figure 6.3 shows the surface topography of modified and unmodified samples with their corresponding contact angles. The untreated SU-8 sample exhibits a very smooth surface with an rms roughness of $0.3 \pm 0.02\ \text{nm}$ and a total height variation of only $4\ \text{nm}$; while the treated sample (8 min O_2 plasma) shows an rms roughness of around $4.5 \pm 0.7\ \text{nm}$ and total height variation of around $27\ \text{nm}$, which confirms that the rms roughness was increased by the plasma treatment [146,149].

Figure 6.3: Contact angle and topographic AFM images of SU-8 ($0.5\ \mu\text{m} \times 0.5\ \mu\text{m}$), (a) untreated surface, (b) treated surface with O_2 plasma for 8 min. The scale was set to the maximum peak-to-valley distance in each image as shown.

X-ray photoelectron spectroscopy (XPS) measurements were also performed to study the chemical composition of the modified and unmodified samples. Analysis was carried out by a PHI 5000 Versa Probe spectrometer (ULVAC-PHI, INC., US) equipped with a 180° spherical capacitor energy analyzer and a multichannel detection system with 16 channels.

The atomic elemental composition is summarized in Table 6.1. For an untreated SU-8 sample, the main two elements were found to be oxygen and carbon, which can be expected from an aromatic polyether material such as SU-8 [146,149]. The presence of small percentages of antimony near the detection limit of XPS was also observed, which normally occurred after plasma treatment [148].

Table 6.1: XPS chemical analysis of the modified and unmodified samples. Concentrations are given in relative atomic percent (at.%).

	Carbon (at%)	Oxygen (at%)	Nitrogen (at%)	Antimony (at%)
Untreated	84.0	16.0	0.0	0.0
Treated (8 min)	67.0	30.0	1.0	2.0

Survey spectra for untreated and treated SU-8 samples are displayed in Figure 6.4. The high-resolution spectrum of the C 1s region in Figure 6.4 (i.e., close-up) displays the changes in the surface chemistry after plasma O₂ treatment. Two main components, aromatic carbon (C-C) at 284.8 eV, ether carbon (C-O) at 286.5 eV can be seen clearly in the Figure 6.4. The presence of additional components at 289 eV is due to the formation of carboxylic acids and aldehyde groups that were generated after plasma treatment [146,150]. It is also evident that the amount of aromatic C-C groups is reduced in the detailed spectrum. Antimony, which was observed in the chemical

analysis, is most probably in the form of Sb_2O_5 which is normally generated by etching of carbonaceous species during the plasma process [148]. The survey spectrum also shows that the peak related to oxygen is also increased after plasma treatment.

Figure 6.4: XPS survey and detail spectra of an untreated (A) and treated (B) SU-8 sample with O_2 plasma. The close-up view shows the C 1s high resolution detail spectra.

6.2.2 Wet chemical treatment

Another approach that we employed to modify the surface chemistry of our polymeric micro-fabricated filters was wet chemical treatment using Ceric ammonium nitride ($(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$) followed by coating with ethanolamine [148,150,152]. Ceric ammonium nitrate (CAN) is a water-soluble inorganic compound, which is used as an oxidizing agent in organic synthesis and have been used extensively for etching of chromium (Cr) in the MEMS industry. CAN is known to act as a catalyst in the

breaking of epoxy rings [152,153], especially in the presence of acetic acid. For the SU-8 membranes CAN attacks and opens the epoxy groups, which then react with O_2 in the environment. This results in the formation of hydrophilic hydroxyl groups on the surface leading to a reduction of the contact angle [152,153]. Treatment of SU-8 membranes with CAN decreased the contact angle from 80° to around $30^\circ \pm 2^\circ$ (see Fig. 6.5). The ethanolamine coating was applied to the membrane surface instantly after the activation with CAN and resulted in a further decrease in the contact angle up to $9^\circ \pm 3^\circ$ and also better stability.

Figure 6.5: Contact angles of samples directly after treatment with CAN and ethanolamine.

Post AFM analysis of the samples also revealed that surface treatment using Ceric ammonium nitrate caused an increase in the rms roughness up to 1.8 ± 0.5 nm in the

treated samples. The surface roughness of the samples after coating with ethanolamine did not change significantly and remained around 1.7 ± 0.6 nm with a total height variation of around 17 nm as can be seen in Figure 6.6.

Figure 6.6: Topographic AFM images of SU-8 ($0.5 \mu\text{m} \times 0.5 \mu\text{m}$) sample after treatment with (a) Ceric ammonium nitrate (CAN) and, (b) with CAN and coated with ethanolamine.

The atomic elemental composition of oxygen and carbon in the treated SU-8 samples with CAN and ethanolamine was found to be 35 and 40, respectively. A significant reduction of the carbon level in the SU-8 sample was a consequence of carboxyl group formation and reaction with the amino group of ethanolamine. The XPS analysis of the samples illustrates that due to the etching process, hydroxyl groups were formed on the SU-8 samples, which is verified by the increase in the oxygen signals while the carbon signals decreased at the same time. High resolution analyses of the C 1s region confirmed the presence of signals related to COO and C=O groups [146,153].

6.3 Surface modification of metallic micro-fabricated filters

Plasma treatment of metallic surfaces has been used extensively for removal of organic contaminants by chemical reaction with highly reactive oxygen radicals and ablation by energetic oxygen ions. Hydrophilic metal surfaces are of increasing interest to enhance adhesion, biocompatibility as well as the heat transfer properties of metals for various industrial applications [154,155]. In this study we employed vacuum plasma treatment under different exposure time to change the surface wettability of our nickel micro-fabricated filters in order to reduce fouling and cell adhesion during microfiltration. For this purpose nickel samples were used as a substrate for plasma treatment using our Harrick plasma cleaner with an oxygen pressure of 0.4 mbar and power of 30 W. Before the treatment the substrate surfaces were cleaned by ethanol and acetone for the removal of contaminants (i.e., degreasing) inside an ultra-sonic bath, and then rinsed with de-ionized water. Figure 6.8 shows the measured contact angle from metallic samples for an untreated reference and a plasma-treated specimen. After the surface treatment by the O₂ plasma, the wettability of the surface enhanced considerably as evidenced by the contact angle change from 97° to 5°. With these results we can confirm that oxygen plasma is a very effective method to make the metallic microfilters hydrophilic.

Figure 6.8: Water contact angles on the metallic sample surface directly after treatment with an O₂ plasma.

Similar to the previous section, the rms roughness of the modified and unmodified metallic samples was also measured by atomic force microscopy (AFM) in a tapping mode using our Dimension 3000 SPM machine. AFM images of a modified and an unmodified sample are shown in Figure 6.9 with their corresponding contact angles. It can be seen that the unmodified nickel sample has an rms roughness of around 4 ± 0.5 nm and a total height variation of only 30 nm, while the treated sample (10 min O₂ plasma) shows rms roughness of around 11 ± 2 nm and a total height variation of around 65 nm, which confirms that the surface roughness was increased with plasma treatment similar to the polymeric one.

Figure 6.9: Contact angle and topographic AFM images of nickel samples ($1\ \mu\text{m} \times 1\ \mu\text{m}$), (a) untreated surface, (b) treated surface with O_2 plasma for 10 min.

XPS analysis of the samples was also carried out to investigate the effect of plasma treatment on the metallic microfilters. The survey spectrum of a sample before and after modification with plasma (10 min O_2 plasma) is shown in Figure 6.10. This figure clearly indicates the differences in the photoelectron peaks of O 1s and Ni 2p between the modified and unmodified samples as well as the O (KVV) Auger signals. A sensible decrease in the C 1s photoelectron peak was also observed [155,156] in the treated samples.

Figure 6.10: XPS survey and detail spectra of an (A) untreated and (B) treated nickel sample with an O₂ plasma. The close-up view shows the Ni 2p high resolution detail spectra.

The atomic elemental composition of the samples is also summarized in Table 6.2. As represented in Figure 6.10 and Table 6.2, the hydrophilic surfaces, which have been treated by the O₂ plasma, contain larger amounts of oxygen molecules (i.e., mainly in form of the nickel oxides Ni₂O₃ and NiOOH) respectively, compared to those of the untreated substrate [157]. Therefore, from the measured results of the contact angle in conjunction with those of the chemical composition analysis, it can be concluded that the nickel oxides (i.e., Ni₂O₃ and NiOOH) are the hydrophilic functional elements [157]. A high level of carbon in the untreated samples is mainly due to the organic

contaminants (i.e., impurities in the metal and air contamination by CO₂ and CO molecules).

Table 6.2: XPS chemical analysis of the modified and unmodified samples. Concentrations are given in relative atomic percent (at.%).

	Carbon (at%)	Oxygen (at%)	Nickel (at%)	Si (at%)
Untreated	50.50	35	12.0	2.5
Treated (10 min)	22.0	58.0	20.0	0.0

6.4 Stability of modifications

In the previous sections surface modification of micro-fabricated filters was investigated using oxygen-plasma and wet chemical-etching techniques. Making the surface of a membrane hydrophilic can be one of the requirements for the desired coating; however, stability of the surface property is also a very important parameter that must be taken into account [22,150]. Therefore, we measured the contact angles of the modified membranes as a function of storage time. The samples were stored in the air environment inside petri dishes without further treatment. After analysis of different samples and measuring their contact angles, it was realized that polymeric samples treated with CAN and coated with ethanolamine have the best stabilities with respect to a moderate recovery to hydrophobic state (θ changed from $9^\circ \pm 3^\circ$ to $29^\circ \pm 1^\circ$) [146]. In contrast, samples that were just treated with CAN solution show a significant recovery to the hydrophobic condition (i.e., over 40° growth in contact angle). For the metallic microfilters which were modified with oxygen plasma, the contact angle changes were not significant, which means samples can remain hydrophilic for a long storage time. Figure 6.11 shows the summary of the measurements. All the

experiments were performed three times and the presented results represent the average.

Figure 6.11: Contact angles as a function of storage time for treated samples stored in air for a period of one month.

6.5 Microfiltration using modified microfilters

In order to investigate the effect of surface modification (i.e., making the membrane surface hydrophilic) on the rate of particulate fouling and flux decline, we performed some microfiltration tests using our micro-fabricated filters with simulated drinking water samples as follows.

6.5.1 Microfiltration with clay particles

Microfiltration (MF) has been used widely as a pretreatment step prior to UF/NF or RO processes in industry for the removal of large particles in the range of 0.1 to 10 μm

[12]. The most important constituents of natural waters are clays such as kaolin (aluminum silicate) and other inorganic and particulate materials with a size in the range of 0.1 to 5 μm , which must be removed by microfilters before further purification with RO/NF processes. For this purpose the clay suspension was prepared by adding approximately 1.0 g of kaolin particles (0.1–5.0 μm , Sigma Aldrich) to 2 L of ultrapure water (18.2 M Ωcm) containing 0.2 Mm NaHCO_3 and 0.3 mM CaCl (i.e., $\text{pH} \approx 7.5$). Then, the solution was filtered through the micro-fabricated filters (i.e., both modified and unmodified, 2 μm pore size) with a cross-flow filtration test rig. The clean water flux of both unmodified modified filters was measured in the pressure range of 20–30 kPa at $20 \pm 2^\circ\text{C}$ before any clay filtration. It should be noted that fouling without any backflushing or air sparging is independent of the membrane surface properties because it normally caused by particle aggregate deposition [22,158,159]. This phenomenon has been observed in other investigations for filtration of clay or proteins via membranes with different properties [2,12,22]. To solve this problem, we applied periodic back-washing using a peristaltic pump at 20 kPa pressure during the filtration process. A schematic of the cross-flow microfiltration system is shown in Figure 6.12.

Figure 6.12: Schematic of the experimental setup used for the fouling investigation tests with different water samples using modified and unmodified membranes.

The cross-flow module was designed and fabricated from PMMA (Polymethyl methacrylate) by laser cutting and thermal bonding technique (see Fig. 6.13). The effective membrane area and height of the crossflow channel were $6.25 \times 10^{-4} \text{ m}^2$ and 0.0015 m, respectively. The microfiltration of the clay suspensions was also performed at 30 kPa pressure with a cross-flow velocity of around 0.2 m/s. Prior to the experiments, the treated membranes were inspected under an optical microscope to check their integrity. Subsequently, they were pre-wetted with pure water for 5 minutes.

Figure 6.13: Optical image of the cross-flow module fabricated from PMMA by a laser-cutting technique.

Figure 6.14 shows the results of microfiltration with a clay feed solution for two different membranes ($2 \mu\text{m}$ pore size, made from nickel) without using intermediate backflushing. As shown in Figure 6.14, the flux decreased quickly for both membranes due to fouling. For both membranes the flux decline rate is almost the same after around 60 min filtration without back-washing. It is expected that the fouling is

primarily due to physical deposition of the clay particles on the membrane surfaces which is not strongly dependent on the membrane surface chemistry [158,159].

Figure 6.14: Normalized flux vs. time for the filtration of a clay solution under 30 kPa pressure without back-washing using metallic micro-fabricated filters (both modified and unmodified).

To determine the effectiveness of surface modification on the membrane fouling reduction, the combination of surface modification and back-washing was employed. Figure 6.15 shows the permeate volume versus time for both modified and unmodified membranes using intermediate back-washing during the filtration process. In all the experiments, back-washing was performed every 3 minutes for 5 seconds using the filtrate side during the course of filtration. It can be seen that a significant improvement in the collected volume of modified membrane was obtained with back-washing. These results suggest that the adhesive hydrophobic interactions are stronger than the hydrophilic ones [14] between the clay particles and the membrane surfaces.

Therefore, the clay particles are more easily lifted off the modified membrane during each back-washing.

Figure 6.15: Permeate volume vs. time for filtration of clay solution under 30 kPa pressure with back-washing of both modified and unmodified metallic microfilters.

Figure 6.16: SEM image of a membrane after filtration of clay particles.

It should be noted that membrane fouling is related to many factors such as the membrane properties (i.e., pore size, material, hydrophilicity/hydrophobicity, etc.), solution properties (i.e., concentration, particle size, etc.) as well as the operating conditions (i.e., pH, temperature, flow rate, etc.) all of which need to be taken into account during a filtration process [1,2]. Figure 6.16 shows an SEM image of a membrane after filtration of clay particles. The deposited particles on the membrane surface can be seen clearly after filtration.

6.6 Summary

If the colloids and the membrane surface have opposite hydrophobicity, the colloids may be repelled by the membrane. Therefore, many membranes for drinking water treatment are made hydrophilic, which has the advantage of high membrane permeability and low affinity with the aromatic foulants. In this chapter we have employed two different strategies (i.e., oxygen plasma and wet chemical treatment using CAN) to change the surface properties of the micro-fabricated filters and render them hydrophilic. The measurement details for the contact angle, surface roughness (AFM analysis) and chemical composition of the surface (XPS analysis), both prior and after the surface modifications, were highlighted. It was found that polymeric samples treated with CAN and coated with ethanolamine have the best stabilities with respect to a moderate recovery to the hydrophobic state. In general, treatment with an oxygen plasma (for both polymeric and metallic membranes) also disclose good results in terms of hydrophilization and stability.

A scanning electron micrograph (SEM) showing a grid of circular pores in a membrane. A single, bright, irregularly shaped object, likely a Cryptosporidium parvum oocyst, is trapped in one of the pores.

Chapter 7

7

Isolation and recovery of *Cryptosporidium parvum* oocysts using micro-fabricated filters

A novel generation of high-flux screen micro/nano filters was introduced in the previous chapters. Investigation of their filtration performance, especially for isolation of waterborne pathogens and proposing efficient strategies for their fouling prevention could be very interesting and promising for future industrial application. In this chapter microfiltration results with both model particles and *C. parvum* oocysts using micro-fabricated filters are demonstrated and quantitative comparison with available commercial microfilters is performed.

Parts of this chapter have been published previously in Journal of Membrane Science and also Journal of Micromechanics and Microengineering [17,116,117,126].

7.1 Introduction

Many problems associated with the physical, chemical, and microbiological studies of the human environment require rapid concentration of small particles suspended in minute concentration within the two foremost environmental vehicles that human involve continuously in large quantities – namely, air and water [1,12]. Waterborne pathogens such as *Cryptosporidium parvum* and *Giardia* that exist in rivers and lakes can cause intestinal illnesses. Infections by these parasites can cause acute gastrointestinal symptoms in normally healthy people, and can lead to life-threatening conditions in individuals with impaired immune systems, such as patients with acquired immune deficiency syndrome [160,161]. Filtration-based methods have been used widely for isolation and recovery of *Cryptosporidium parvum* and *Giardia* (oo)cysts over the past decades [17,160]. Many membrane-filtration techniques have been developed and employed to quantify the presence of these parasites in surface and treated water samples, including polycarbonate track-etched membrane filters [162], compressed-foam depth filters [163], and yarn-wound cartridge filters [164]. Efficiency assessment of these membranes has shown various degrees of recovery for *Giardia* and/or *Cryptosporidium* [163-165]. The major drawbacks that normally lead to poor cell recovery in current techniques are mainly associated with the structure of these filters. Depth filters such as cellulose, yarn-wound and glass fibers, which normally are made of a thick bed of fibers or other materials, capture target cells (i.e., (oo)cysts) with other bigger particles because their pore diameter is an average value in a certain scale range (see Fig. 7.1). Therefore, it is often difficult to realize an absolute separation and a full collection of (oo)cysts using these filters. In addition, it is normally required to apply a high transmembrane pressure (TMP) to facilitate flow through the depth filters due to their large thickness and tortuous pore path [2,10].

Screen-type filters such as track-etched membranes (i.e., used in many USEPA recommended filters like Envirochek HV and CrypTest) [165,166] are another well-known type of filter that employ relatively thin membranes in contrast to the depth filters. Although they have nominal pore sizes and have been used in a wide variety of industrial and medical applications, they tend to have a limited porosity (i.e., $\approx 5\text{--}10\%$) [115], which normally leads to a small throughputs due to the blockage of a large part of the membrane surface area.

Figure 7.1: Schematic of pore-size distributions in commercial and micro-fabricated filters.

A novel generation of high-flux screen microfilters was introduced in the previous chapters. The micro-fabrication process allows enough flexibility to control the porosity, pore size and pore shape of the filter according to desired application in order

to reach higher flow rate, lower clogging ratio, better recovery and enough reliability. For example, for 100 % capture of *C. parvum* oocysts, which has a spherical shape with diameter of 3-6 μm , micro-fabricated filters with pore size of 2-3 μm can be used, while commercial filters use an average pore size around 1 μm (e.g., Envirochek HV). This ability to use a bigger pore size for a similar isolation efficiency increases the throughput of the micro-fabricated filter significantly in comparison to the commercial ones. Likewise, the narrow pore-size distribution of the micro-fabricated filters allows the small and unwanted particles ($<$ pore size) to pass from the filter. This will help to achieve a lower turbidity level in the eluent during the back-flushing step, which is crucial for the detection limit of bio-sensors. This property also helps to have a lower deposition rate of small particles on the membrane surface (i.e., lower fouling rate). In addition, a straight rather than a tortuous pore path prevents accumulation of particles inside the pores (see Fig. 7.1). The extremely smooth surface of the micro-fabricated filters will also help in better recovery of trapped cells.

In order to prove the aforementioned hypotheses, a number of experiments were performed to fully characterize the micro-fabricated filters. These experiments comprised of flux, recovery and turbidity measurements.

7.2 Materials and methods

7.2.1 Polymeric and metallic micro-fabricated filters

Polymeric and metallic micro-filters with the membrane diameters of 25 mm and 47 mm (pore size, 2 μm) were fabricated by the dissolving mold technique, multi-level lithography and electroplating techniques. The structural characteristics of the membranes used are described in detail in Chapters 3, 4 and 5.

7.2.2 Commercial microfilters

In order to compare and evaluate the performance and sieving characteristics of our micro-fabricated filters, we used the following commercial membranes:

i) Envirochek high volume (EC-HV) membrane, **ii)** Envirochek (EC) standard membrane filter, **iii)** Mixed cellulose esters membrane filter, and **iv)** Track-etched polycarbonate membrane filter. These membranes are normally used in industry for clarification of aqueous solutions and microorganism removal. They were procured and used as provided by the supplier without further modification/preparation. Some important characteristics of the commercial membranes are summarized in Table 7.1. Figure 7.2 also shows SEM photos of them.

Table 7.1*: Properties of commercial and micro-fabricated membrane filters used in microfiltration tests.

	Envirochek high volume membrane	Envirochek standard membrane	Mixed cellulose membrane	Track-etched polycarbonate membrane	Micro-fabricated membrane
Pore size	1 μm	1 μm	1.2 μm	2 μm	2 μm
Tolerance	not available	not available	$\pm 40\%$	$\pm 15\%$	$\pm 3\%$
Thickness	10 – 30 μm	150 – 200 μm	150 μm	10 – 30 μm	3 – 20 μm
Structure	Cylindrical	Tortuous	Tortuous	Cylindrical	Cylindrical (Straight-pore)
Porosity	25 – 40%	50 – 60%	82 %	16%	25%– 40%
Material	Polyester	Polyethersulfone	Cellulose acetate	Polycarbonate	SU-8, Nickel, Polyurethane
Supplier	Pall Co	Pall Co	Millipore®	Whatman®	

* All the data presented here have been measured or extracted from the product catalogs.

Figure 7.2: SEM images of commercial filters and our micro-fabricated filter (a) Envirochek high volume (EC-HV) membrane filter, (b) Envirochek (EC) standard membrane filter, (c) Mixed cellulose esters membrane filter, (d) Track-etched polycarbonate membrane filter, (e) Polymeric micro-fabricated membrane with circular pores, and (f) Metallic micro-fabricated membrane with slotted pores [17].

7.2.3 Filter holder for filtration and backflush

In order to test and compare the results of our micro-fabricated filter with commercial filters, a filter holder was designed and fabricated from PMMA (Polymethyl methacrylate) by a laser cutting and thermal bonding technique. It comprises a base for sample inflow and eluent outflow, a cap for outflow of the filtered sample and for intake of eluent, a gasket for leakage prevention and also upper and lower perforated supports for keeping the microfilters firmly during the test. The major parts of this holder are depicted schematically in Figure 7.3.

Figure 7.3: A cut-away view of the filter holder.

7.2.4 Water supply simulation station

To facilitate the testing of oocyst isolation from a large volume of water, we built a water supply simulation station. The station contains a water tank, two pumps, and a regulator to set the water pressure as shown in Figure 7.4. The water tank allows us to

spike *C. parvum* oocysts into a large volume of water for the isolation test. The installed pumps have the ability to pump 2000 L of water in 10 minutes from the 50 mm diameter pipe, thus allowing us to test at high flux, which cannot be provided from the water taps in the lab. In addition, the water pressure can be adjusted from 1 to 7 bars with the regulator that facilitates tests at various pressures.

Figure 7.4: Water-supply simulation station provides high flux, various pressures and facilitates oocyst spike.

7.2.5 Filter array

An array of filters can multiply the flux ability of a single piece of filter. For preliminary tests using large volumes of water, we made an array of filter holders, which can hold 30 pieces of 47 mm diameter commercial/micro-fabricated filter membranes, as shown in Figure 7.5. This filter array together with the water supply station allows us to test our proposed filtration method at a real, high flux level.

Figure 7.5: A platform filter manifold for holding 30 microfilters in a field test.

7.2.6 Turbidity condition of the tap-water

For all the tests involving tap-water, the turbidity of the water influences the results for the flow rate, flux and also the oocyst capture and collection with filters. Turbidity values of the water samples from 3 taps in our lab were measured (Oakton® T-100) hourly through a whole day as shown in Figure 7.6. The turbidity falls into a range of 0.2 to 0.7 NTU (Nephelometric Turbidity Units). In our test water from the same tap with a turbidity around 0.36 NTU was used in order to test and evaluate different filters. In some tests samples with higher or lower turbidities have been used, which is stated in the details of the experiment.

Figure 7.6: Measured turbidity of tap water in the Bio-MEMS laboratory.

7.2.7 *Cryptosporidium* strains and species

Viable *C. parvum* oocysts (bovine, Iowa isolate) and inactivated *C. parvum* oocysts (bovine, Iowa isolate) from Waterborne Inc. (New Orleans, LA, USA) were used to test the performance of the microfilters (i.e., isolation and recovery rate). Purified oocysts suspension was supplied in deionized water and stored at 4 °C. For all the experiments oocyst suspension enumeration was performed manually according to the USEPA protocol, method 1623 [167], in which the purified stock suspension was diluted initially to yield a suspension at the appropriate oocyst concentration for spiking. Then 10 aliquots of spiking suspension were prepared using well-slides to calculate a mean spike dose.

7.2.8 Water samples

Ultrapure water for the flux measurements was obtained from a Millipore purification unit (MilliQ plus). Tap-water samples were obtained on the day of testing from the laboratory faucet, which was flushed for 5 minutes before sample collection to reach a relatively stable turbidity. In order to simulate the condition of the impurities in water during filtration and recovery of *C. parvum* oocysts from a large amount of tap water while avoiding a large quantity of bio-hazardous waste, water samples with condensed impurities were prepared. For this purpose, 10 L of tap-water were filtered using a 1.2 μm pore sized cellulose membrane (Millipore Cat No: RAWP1904700) followed by back-flushing using 10 ml deionized water to collect all the impurities present in the tap-water sample. *C. parvum* oocysts were later spiked in this water sample for tests. In some cases we used bentonite and clay particles to make feed water with different turbidities.

7.2.9 Microfilter inspection

Inspection of the polymeric/metallic micro-fabricated filters was performed before and after the flux measurements using a Nikon optical microscope. For a more detailed inspection, microfilters were visualized by Scanning Electron Microscopy (SEM, HITACHI 3500S, at 10-15 kV).

7.2.10 Integrity test with bubble-point technique

We employed the bubble-point test for integrity testing the micro-fabricated filters after each experiment. The relationship between the minimum pressure (ΔP) to be applied on the liquid to enter the pores of a membrane is given by the Laplace-Young equation [2] as follows:

$$\Delta P = \frac{-2\gamma_L \cos \theta}{r}$$

where γ_L is the surface tension of the liquid, θ the contact angle of the liquid with the surface and r the membrane pore radius. This equation was employed to estimate the bubble point of micro-fabricated filters. For this purpose the surface treated microfilters were immersed in isopropanol ($\gamma = 24 \times 10^{-3}$ N/m) for 5 minutes after which the bubble-point tests were carried out by applying pressure beneath the membrane in a fabricated test rig. The measured values of the bubble point for the micro-fabricated membranes were compared with those obtained from the Laplace-Young equation. If the measured pressure was 10-15 % lower than calculated one, the micro-fabricated filter was discarded, assuming it had defects.

7.3 Water-flux measurement

The clean water permeability of the micro-fabricated filters was measured in the pressure range of 0.2-1 bar at $20 \pm 2^\circ\text{C}$ in the Dead-End Filtration (DEF) mode shown in Figure 7.7.

Figure 7.7: Schematic representation of Dead-End Filtration (DEF) setup.

Ultrapure water was obtained from a Millipore purification unit (MilliQ plus) in Bio-MEMS laboratory. Figure 7.8 shows the flow rate versus pressure for a polymeric micro-fabricated membrane with a 2 μm pore size and different porosities. It can be seen that by increasing the porosity, higher flow-rates can be achieved. It should be noted that membranes with a higher porosity have lower strength; therefore, the required pressure and flux during microfiltration can be a good criterion to determine the optimum thickness and porosity during micro-fabrication using polymeric/metallic microfilters.

Figure 7.8: Flow-rate vs. pressure for a micro-fabricated filter with a 2 μm pore size and different porosities (membrane thickness was around 10 μm).

In order to calculate the throughput of the micro-fabricated filters, microfiltration tests were performed using real water samples with different turbidities. Similar tests were also carried out using the aforementioned commercial microfilters to compare the

performance of our microfilters. For all the experiments, microfiltration tests were performed in the dead-end filtration mode (DEF) with constant pressure (i.e. 1 bar). During all measurements, initially a constant pump rate fluid delivery was implemented by DEF system until a user-defined pressure limit was reached. At this point, the fluid handling system automatically switched to a constant pressure delivery, i.e., modulating the pump output, until a user-defined lower flow limit (i.e., 1 ml/min) was reached and the pump stops. The permeate was collected in a container located on an electronic balance interfaced to a computer. The microfiltration stand was equipped with a data-acquisition unit. The presented data have the average of at least three separate experiments.

Figure 7.9: The collected filtrate weight for three commercial membrane filters and a micro-fabricated filter for the filtration of tap-water at a pressure of 1 bar and turbidity of 0.36 NTU.

Figure 7.9 shows the filtrate collection weight data for filtration of tap-water with turbidity of 0.36 NTU for three commercial microfilters and a polymeric micro-fabricated filter (2 μm pore size, 30 % porosity). It can be clearly seen that the micro-fabricated membrane has much higher throughput in comparison to the other microfilters. This can be attributed to its low flow resistance (due to the small thickness), high porosity and a very narrow pore-size distribution. The Envirochek filter ($\approx$ 40% porosity) have slightly better performance in the first 10 minutes of filtration because its porosity is higher than the micro-fabricated filter, but its flow rate gradually decreased and stopped within 40 minutes when it reached the limit of 1 ml/min. By increasing the porosity and decreasing the thickness of the micro-fabricated membrane, a flow rate of about 2000 ml/min/cm² has been achieved in our filtration testes. The specific characteristics of all the microfilters can be found in Table 7.1. The bar chart (Fig. 7.10) shows the total collected volume passing through a 90 mm diameter metallic micro-fabricated filter for the filtration of water having different turbidities. It can be seen that a 90 mm diameter membrane ($\approx$ 36% porosity) with a 9 μm thickness can pass around 90 liters of tap-water in less than 20 minutes before complete plugging. Figure 7.11 shows the flux comparison of two commercial microfilters with a metallic microfilter for filtration of high turbidity water samples ($\approx$ 15 NTU). It can be seen that even at high turbidity conditions, the micro-fabricated filter gives better results than the commercial microfilters.

Figure 7.10: Total collected volume for a micro-fabricated microfilter with a 90 mm diameter ($2 \times 8 \mu\text{m}$ pore size and 36 % porosity) for filtration of water having different turbidities.

Figure 7.11: The flux of two commercial microfilters and a micro-fabricated microfilter for filtration of a water sample at a pressure of 1.5 bar and turbidity of 15 NTU.

Figure 7.12 shows the flux of two metallic micro-fabricated microfilters with different thicknesses for the filtration of tap-water at a pressure of 1 bar. It can be seen that by decreasing the membrane thickness (i.e., without compromising the membrane strength) the filtration throughput increased significantly. Figure 7.13 also shows the effect of pressure during filtration for two different micro-fabricated filters. This bar chart also shows that by increasing the filtration pressure, the throughput can be increased. It should be noted that higher pressure during filtration will cause faster membrane fouling, which is not recommended in industry [1,2,12]. These experiments confirmed that polymeric/metallic micro-fabricated membranes with smooth surfaces and a perfectly ordered array of pores show higher flow rates in comparison to the commercial microfilters. These findings are in good agreement with previous studies [10,20].

Figure 7.12: The flux of two micro-fabricated microfilters with different thicknesses for the filtration of tap-water at a pressure of 1bar and turbidity of 0.36 NTU.

Figure 7.13: Total collected volume for a micro-fabricated microfilter with a 90 mm diameter ($2 \times 8 \mu\text{m}$ pore size and 36% porosity) for the filtration of tap-water at two different pressures (i.e., filtration was performed in dead-end mode for equal times until reach complete clogging).

7.4 Isolation and recovery tests

7.4.1 Isolation and recovery of latex particles

Microfiltration with latex particles having a specific size can reveal how a pore is blocked and what the performance of a membrane will be for feeds having different particle concentrations. In addition, a direct integrity test of the micro-filters before any biological test can be done using appropriate surrogates (such as latex particles) by directly assessing the removal of the surrogates [166]. Therefore, we evaluate the performance of our micro-fabricated filters using challenge suspensions containing latex particles with a $3 \mu\text{m}$ diameter (i.e., $3 \mu\text{m}$ latex particles are the recommended surrogates by USEPA for integrity test of microfilters [166]). For this purpose two test solutions with different concentrations (1 g/L and 0.1 g/L mixed with pure water) were

prepared and filtered through the micro-fabricated filters (both circular and slotted pore membranes, 25 mm diameter, and 2 μ m pore size) by a dead-end filtration setup under a constant pressure of 1 bar. Then, the permeate solutions were filtered for a second time through an Anopore™ aluminum membrane (Cat No: 6809-5022) with a nominal pore size of 0.2 μ m to capture any latex particles that might have passed through the micro-fabricated filters. Subsequently, when the full surface of the aluminum membrane was observed under the microscope, it was realized that no latex particles passed through the micro-fabricated microfilters (i.e., 100 % capture).

In order to check the recovery rate of the micro-fabricated filters, they were loaded inside a tube containing 5 ml of washing buffer (0.1% Tween 80, 0.1% NaPP and PBS) and were shaken with a vortex shaker (Cole-Parmer, 3400 rpm) for 10 minutes. Then, their surface was back-flushed (under 0.5-1 bar pressure) using 1.0 L ultra-pure water to remove the layer of beads from the surface of the membranes. The results of the optical observations revealed that more than 90-97% of the latex particles were recovered.

Figures 7.14 and 7.15 shows SEM photos of two micro-fabricated filters after filtration of a latex solution and after back-flushing. Unique features of the micro-fabricated filters such as their smooth surface and uniform pore-size greatly reduced the latex adhesion to the filter surface and enabled a very high recovery rate.

Figure 7.14: SEM images of a polymeric micro-fabricated membrane (i.e., 25 mm diameter, 2 μm pore size) with circular pores, (a) after filtration of the latex solution, (b) after recovery step.

Figure 7.15: SEM images of a metallic micro-fabricated membrane (i.e., 25 mm diameter, 2 μm pore size) with slotted pores, (a) after filtration of the latex solution, (b) after recovery step.

7.4.2 Isolation and recovery of *C. parvum* oocysts

7.4.2.1 Filtration and recovery of heat-inactivated *C. parvum* oocysts

The capturing capability of our micro-fabricated filters was also verified by a two-step oocysts filtration method. In the first step 10 L of sample, which was pure water (collected from a Millipore purification unit) spiked with 1×10^5 heat-inactivated *C.*

parvum oocysts (Waterborne Inc. New Orleans, LA, USA, Cat No: P102C@1×10⁷) with fluorescent tagged antibodies, was filtered using a polymeric micro-fabricated filter (i.e., 25 mm diameter, 2 µm pore size). The filtration was performed in the dead-end mode using a peristaltic pump under 1.5 bar pressure. Upon completion of the filtration, the containers were rinsed with 2 L of pure water that was additionally filtered. Then the filtered water went to a subsequent filtration step using an Anodisc membrane (Cat No: 6809-5022) filter to capture any oocysts that might passed through the micro-fabricated filter. *C. parvum* oocysts attached to both two filters were observed under a fluorescence microscope by a FITC (fluorescence iso-thiocyanate) technique (Waterborne. Inc., New Orleans, LA, Cat no. A400FLK). It was observed that all oocysts were captured by the micro-fabricated filter and none was found on the second filter (i.e., the Anodisc membrane). Figure 7.16 (a) and (b) shows microscopic images of a polymeric membrane with circular pores after filtration of *C. parvum* oocysts and after recovery, respectively. The captured oocysts on one part of membrane surface can be seen easily (i.e., green dots).

To check the recovery rate, the micro-fabricated filter was loaded inside a tube (i.e., similar to that of the previous section) containing 5 ml washing buffer (0.1% Tween 80, 0.1% NaPP and PBS) and was shaken with a vortex shaker for 10 minutes. Then, the membrane surface was back-flushed with 1.0 L of ultra-pure water under 1 bar pressure. The optical observation results (i.e., under a fluorescence microscope by FITC (fluorescence iso-thiocyanate) technique, USEPA protocol, method 1623 [167]) revealed that more than 95% of *C. parvum* oocysts were recovered. The reproducibility of enrichment and recovery was determined for a variety of the *C. parvum* oocyst concentrations (i.e., 10⁶, 10⁵, 10⁴ *C. parvum* oocysts). It was realized

that the recovery was $95\pm2\%$ *C. parvum* oocysts according to three individual concentrations enriched in triplicate experiments.

Figure 7.16: Microscopic images of a polymeric micro-fabricated membrane (i.e., 25 mm diameter, 2 μm pore size), (a) after filtration, where the green particles are the fluorescently labeled oocysts captured, (b) after backflush.

Furthermore, from the images of the micro-fabricated filter after back-flushing we can observe that the filter surface is clean and had been nearly restored to its original status before sample loading, which indicates the high reusability of the filter.

7.4.2.2 Filtration and recovery of live *C. parvum* oocysts and comparison with commercial microfilters

The recovery performance of the micro-fabricated filters (25 mm diameter, 2 μm pore size) was also evaluated by comparing with two different types of commercially available filters, which were a Millipore multi-cellulose acetate membrane (pore size of 1.2 μm , Cat No: RAWP01300) and an Envirochek filter (pore size of 1 μm , Cat No: 121110) membrane, which is now serving as the US EPA recommended standard for

enrichment of *C. parvum* oocysts [167]. For the recovery evaluation test three similar samples of live *C. parvum* oocysts were prepared by labeling 10^3 viable *C. parvum* oocysts (Waterborne Inc. New Orleans, LA, USA, Cat No: P102C@ 1×10^6) with fluorescent tagged antibodies and spiking them into 10 L of tap-water (collected from the tap in our laboratory, turbidity ≈ 0.5 NTU, pH = 7.6, conductivity = $369 \mu\text{Scm}^{-1}$) on the day of the experiments. The microfiltration was carried out using a peristaltic pump under 1.5 bar pressure in dead-end mode. Similar to the previous section, upon completion of filtration, the containers were rinsed with 2 L of pure water that was additionally filtered. For the recovery of *C. parvum* oocysts from the membrane surface we have done the following procedures:

A) Recovery by lateral shaking

After samples were filtered by the microfilters, they were soaked inside a tube containing 5 ml elution buffer (0.1% Tween 80, 0.1% NaPP, 1g NaCl and PBS). The tube was then shaken with a vortex shaker for 10 min at maximum speed. Then eluent was removed from the tube for subsequent analysis. The *C. parvum* oocysts attached to the filters before and after the recovery were visualized by staining the membranes using the FITC technique followed by observation under a fluorescence microscope. Careful optical observations of the microfilters revealed that more than $65 \pm 5 \%$ of the *C. parvum* oocysts were recovered from the micro-fabricated microfilter, which was significantly higher than the recovery obtained with the Envirochek HV ($55 \pm 8 \%$) and cellulose acetate ($30 \pm 5 \%$).

B) Recovery by back-washing

In another strategy for recovering of *C. parvum* oocysts from the surface of microfilters, they were back-flushed with the elution buffer (0.1% Tween 80, 0.1% NaPP, 8g NaCl in 1.0 L PBS) using a peristaltic pump under 1 bar pressure. The eluate was collected in a sterile 2 L bottle for further analysis. Again, the *C. parvum* oocysts attached to the microfilters before and after the recovery were visualized by staining the membranes using the FITC technique followed by observation under a fluorescence microscope. For this case the recovery rate for the micro-fabricated filter was higher (i.e., $80 \pm 5 \%$) while no significant changes for the other microfilters were observed (i.e., Envirochek: $50 \pm 5 \%$; Cellulose acetate: $35 \pm 5 \%$). For the case of micro-fabricated filters it was found that by the combination of lateral shaking and back-flushing a high recovery rate (i.e., up to 95 %) can be achieved. The resulting microscopic pictures are shown in Figure 7.17. It can be seen that some *C. parvum* oocysts still adhere to the other two types of commercial microfilters after lateral shaking and back-flushing with washing buffer while nearly all the oocysts were recovered from the micro-fabricated filter. Unique features of the micro-fabricated filter such as the smooth surface, straight pore path and uniform pore-size greatly reduced the oocyst adhesion to the filter surface and enabled us to achieve a very high recovery rate. Figure 7.18 shows the summary of the recovery rate for the different microfilters that was obtained by careful microscopic observation and counting.

Figure 7.17: Fluorescence microscopic images showing the surfaces of micro-filters after filtration of 10 L of sample (tap-water spiked with 10^3 viable *C. parvum* oocysts) (Left), after back-flushing and lateral shaking (Right) using a micro-fabricated filter (a and b), Envirochek filter (c and d), and cellulose membrane (e and f).

Figure 7.18 Recovery of *C. parvum* oocysts from tap-water (10L) using micro-fabricated, Envirocheck and cellulose microfilters. The filtration was performed under 1.5 bar pressure at a turbidity of 0.5 NTU with 25 mm microfilters.

A SEM photo of a polymeric membrane with a honeycomb support mesh after filtration is also depicted in Figure 7.19.

Figure 7.19: A SEM image of a polymeric micro-fabricated membrane with a honey-comb support mesh after filtration. Close-up view shows the membrane surface with the captured oocysts.

The close-up view shows the trapped *C. parvum* oocysts between the pores, which confirms that the isopore and the smooth surface of the micro-fabricated membrane assisted in better performance in comparison to the commercial membranes for concentrating the oocysts.

7.5 Regeneration of micro-fabricated filters

Commercial filters are not designed to be reusable; moreover, they can hardly retain their initial properties after a filtration and recovery process. In contrast, micro-fabricated filters can easily retain their original properties after a specific treatment. To prove this hypothesis, a metallic micro-fabricated filter (25 mm diameter, 2 μm pore size) was used 10 times to filter 7 L of tap-water (collected from the Bio-MEMS lab, $\text{pH} \approx 7.5$, $\text{NTU} \approx 0.5$). The filtration was performed in the dead-end mode under 1.5 bar pressure.

In order to regenerate the microfilters, they were removed instantly after each test from the filter holder and soaked in a strong alkaline solution (40% NaOH or KOH) for around 5 min. After washing with DI water, they were immersed again inside a tube containing 5 ml of washing buffer (15% Tween 80 and PBS). The membranes were shaken vigorously with a vortex shaker for 10 min to wash all the retained deposits from the filter surface. Subsequently, the microfilter surface was back-flushed with 2 L of pure water under 1.5 bar pressure. High pressure back-washing helps to re-open the clogged pores. This procedure was performed 10 times. Figure 7.20 shows the collected volume versus time for the filtration of tap-water using a 25 mm microfilter. It can be seen that the micro-fabricated filter after 10 filtrations shows a

good performance on that only a moderate decline in throughput occurred. In order to ensure no viable oocyst remain on the microfilter after filtration, the NaOH solution can be heated to 70°C (i.e., by keeping the microfilter inside the solution for 5 minutes) to kill all the living *C. parvum* oocysts [168,169].

Figure 7.20: The collected filtrate volume vs. time for a 10-time filtration of tap-water under 1.5 bar pressure and turbidity of 0.5 NTU using a 25 mm metallic micro-fabricated filter (2 μ m pore size).

Figure 7.21 shows optical images of the microfilter after several filtrations and cleaning. It can be seen that a large volume of deposits that exists in the tap-water is retained by the micro-fabricated after each test (See Fig. 7.21(b)). Figure 7.21 (c) shows the regenerated filter. It can be seen that a majority of the deposits has been removed during the cleaning process. By performing the aforementioned regeneration

technique, we could successfully use each microfilter for several filtration processes.

Figure 7.21: Microscopic images of a micro-fabricated filter with a 2 µm pore size, (a) before filtration, (b) after first filtration (c) after first regeneration, (d) after second filtration, (e) after five regenerations, and (d) after ten regenerations (Note: For all the filtrations, 7 L of tap-water was used).

It conclusion, the long lifetime and easy cleanability make the micro-fabricated filters a good option for large-scale applications where conventional filters must be replaced frequently such as in the water purification industry or in the breweries. The black

spots on top of the microfilter (Fig. 7.21(f)) are mainly oily particles that are present in the water and adhere strongly to the membrane surface.

7.6 Turbidity of eluent

Fast detection of pathogenic microorganisms in potable water with high sensitivity is still a scientific challenge. Small biosensors only detect bacteria that are in contact with or in the perimeter of the sensitive area [161]. Therefore, detection of bacteria with low concentration in a large volume is difficult because the probability of the bacteria to interact with the sensitive area is very small. Filtration-based concentration techniques have been widely used to concentrate bacteria into small volume for downstream analysis [170]. The bacteria on the filter surface can be recovered and transferred to a biosensor with a microfluidic system. Thus, the biosensor is now exposed to a higher bacteria concentration than without the filtering step and can detect the bacteria. During the back-flushing step, different types of debris and particles (i.e., in the range of 100 nm –10 µm) that were trapped on the surface of the microfilter (or within the pores) will float inside the eluent. This can severely compromise the efficiency of the biosensor in detecting the bacteria, or inhibit the PCR process in subsequent steps [161]. In contrast to commercial microfilters, a micro-fabricated filter with slightly larger pores and also a smoother surface shows better results. For this purpose, we performed a series of experiments to calculate the turbidity level in the eluent of each microfilter as described in the following section.

7.6.1 Turbidity of eluent in filtration of tap-water

The turbidity of the eluent of the micro-fabricated filters was evaluated by comparing with three different commercially available microfilters. In all experiments 5 L of tap-

water filtered by the microfilters (25 mm diameter). Then 500 ml of buffer (containing 1% NaPP, 0.1% Tween 20 and PBS) were back-flushed through the membranes with an air pressure of 1.5 bar. Table 7.2 shows the summary of the experiments. All the experiments were performed three times in the dead-end mode and presented results are an average of the experiments. After each experiment, inspection of the micro-fabricated filters was performed using a Nikon optical microscope and also SEM analysis.

Table 7.2: Measured turbidity of eluent for different types of microfilters.

	Envirochek high volume membrane	Envirochek standard membrane	Mixed cellulose membrane	Track-etched polycarbonate membrane	Micro- fabricated membrane
Turbidity	4.5 NTU	6 NTU	7.5 NTU	5 NTU	1.9 NTU

The results indicate that the micro-fabricated membrane gives much lower turbidity of eluent during the back-flushing step. This is attributed to the smooth surface of the micro-fabricated filter and also passage of small particles (i.e., unwanted) through the pores during the filtration process. Figure 7.22 shows the SEM photos of different microfilters after filtration of 5 L water in the dead-end mode. For the case of the cellulose and Envirocheck standard microfilters, which are both depth filters, a severe clogging occurred because the majority of the small particles in the tap-water were trapped in their tortuous pore structures (Fig. 7.22 (a) and (b)). Pore narrowing and finally severe internal clogging and cake formation (i.e., deposition of particles inside the pores) were the major fouling mechanism in the Envirocheck HV filter (Fig. 7.22 (c)). The micro-fabricated filter has larger pores than the other microfilters; therefore, unwanted particles in tap-water (i.e., with few hundred nanometers) could pass easily through the membrane. Hence, a much lower rate of particle deposition and therefore,

lower turbidity level in the eluent was observed for this case. These photographs clearly show that the micro-fabricated microfilter still has many open pores for water to penetrate, while other microfilters are completely blocked. These images also confirm that major micro-structural features in commercial microfilters such as tortuous pore path and rough surfaces caused the lower throughput during filtration as well as poor cell recovery and high turbidity of the eluent after back-flushing.

Figure 7.22: SEM photos of microfilters after filtration of 5 L of tap-water (a) a cellulose membrane filter (i.e., fully clogged with tapped particles), (b) an Envirochek standard filter (i.e., fully clogged with tapped particles), (c) an Envirochek HV filter (i.e., fully clogged with tapped particles on the surface as well as inside the pore) (d) a Micro-fabricated filter (i.e., partially clogged with tapped particles on the surface or within the pores. Note: many pores are still open for water to penetrate).

7.7 Summary

Three important characteristics of micro-fabricated filters (i.e., flow rate, recovery ratio and turbidity level of eluent) were investigated in this Chapter. Several tests were performed to compare and evaluate the throughput of the micro-fabricated filters with available commercial microfilters for the same purpose of pathogen removal and recovery. The results revealed that micro-fabricated filters present a much higher flow rate in comparison to the commercial membrane filters. This unique feature attributed to their smooth surface, high porosity, low flow resistance (i.e., because of low thickness) and straight pore path.

Sample loading and back-flushing using the micro-fabricated filters resulted in nearly 95% recovery of the spiked *C. parvum* oocysts. The micro-fabricated filters showed better performance than the current commercial filters in terms of sample throughput, recovery ratio, and reusability. This research demonstrated the potential application of micro-fabricated filters in monitoring *C. parvum* oocyst contamination in a tap-water supply system. Measurement of eluent turbidity revealed that the micro-fabricated filter achieved the lowest turbidity in comparison to the commercial microfilters.

Chapter 8

8

Investigation of membrane fouling at the pore scale using micro-fabricated filters

In the previous chapter three important characteristics of micro-fabricated filters were addressed. Polymeric micro-fabricated filters made from SU-8 are transparent and can be employed for online fouling investigation using direct observation through the membrane (DOTM). In this chapter we investigate the effect of membrane pore geometry on the fouling mechanism using micro-fabricated filters with different pore shapes, and then we use them as a tool for DOTM applications.

Part 1: Effect of membrane pore morphology on the fouling behavior of micro-fabricated filters

8.1 Introduction

Microfiltration is a pressure-driven separation process to concentrate fine colloidal suspensions or to remove suspended solids such as yeasts, bacteria, etc. from fluids. It is also widely used as a pretreatment for surface water, seawater, and biologically treated municipal effluent before reverse osmosis and other membrane systems [1,12]. During microfiltration, a deposit cake layer tends to form on the membrane, which usually controls the performance of the filtration process [12,16]. Recent studies demonstrated that the membrane pore morphology could have a significant effect on the rate of flux decline (i.e., initial stages of filtration), where the dominant fouling mechanism is pore blockage [76,171]. For instance, membranes with slotted openings and a high porosity would be less likely to get blocked during filtration of typical cell or particle suspensions [77,78]. Such membranes can provide the adequate selectivity needed for sterile filtration and microorganism removal, while minimizing the rate of flux decline. Investigations of membrane fouling at the pore scale (not at the membrane scale) have long been of limited interest due to micro-structural defects of the commercial membranes (i.e., tortuous pore paths, rough surfaces, etc.) that prevent any quantitative analysis of the experimental results [172]. In this Chapter we demonstrate the benefit of isopore micro-fabricated membranes with identical pore sizes as a promising tool for studying fouling phenomena for different pore geometries by post-SEM analysis. The observations were validated by analysis of the filtration data using the available classical filtration models for this purpose. Furthermore, we

employed polymeric micro-factored filters as a promising tool for online particle observation on the membrane surface using our DOTM facilities.

8.2 Material and methods

8.2.1 Micro-fabricated membranes

Micro-fabricated membranes with circular and slotted openings were prepared with our techniques previously described. The resulting membranes had a pore dimension (diameter or slit width) of 3 μm and thickness of around 6 μm . The slotted pore membrane had a pore length of 8 μm and a porosity of approximately 33% while this value is around 27% for the circular membrane. Figures 8.1(a) and (b) show SEM images of the polymeric micro-fabricated membranes with circular and slotted openings, respectively.

Figure 8.1: SEM images of the micro-fabricated membranes with a pore dimension (diameter or slit width) of 3 μm , (a) circular and (b) slotted.

8.2.2 Surface modification of the membrane

For all the experiments that we performed in this chapter, micro-fabricated filters were modified using an oxygen plasma according to the procedures that were described in the Chapter 6. Prior to the experiments, the treated membranes were inspected under an optical microscope to check their integrity. Subsequently, they were pre-wetted with pure water for 5 minutes.

8.2.3 Preparation of latex solution

White polystyrene latex particles with diameters of 1, 3 and 6 μm were obtained from Sigma Aldrich (Sigma-Aldrich®). Feed solutions were prepared by diluting the stock solution with ultra-pure water (18.2 $\text{M}\Omega\text{ cm}$) from a Millipore purification unit (MilliQ plus). The latex stock suspension was sonicated for 2 minutes in an ultrasonic bath before each test. The suspension was kept homogenous by stirring the solution with a magnetic stirrer during experiments.

8.2.4 Experimental setup

The filtration experiments were performed in both cross-flow and dead-end modes using our setups, which have been used in the previous chapters for microfiltration tests (i.e., Chapter 7, section 7.3 and Chapter 6, section 6.5.1). The clean water permeability of the membranes was measured in the pressure range of 5–20 kPa at 20 °C. The average clean water permeability of the circular pore membrane was around $0.38 \times 10^6 \text{ l/m}^2\text{hbar}$ while this value was around $2 \times 10^6 \text{ l/m}^2\text{hbar}$ for the slotted membrane. This is a difference of 5x in favour of the slotted membrane, which will be discussed below. Direct observation of particle deposition on the membrane surface

was also performed with a Zeiss microscope and a CCD camera during microfiltration tests. The micro-fabricated membranes were glued onto a metallic mesh with 600 μm openings to enhance their mechanical strength [17].

8.3 Analytical models used

8.3.1 Flux

Kuiper and coworkers [10,21] used the following equation for calculation of flow through a microsieve with short cylindrical channels:

$$J = \frac{\Delta p \cdot n \cdot d_{pore}^3}{24 \mu \cdot A_m} \left[1 + \frac{16L}{3\pi \cdot d_{pore}} \right]^{-1} [1 - f(\varepsilon)]^{-1}$$

where J is the flux, n the number of pores, L and d_{pore} the length and diameter of a pore, respectively. Δp is the applied pressure, μ the solution viscosity, A_m the membrane area, and ε the membrane porosity. The porosity function $f(\varepsilon)$ can be obtained as follows:

$$f(\varepsilon) = \sum_{i=1} a_i \varepsilon^{(2i+1)/2}$$

with $a_1 = 0.894$, $a_2 = 0.111$ and $a_3 = 0.066$ [10,21]. The calculated permeability determined from the above equation for the circular pore micro-fabricated membrane was around $0.32 \times 10^6 \text{ l/m}^2\text{hbar}$, which is in good agreement with measured value (0.38×10^6). This equation is not valid for high aspect ratio microfilters, where the pore length is much larger than the pore width.

8.3.2 Blocking model

In order to describe the blocking phenomena during microfiltration with latex particles, the Hermans and Bredee's blocking model [173], which describes the relationship between the filtrate volume and the filtration time, has been used. This membrane blocking model is commonly a useful tool to explain how and when the particles to penetrate into or block the pores.

$$\frac{d^2t}{dv^2} = k \left(\frac{dt}{dv} \right)^i$$

where t is filtration time, v filtrate volume, and the index i and the constant k depend on the blocking models. In this equation $i = 2, 1.5, 1$ and 0 apply to the complete blocking, standard blocking, intermediate blocking and cake filtration models, respectively [173]. It should be noted that this model is a single pore blocking model, which is rarely a good description of a filtration process and can be employed only for constant-pressure filtration. Recently, combined pore blocking and cake filtration models have been developed [71], which can be employed for the complex conditions where several mechanisms are involved.

8.4 Results and discussion

Microfiltration with different particles sizes can reveal how a pore is blocked and how the performance of a membrane will be with feeds having different particle concentrations. The performance of a micro-fabricated membrane with perfectly ordered pores was studied using latex particles of different sizes and concentrations as a model feed for microfiltration. Direct observation of the latex deposition on the

membrane was used to reveal the mechanism of membrane pore lockage. When the pore and particle sizes are known, the mechanism for particle deposition can also be determined and compared with available analytical models [137,174].

8.4.1 Fouling studies with a model particle smaller than the pore size

In order to evaluate the performance of the micro-fabricated membranes, latex suspensions containing 1 μm particles at two different concentrations (0.1 g/L and 1g/L) were tested. The goal was to determine the flux decline rate as a function of particle concentration. The membrane blockage was also studied by SEM analysis. The filtrations were performed in dead-end mode at a constant pressure of 20 kPa for both circular and slotted pore membranes, and the permeate volume was measured over time. The results are expressed as normalized flux (i.e., J/J_w), where J is the recorded flux from the experiments, and J_w the flux obtained from the tests with ultra-pure water. Figure 8.2(a) and (b) show the results for a circular membrane with a 3 μm pore size. It can be seen that severe flux decline occurred with a more concentrated feed, producing a permeate volume of only 75 ml during 30 min of filtration. For the diluted feed (0.1 g/L), the flux decline occurred more gradually. Since the particles are smaller than the membrane pores, a few particles can deposit on the membrane surface, while the remainder are carried by the filtrate, entering and plugging the membrane pores. Post-filtration SEM analysis of the samples revealed that blocking has occurred within the membrane pores (see Fig. 8.3).

Figure 8.2: Normalized flux of two different latex solution concentrations as (a) a function of time, and (b) permeated volume for a circular pore membrane with 3 μm pore size.

It can be seen that up to three particles of 1 μm can block a single circular pore, while for the slotted pore membrane, shown in Fig 8.3 (b), a relatively large open area is still available for water to penetrate although several particles have been deposited inside the pore.

Figure 8.3: SEM images of two polymeric micro-fabricated filters with deposited latex particles with a diameter of 1 μm , (a) circular pore and (b) slotted pore.

Figure 8.4 shows a flux comparison of the circular and slotted pore membranes for filtration of a latex suspension with a concentration of 1 g/L. It can be seen that the slotted pore membrane gives a higher flux and therefore, a larger collected volume than the circular pore membrane under equal conditions, thereby confirming its superior performance.

Figure 8.4: Normalized flux vs. time for the circular and slotted pore membranes. The concentration of the solution was 1 g/L for the experiments and the pressure was 20 kPa.

In order to evaluate the experimental results with Herman's model [173], the slope of the logarithmic plot of d^2t/dV^2 versus dt/dV (i.e. i value) was calculated as 1.55 ± 0.02 for the circular pore and 1.60 ± 0.07 for the slotted pore membrane, indicating that the main fouling mechanism is more likely to be standard blocking or pore narrowing.

8.4.2 Fouling studies with a model particle equal to the pore size

Filtration tests with 3 μm diameter particles were also performed to examine how particles with a similar size to the membrane pores would deposit on the surface.

Experiments were performed under both cross-flow and dead-end modes with various feed concentrations similar to those described in section 8.4.1. Direct microscopic observation during microfiltration showed that a very rapid flux decline took place within a couple of minutes for high concentration suspensions until a steady-state condition was achieved, resulting in a very low permeate flux. Since the cake formed by the latex particles is not completely dense, water can still penetrate through the micro-voids between particles and a residual flux can be measured.

Figure 8.5: (a) Normalized flux vs. time for the circular and slotted pore membranes. (b) Total resistance vs. time for the circular and slotted pore membrane. The concentration of the solution was 1g/L and pressure was 20 kPa for the experiments.

Figure 8.5(a) shows a flux comparison of the circular and slotted pore membranes. The flux decline data were consistent with an initial fouling mechanism due to intermediate blockage followed by cake filtration for both membranes. The initial rate of flux decline was slower for the membrane with slotted pores compared to the membrane with circular pores since the initial particle deposition only covered a fraction of the slotted pore area. Figure 8.5(b) depicts the total resistance obtained from the flux data shown in Figure 8.5(a). For the slotted pore membrane the flow resistance is much

smaller than the circular one because a slotted pore has a smaller perimeter than several circular pores with the same total surface area (i.e., if a/b , which is the ratio of slit length to the slit width, be larger than 3). Hence, this smaller perimeter gives less flow resistance. This behaviour has been also reported by other researchers [22,175]. Figure 8.5(b) also shows that the total resistance curves have a concave down trend suggesting fouling by pore blockage and cake filtration [176]. SEM images of the membrane surface shown in Figure 8.6 also clearly corroborate this.

Figure 8.6: SEM images of two polymeric micro-fabricated filters with deposited latex particles with a diameter of 3 μm , (a) circular pore and (b) slotted pore.

In this case the slope of the logarithmic plot of d^2t/dV^2 versus dt/dV for the circular pore membrane was around 1.1 ± 0.1 for the initial stage of filtration indicating the intermediate pore blockage condition followed by a zero slope on the log-log plot, consistent with the classical cake filtration model. The slope on the log-log plot for the slotted pore membrane was 1.2 ± 0.5 at the early stages (i.e., intermediate pore blockage) followed by a small region with a slope equal to 2 (i.e., pore blockage) and finally with a zero slope, which is consistent with the presence of an integral latex cake on the surface of the slotted pore membrane.

8.4.3 Fouling studies with a model particle bigger than the pore size

For comparison with the previous cases, we also examined how particles with a diameter (i.e., 6 μm) larger than the membrane pore size would deposit on the membrane surface. For both membranes, the microfiltration was performed with various feed concentrations under 20 kPa pressure in dead-end mode. Figure 8.7(a) shows typical normalized flux data versus filtration volume for 6 μm latex particles at three different concentrations. It can be seen that rate of flux decline for the most concentrated feed (i.e., 1g/L) is much more severe than two others, producing a permeated volume of only 37 ml during filtration; while this value is around 150 and 400 ml for the feed with the concentrations of 0.5 g/L and 0.1 g/L, respectively. A better picture of the fouling mechanism during filtration of 6 μm latex particles through circular and slotted pore membranes was achieved using the log-log plot as shown in Figure 8.7(b). The derivative plot for the circular pore membrane shows an initial slope of 2 ± 0.05 , which is similar to what is predicted by Herman's pore blockage model. The slope of d^2t/dV^2 versus dt/dV approaches a value of zero for long filtration times (i.e., large values of dt/dV), consistent with the classical cake filtration model [76,137]. The almost linear behavior of normalized flux versus filtration volume (Fig 8.7(a)) also confirmed that pore blockage is the dominant mechanism at the early stage of filtration [76]. For the slotted pore membrane, a single 6 μm particle cannot cover (see Fig. 8.8) a pore completely; therefore, the fluid still has this ability to flow through the open (uncovered) region of the pore (i.e., analysis of the SEM images also confirmed this). By continuing the filtration, the latex particles will begin to cover all the open areas causing a rapid flux decline during the final stages of the filtration.

Figure 8.7: Normalized flux of three different latex solution concentrations as a function of the permeated volume for a circular pore membrane with 3 μm pore size, (b) derivative plot for 6 μm latex filtration (0.1 g/L) through the circular and slotted pore membrane under 20 kPa pressures in dead-end mode.

Figure 8.8: SEM images of two polymeric micro-fabricated filters with deposited latex particles with a diameter of 6 μm , (a) circular pore and (b) slotted pore.

The derivative data for the slotted pore membrane show a small region with slope of 1.25 ± 0.4 initially (i.e., intermediate pore blockage), followed by another small region with slope of 2.1 ± 0.5 (i.e., pore blockage) and finally, a constant value for the d^2t/dV^2 (i.e., zero slope) for the rest of filtration. In the transition between the regions descend by pore blockage and cake filtration mechanisms in the log-log plot, a negative slope

was observed, which cannot be explained by classical fouling models, but has been described in detail by Ho and Zydney [76] for protein filtration.

Part 2: Direct observation of particle deposition on the surface of micro-fabricated filters

8.5 Polymeric micro-fabricated filters as a tool for investigation of “critical flux”

8.5.1 Introduction

Tangential flow (or cross-flow) micro-filtration is a widely used technique for processing particulate suspensions in different areas such as wastewater treatment, microorganism removal, and mineral processing [1]. In cross-flow micro-filtration, a cake layer deposit (i.e., fouling) tends to form on the membrane that usually controls the performance of the filtration process. The idea of hindering fouling by low flux operation was suggested by researchers in the 1980s. Field et al. [177] proposed that there is a flux value (so called “critical flux”), below which there is no or negligible particle deposition on the membrane surface and above which the deposition is significant during the filtration of suspensions.

A common non-invasive technique that can be used to identify the critical flux is direct observation through the membrane (DOTM) to view particle deposition on a transparent membrane [178,179]. By employing this method during cross-flow filtration, it is possible to see the cake formation on the membrane surface as the flux transits across the critical flux [179].

A well-known transparent membrane which has been used widely for DOTM tests is the AnoporeTM inorganic membrane (Anodisc, Whatman, UK) with a nominal pore

size of 0.2 μm (Fig. 8.9). This membrane has a high porosity and narrow pore-size distribution, and is visually transparent when it is wet because of the capillary honeycomb pore structure which allows light transmission. However, this membrane has two major drawbacks. Firstly, the pore-size distribution is limited due to the nature of the fabrication process (i.e., Aluminum anodization). Secondly, this membrane is so fragile that extreme care is required during handling and characterization.

Figure 8.9: SEM image of an Anopore inorganic membrane with average pore size of 0.2 μm [180].

In this section we used polymeric micro-fabricated membranes that were made by the dissolving mold technique (or direct lithography techniques) to study the mechanism of particle deposition on the membrane surfaces using the DOTM technique. We employed membranes with different pore shapes (i.e., circular, square-shape and slotted) in order to study the effect of pore morphology on the critical flux.

8.6.1 Test set-up and protocol

In this study all the experiments were conducted in the constant flux mode. The protocol for critical flux determination includes operation with different flux steps, initially with an increment of 15-20 l/m² h, which was achieved by a peristaltic pump. The flux normally was kept constant for 15 min during each step. If there were no or negligible particles deposited on the membrane surface during this period, the flux was considered to be below the critical flux [179]. The experiment was continued to the

next higher flux step until particle deposition became significant at a specific flux. After observation of severe particle deposition, the flux was then dropped and stepped up again in smaller increments ($3\text{--}5 \text{ l/m}^2 \text{ h}$) to more closely identify the critical flux. Since particle deposition on the membrane surface is non-uniform during each step, the experiments were repeated at least three times to identify the critical flux value.

8.6.2 Feed suspensions

Polystyrene latex beads with mean diameters of $4 \mu\text{m}$ and $6 \mu\text{m}$ were used as the model particles. Feed suspensions were prepared by diluting the stock solution with ultra-pure water at different concentrations (i.e., $0.1\text{--}0.5 \text{ g/L}$). The latex stock suspension was sonicated for 2 minutes in an ultrasonic bath before each test, and kept homogenous with a magnetic stirrer during experiments. The average cross-flow velocity was fixed at 0.2 m/s . The corresponding Reynolds number was around 800, thus the flow regime was laminar. The micro-fabricated membranes that we used are similar to those in the section 1 (i.e., circular and slotted with $3 \mu\text{m}$ pore size). We also performed some tests with membranes with square-shaped pores with a $3\times 3 \mu\text{m}$ pore size for comparison.

8.7 Results and discussion

In order to compare the filtration behavior between membranes with different pore geometries, the critical fluxes of all the membranes determined using DOTM technique and deposition rates in terms of particle coverage versus time on the membrane surface were also compared.

8.7.1 Critical flux of slotted pore membrane

The critical fluxes of sample particles for the slotted pore membrane were determined at various concentrations at the cross-flow velocity (CFV) of 0.2 m/s. Figure 8.11 shows the images of particle deposition for 6 μm latex particles at a concentration of 0.1 g/L during the test. Figures 8.11(a) and (b) were captured after 30 and 75 minutes during the cross-flow filtration process at an imposed flux of 35 and 95 $\text{l/m}^2\text{h}$, respectively. During this period, the particle deposition was sporadic on the membrane surface at an imperceptible rate. Therefore, the flux was considered to be below the critical flux in this case. The filtration was continued for 15 min at this flux, but no significant fouling was observed on the membrane surface. Then, the imposed flux was increased to 115 $\text{l/m}^2\text{h}$ using a peristaltic pump at the permeate side. Only 3 min after increasement of the flux, particles began to deposit at a considerable rate, as can be seen in Figure 8.11(c). Hence, the flux was judged to be above the critical flux in this case. During this period latex particles were dragged toward the membrane surface due to the convection and accumulated individually or in clusters on the pores and membrane surface. At the end of this step (110 min), the membrane area was almost totally covered by the deposited particles (Fig. 8.11 (d)).

Online monitoring of the transmembrane pressure during this period also confirmed that the TMP stayed relatively constant during first stage (≈ 0.375 kPa), but began to increase slowly in the second step (≈ 0.8 kPa), which was consistent with the observations of the images in Figure 8.11 (a)–(d).

The critical fluxes of the 4 μm particles were also measured in the same way. Figure 8.12 summarizes the critical fluxes of 4 and 6 μm latex particles at different concentrations (0.1–0.5 g/L) for a cross-flow velocity of 0.2 m/s. The overall trends were consistent with the finding of other researchers. For the case of the micro-

fabricated membranes it was observed that the critical flux decreases with increasing concentration [178,179]. In addition, it was found that larger particles give a higher critical flux [179]. The effect of cross-flow velocity on the critical flux was also investigated for the slotted pore micro-fabricated filter (see Fig. 8.13).

Figure 8.11: DOTM images of particle deposition during the filtration of 6 μm latex particles (0.1 g/L) at CFV = 0.2 m/s. (a) and (b) were taken under an imposed flux of 38 and 95 $\text{l/m}^2\text{h}$, respectively, while (c) and (d) were with a higher flux of 110 $\text{l/m}^2\text{h}$.

Figure 8.12: Critical flux vs. concentration during the filtration of latex particles at a CFV = 0.2 m/s using a slotted pore membrane with a 3 μm pore size.

Figure 8.13: Critical flux vs. cross-flow velocity measured in the filtration of latex particles using a slotted pore membrane with a 3 μm pore size.

This figure shows that by increasing the cross-flow velocity, the critical flux increases as well [179]. These observations are in good agreement with the predictions of a number of microfiltration models based on shear-induced diffusivity and also those in previous studies [179].

8.7.2 Critical flux of circular and square-shape pore membranes

The critical fluxes of sample particles for circular and square-shape pore membranes (3 μm pore size) were also determined at various concentrations at a cross-flow velocity (CFV) of 0.2 m/s similar to the previous section. It should be noted that the critical fluxes for the circular and square-shape membranes were almost the same at different particle concentration and velocities. Therefore, we just present the results for the membrane with the square-shape pore in this section and will compare them with the slotted one.

Figure 8.14 shows the images of the latex deposition on the membrane with square-shape pores at a concentration of 0.1 g/L and cross-flow velocity of 0.2 m/s. The images in Figure 8.14 (a) and (b) were captured at the 15th and 60th minute, during the filtration of 6 μm latex particles at an imposed flux of 30 and 65 $\text{l/m}^2\text{h}$, respectively. It can be seen from these photos that the particle deposition rate was not significant at these stages; therefore, the flux was considered to be below the critical flux. The filtration process was continued for 15 min at this flux, but no significant fouling was observed on the membrane surface. Then, the imposed flux was increased to 90 $\text{l/m}^2\text{h}$ to check the effect of higher imposed flux. At this stage it was observed that rate of latex particles deposition was increasing at a considerable rate (see Fig. 8.14(c)). Thus, the flux was judged to be above the critical flux in this case. Figure

8.14(d) shows that the surface of membrane at the end of this step was fully covered by latex particles.

Microfiltration tests with different feed concentrations and cross-flow velocities were also performed for the circular and square-shape pore membranes. Similar results to the previous section were obtained for these membranes.

In order to compare the results of the slotted pore membrane with square-shape one, we plotted the surface coverage and TMP changes for both membranes for different imposed fluxes to have better understanding of critical flux.

Figure 8.14: DOTM images of deposition during the filtration of 6 μm latex particles (0.1 g/L) at a CFV = 0.2 m/s. (a) and (b) were taken under an imposed flux of 30 and 65 $\text{l/m}^2\text{h}$, respectively, while (c) and (d) were with a higher flux of 90 $\text{l/m}^2\text{h}$.

Figure 8.15 shows the $d(\text{TMP})/dt$ versus different imposed fluxes for both micro-fabricated membranes. It can be seen under identical conditions, the rate of TMP increase for the membrane with square-shape openings is much higher than the slotted one. This is most probably due to the fact the a single size particle (4 or 6 μm) can block a single $3 \times 3 \mu\text{m}$ pore, while for the slotted pore membrane some open areas still available for water to penetrate. This picture confirms that critical flux for a slotted pore membrane is considerably higher than a membrane with square-shape (or circular) pores.

Figure 8.15: Comparison of TMP changes between two different micro-fabricated membranes (slotted and square-shape pore) during cross-flow filtration of 6 μm latex particles at a concentration of 0.1 g/L.

Figure 8.16 shows the extent of membrane coverage by the deposition during the filtration of 6 μm latex particles at a concentration at concentration of 0.1 g/L and CFV of 0.2 m/s.

For the membrane with square-shape pores it can be seen that the surface coverage is below 25 % for the imposed flux of $65 \text{ l/m}^2\text{h}$ and lower, but this value increased up to 60% near the flux of $90 \text{ l/m}^2\text{h}$. For the case of slotted pore membrane, the surface coverage was below 20% at an imposed flux of $100 \text{ l/m}^2\text{h}$ and lower, but the coverage rate start to increase after this value and reached to 40% at a flux of $110 \text{ l/m}^2\text{h}$. Surface-converge analysis of the membranes shows that under the same conditions, the particle deposition rate (fouling) for the square-shape membrane is higher than the slotted one.

Figure 8.16: Comparison of membrane coverage between two different micro-fabricated membranes (slotted and square-shape pore) during cross-flow filtration of $6 \mu\text{m}$ latex particles at a concentration of 0.1 g/L .

8.8 Summary

In the first section of this chapter we demonstrated the benefit of high-flux micro-fabricated membranes with identical pore sizes as a promising tool for studying fouling phenomena for different pore geometries. The regular pores of the membranes enabled us to study the mechanism of particle deposition at the pore scale and also analyze the experimental results with available classical models. The results in this study revealed the significant effects of pore morphology on the membrane performance during the filtration of micron size particles. The initial rate of flux decline was significantly lower for membranes with slotted pores compared to membranes with circular pores, since the initial particle deposition only partially covered the slotted holes. These results confirm that the membranes with slotted pores are less likely to suffer from hydrodynamic particle bridging on the pores and are intrinsically less prone to fouling and easier to clean. It was also found that the flow resistance of the slotted pore membrane is much lower than the circular one because a slotted pore has a smaller perimeter than several circular pores with the same total surface area.

In the second part we demonstrated the use of polymeric micro-fabricated filters as a great tool for DOTM applications. These membranes can be a good alternative to Anodisc membranes. It was found that under identical conditions, the critical flux of slotted pore membrane was higher than that of a circular (or square-shape) pore membrane, suggesting the better performance of the slotted pore filters. Lastly, we conclude that by proper selection of membrane pore geometry, flux decline can be hindered while maintaining a high selectivity during microfiltration.

A fluorescence micrograph showing numerous bright green, oval-shaped oocysts of *Cryptosporidium parvum* against a dark background. A white scale bar in the lower-left corner indicates 10 μm.

10 μm

Chapter 9

9

Automation and genetic testing

In chapter 7 we have shown the isolation and recovery of *Cryptosporidium parvum* oocysts from tap-water with high efficiency and reliability using our novel micro-fabricated filters. Also, pathogens can directly be detected on the surface of the micro-fabricated filters using fluorescently labeled antibodies, but other methods to detect *Cryptosporidium* with higher speed, specificity and sensitivity are mandatory. For this purpose, micro-fabricated filters were tested on two in-house automated waterborne pathogen isolation and identification instruments, which will be described in this chapter.

Note: Major parts of this chapter have been done with help of my co-workers at the Star-array and Fluigen Pte Ltd.

9.1 Introduction

Cryptosporidium is an intracellular parasite that has emerged as a common cause of diarrhea among humans and animals [181]. The infection caused by this pathogen is more severe in immuno-compromised patients and can become chronic and sometimes fatal [182]. The conventional diagnosis is based on microscopic observation of oocysts, which provides no information on the infected species and presents a challenge even to the most experienced laboratory technician [183]. Therefore, a method to detect *C. parvum* oocysts with higher speed, specificity and sensitivity is needed for pathogen detection in environmental and clinical samples.

In Chapter 7 micro-fabricated filters have been used for isolation and recovery of oocysts from tap-water with high efficiency and reliability. Also, pathogens can be detected directly on the surface of the micro-fabricated filters using fluorescently labeled antibodies, but other methods to detect *C. parvum* oocysts with higher speed, specificity and sensitivity are mandatory. Since water reservoirs are contaminated by numerous microorganisms, strict specificity in the detection system is required. Populations of *C. parvum* oocysts in reservoir water are supposedly sparse, sometimes below the detectable level by conventional methods. Unfortunately, uptake of even a single *C. parvum* oocyst may cause cryptosporidiosis, which requires extra high sensitivity in the detection system. These demands have been more satisfied by molecular detection techniques, such as real-time PCR, than traditional microscopic methods.

To use the micro-fabricated filters in a real scenario (i.e., filtration of large volume of water sample (1000 L)), an automated filtration system for isolation and recovery of pathogens and an integrated genetic analyzer have been developed by the Fluigen/EWI

project team. The aim was to test the feasibility of integrating the micro-fabricated filters with an automated waterborne testing instrument for detecting *C. parvum* oocysts in tap-water through genetic testing. The micro-fabricated filters were mounted and tested in an in-house waterborne pathogen isolation and identification instrument developed by our group.

9.2 Automated filtration system

The filtration and recovery module was designed to capture rare *C. parvum* oocysts in a large amount of water (e.g., 1000 L) and concentrate them into a small volume (e.g., less than 1 ml) for the subsequent genetic analysis by a separate group of researchers under an EWI project (project grant MEWR C651/06/149) at Nanyang Technological University. These two machines were used in this thesis work. To achieve such a high concentration ratio in a short time (e.g., less than 30 min), high porosity (40%) metallic micro-fabricated filters were employed. The fabrication and performance of the membrane has been reported in the previous chapters. To take full advantage of the superior nickel membrane developed, a filtration and recovery module was designed based on these membranes. The module is to automate the whole capture and concentration process, and then transfer the sample to the downstream analysis instrument. The nickel membrane has a diameter of 90 mm, which can pass through 90 L of tap-water before it is blocked by the deposited particles, under a dead-end filtration condition. In addition, the nickel membrane is easily cleanable with pulsed back-flush. By back-flushing the membrane after filtering a specific volume (e.g., 20 L), a high filtration capacity was achieved. However, on the other hand there is also a speed requirement. If one relies on only one membrane, it will take a few hours to filter 1000 L of tap-water. Thus, considering the balance of the capacity and speed

requirements as well as cost, 8 membranes was used for the purpose of filtration and recovery. The eluent collected from multiple back-flushing of the arrayed microfilters (8 membranes) is relatively high for genetic analysis (i.e., 2 to 6 L). Therefore, a second-stage filtration was designed to further concentrate the sample to a small volume (i.e., around 1 ml). The second filtration membrane

is the same nickel micro-structure but has a diameter of 25 mm. This small membrane can pass all the eluent from the first-stage only because the first-stage membrane is so selective that most of the particles in the 1000 L of water are not collected in the eluent, and the selectivity is further improved by using a micro-fabricated nickel membrane with a larger pore size (e.g., 9 μm) as a pre-filter. The back-flushed eluent from the second-stage filter is small in volume (e.g., less than 1 ml) and thus suitable to be analyzed in the downstream genetic test process. Figure 9.1 schematically shows the filtration unit for concentration of 1000 L of tap-water to a 1 ml condensed sample.

Figure 9.1: Schematic of the filtration unit for concentration of 1000 L of tap-water to a 1 ml condensed sample for genetic analysis.

Figure 9.2: A photo of the filtration and recovery module with its PC controlling user interface.

Figure 9.3: System diagram of the filtration and recovery module.

In addition to the above concerns regarding the filter membranes, another important aspect is the automation of the filtration and recovery process. Air pressure of 2 bar can be used as the driving force. Solenoid valves and house-made motorized pinch valves were also employed to control the liquid and air flow. In addition, a peristaltic pump was used to measure the elution buffer volume. A photo of the filtration and recovery module is shown in Figure 9.2. The major components of the module include a pre-filter, 8 first-stage filters, a second-stage filter, air pump, air pressure reservoir, air filter, pressure regulator, buffer tank, first-stage eluent tank, second-stage buffer reservoir, second-stage eluent reservoir, peristaltic pump, input valve and tubing, waste valve and tubing, liquid manifolds, liquid pinch valves, and an electronic control unit. A system diagram of the filtration and recovery module is shown in Figure 9.3, which illustrates how all the components are connected.

9.3 Genetic analysis

All the genetic analysis processes of the liquid sample were conducted inside the bio-chip as shown in Figure 9.4. The bio-chip is comprised of reaction chambers (reactors), reagent reservoirs, microchannels, pinch valves, and connectors. In particular, inside reactor 1 of the bio-chip, the live/dead oocysts are differentiated by a chemical PMA and blue lighting. Also in reactor 1, the *C. parvum* oocysts are lysed to release their DNA.

Figure 9.4: Structure of the bio-chip.

lysis process is conducted by the combined effect of chemical buffer, heat, and mechanical shaking with glass beads. The DNA sample formed in reactor 1 (R1) is then sent to the reactor 2 (R2). Inside R2 the DNA sample is purified and concentrated by DNA magnetic beads and wash buffers. Also PCR reagents are added to R2 to form a sample ready for PCR. The PCR sample together with the other two control samples are analyzed in three PCR reactors (Rp) with a real-time PCR process to qualify and quantify the *C. parvum* oocysts. The transport of liquid inside the machine conducted by air pressure from a pressurized manifold controlled by an air valve outside the machine.

The structure of the integrated genetic analysis instrument is illustrated in Figure 9.5. Besides the bio-chip, other components of the instrument include the chip shaker (a vortex motor), chip locks, blue LED, heater, magnet, solenoid plungers, air pressure manifold, PCR thermal cycler, fluorescence excitation source, camera, and air valves. There are also a power supply, micro-controller unit (MCU) circuit board, and air

pressure source located behind the instrument, as shown in Figure 9.5. The prototype of the integrated genetic analyzer has been made in our lab (by my colleagues) and is currently under biomedical tests. Figures 9.6 and 9.7 show photos of the fabricated biochip and genetic analyzer made in our laboratory, respectively.

Figure 9.5: Components in the front part of the integrated genetic analysis instrument.

The genomic DNA of *Cryptosporidium* was extracted either manually or by the extraction part of the genetic analyzer and used as the PCR template. Briefly, 400 μ l Lysis buffer, 50 μ l Proteinase K solution and 50 μ l magnetic beads were added to 2 ml of a *C. parvum* oocysts suspension. The mixture was incubated at 60 °C for 5 min followed by the addition of 800 μ l isopropanol. After vortex mixing, the magnetic beads were pelleted by applying a magnetic field using a magnet. After aspirating the supernatant, the magnetic beads were washed several times using a washing buffer to obtain a pure pellet. Subsequently, the magnetic beads were heat dried at 60 °C for 5 min and DNA was eluted with elution buffer for real-time PCR amplification.

Figure 9.6: A prototype of the bio-chip.

Figure 9.7: A photo of the prototyped integrated genetic analysis instrument.

Real-time PCR amplification was performed in a 25 μ l volume tube, containing the template DNA (either standard concentration of *C. parvum* genomic DNA, or genomic DNA extracted from standard concentration of *C. parvum* oocysts suspended in 2 ml of tap water), 10 mM Tris-HCl (pH 8.3), 5.0 mM MgCl_2 , 200 μ M each of dATP,

dCTP, dGTP, and dTTP, 25 pmole of each primer, 12.5 pmole of each Taqman probe and 2.5 U Taq DNA polymerase respectively. All PCR reactions were performed in the thermal cycler of the genetic analyzer with an initial denaturation at 95 °C for 200 s, followed by 45 cycles of denaturation for 20 sec at 95 °C, annealing for 20 s at 50 °C and extension for 20 s at 72 °C. Amplified DNA fragments were analyzed by the analysis function of the genetic analyzer.

The cycling temperature curve of the thermal cycler is shown in Figure 9.8(a), which is in line with the settings of the PCR program and the temperature change rate of 2 °C/s. Figure 9.8 (b) shows a series of fluorescence images taken every 3 PCR cycles throughout the PCR amplification. From top to bottom, the three rows of wells were respectively positive control, tap water sample and negative control. Figure 9.9 shows the real-time PCR amplification curves of *C. parvum* oocysts genomic DNA extracted by the genetic analyzer from 100 copies of *C. parvum* oocysts suspended in 2 ml volume of tap water (green line).

(a)

(b)

Figure 9.8: (a) Cycling temperature curve of the thermal cycler and (b) a series of fluorescence images taken every 3 PCR cycles.

Figure 9.9: Real-time PCR amplification curves of *C. parvum* oocysts genomic DNA extracted by the genetic analyzer from 100 copies of *C. parvum* oocysts suspended in 2 ml volume of tap-water.

100 copies of standard *C. parvum* oocysts genomic DNA dissolved in the same volume of elution buffer were used as positive control (blue line), whereas blank elution buffer was employed as negative control (red line). The threshold cycle number of DNA extracted from the tap-water sample containing 100 oocysts was 35.46, comparable to that of the manually extracted DNA. Hence, it has been proven that the detection of *C. parvum* oocysts suspended in tap-water can be fulfilled by the integrated genetic analyzer.

9.4 Summary

To demonstrate feasibility of integrating the micro-fabricated filters with an automated waterborne testing instrument for detecting *C. parvum* oocysts in tap-water through genetic testing, the microfilters were mounted in an in-house developed waterborne pathogen isolation instrument (i.e., which has been designed and fabricated by Fluigen/EWI project team) and successfully used for concentration of *C. parvum* oocysts from large volume water samples (e.g., 1000 L). In addition, a fabrication process for a novel bio-chip and an integrated genetic analyzer for extraction of DNA from *C. parvum* oocysts and the subsequent PCR process has been described briefly. By performing appropriate experiments, the DNA extraction and thermal cycling function of the integrated genetic analyzer have been verified, and it has been proven that the detection of *C. parvum* oocysts suspended in tap-water can be fulfilled by the subsequent molecular analysis.

Chapter 10

10

Conclusions and outlook

The best way to predict the future is to create it.

Peter F. Drucker

10.1 Introduction

In this thesis some novel techniques for fabrication of isopore micro/nano filters with identical pore size have been addressed. Micro-fabricated filters have been used successfully for isolation and recovery of *Cryptosporidium parvum* oocysts from large volumes of water samples. The main conclusions and an outlook on the most remarkable issues for future applications will be discussed in the following sections.

10.2 Concluding remarks and research contributions

The major findings and contributions of this study are summarized in the following points:

- ❖ Major drawbacks of available commercial micro/nano-filters such as tortuous pore paths, large pore variations and rough surfaces were addressed. These micro-structural defects can severely affect their performance and compromise their efficiency during isolation and recovery of waterborne pathogens. It was concluded that micro-fabricated filters with identical pore size and a smooth surface can be a good alternative for the commercial filters. Existing techniques for design and fabrication of screen-type microfilters (i.e., microsieves) suffer from major impediments such as:
 - Membrane folding (curling) upon release from the master mold.
 - Membrane failure and breakdown upon release from the master mold.
 - Adhesion of the membrane to the master mold.
 - Limitation of the material in use.
 - High-cost and low-yield processes.
- ❖ In order to solve aforementioned impediments in existing techniques, a novel technique, which called the “*dissolving mold technique*”, was introduced and described in detail in **Chapter 3**. This method has some advantages over the existing membrane microfabrication methods. Firstly, it resolves thoroughly the demoulding problem by dissolving an interim polymer pillar mold and secondly, it solves the membrane folding (curling) problem upon releasing the membrane from the mold by integrating a support mesh bonding layer before dissolving of the interim pillar mold. The major steps in fabrication of polymeric micro/nano filters with this method are: (1) fabrication of a silicon micro/nano pillar mold, (2)

fabrication of a dissolvable polymer pillar mold, (3) UV embossing of a polymer membrane using the dissolvable mold, (4) bonding of the membrane with a support grid, and (5) dissolving of the polymer pillar mold to obtain the final filter membrane with a support grid.

- ❖ In the **Chapter 4**, two different methods for the fabrication of polymeric micro/nano filters with integrated back-support were investigated. Both methods use the advantages of conventional UV-lithography and MEMS techniques. With the first technique we could successfully make polymeric microfilters with identical pore size and shape on a single layer of SU-8 resist. Due to the limitation of contact mask lithography, it was impossible to project small features ($< 2 \mu\text{m}$) inside a thick SU-8 film. To overcome this obstacle, a novel multi-layer technique to fabricate slotted pore polymeric filters with micron and potentially sub-micron size using several layers of SU-8 was introduced. The resulting membranes have smooth surfaces, narrow pore-size distribution, high porosity, and good mechanical strength. Concerning scaling-up, these methods are promising because the photolithographic techniques are well-known; therefore, not much further research is needed.
- ❖ A new technique for fabrication of metallic micro/nano filters with integrated back-support was also explained in **Chapter 5**. By employing conventional lithography and electroplating techniques, high-throughput micro/nano filters with narrow pore-size distribution were fabricated. Using electroless plating, the pore size of the microfilters was decreased to sub-micron size evenly. The resulting micro/nano filters were used successfully for various microfiltration tests.
- ❖ The performance of micro-fabricated filters in aqueous solutions strongly depends on their surface properties, as well as the feed characteristics. To improve

wettability of the polymeric/metallic micro-fabricated filters, we employed different strategies (**Chapter 6**) such as plasma treatment and wet-chemical modification in order to enhance the membrane performance during microfiltration tests.

- ❖ In **Chapter 7**, three important characteristics of micro-fabricated filters were addressed. The results revealed that the micro-fabricated filters present much higher flow rates in comparison to the commercial filters (*first characteristic*). Sample loading and back-flushing using micro-fabricated filters resulted in nearly 90-95% recovery of the *C. parvum* oocysts spiked. The micro-fabricated filters showed better performance than current commercial filters in terms of the recovery rate (*second characteristic*). Careful analysis of turbidity in the eluent from different microfilters revealed that micro-fabricated filters have a lower turbidity level in comparison to the other filters under the same conditions (*third characteristic*). This finding suggested that downstream analysis of the samples from the micro-fabricated filter is much easier than the commercial ones.
- ❖ In the first section of **Chapter 8** the benefit of micro-fabricated membranes with identical pore size as a promising tool for studying the fouling phenomena for different pore geometries was demonstrated. The regular pore geometry of the membranes enabled us to study the mechanism of particle deposition at the pore scale and also to evaluate the experimental results with available classical models. The results in this study revealed the significant effects of pore morphology on the performance of membrane during filtration of micron-size particles. In the second part polymeric micro-fabricated filters were employed as a useful tool for DOTM applications. It was shown that these membranes can be a good alternative for the Anodisc membrane. DOTM experiments revealed that under comparable

conditions, the critical flux of the slotted pore membrane is higher than the circular (or square-shape) pore membrane, suggesting better performance for the slotted pore microfilters. Moreover, we have shown that micro-fabricated membranes are excellent candidates for the experimental verification of filtration models. Their identical properties such as a smooth surface and uniform pore distribution give low particle adhesion and reproducible results. Lastly, we can conclude that by proper selection of membrane pore geometry, flux decline can be hindered while maintaining a high selectivity during microfiltration.

- ❖ In the **Chapter 9** micro-fabricated filters have been employed inside a fully automated filtration system (i.e., developed by Fluigen/EWI project team) for isolation and recovery of *C. parvum* oocysts from large volume of water samples (e.g., 1000 L). Genetic analysis was also performed using a novel bio-chip and genetic analyzer. It has been proven that the detection of *C. parvum* oocysts suspended in tap-water can be fulfilled by the subsequent molecular analysis.

10.3 Outlook

10.3.1 Other applications

Micro-fabricated filters have the potential to cause a revolution in the filtration industry. These membranes with their identical properties such as a smooth surface, high porosity and uniform pore size can be employed for a wide variety of applications such as microorganism removal (i.e., demonstrated in this thesis), protein filtration, emulsification, circulating tumor cells (CTCs) isolation, yeast harvesting, micro-patterning of cells and healthcare.

10.3.2 Cleaning and regeneration of membranes

An important topic for future research concerns cleaning of the micro-fabricated filters. Especially for a durable membrane such as the micro-fabricated filters a good cleaning process is crucial for implementation in a filtration application. We have done some preliminary research on cleaning of metallic micro-fabricated filters using strong alkaline media (e.g., NaOH), but further investigation needs to be done by expertise in this area for both metallic and polymeric filters.

10.3.3 Optimization of the fabrication process

The dissolving mold technique, which has been explained in this thesis, is a promising method to fabricate low cost polymeric filters from a wide variety of polymers. This method has potential to scale-up for mass production. The only concern about this method is the presence of a residual layer (i.e., during fabrication process) that needs to be removed using an oxygen reactive-ion etching (RIE) process. This extra step increases the cost and lowers the overall throughput in the fabrication process. Additional research needs to be performed to solve this hurdle too.

10.3.4 Fabrication of membranes with tapered pores

In Chapters 3, 4 and 5 three different methods for fabrication of isopore polymeric and metallic micro/nano filters with identical pore sizes and arbitrary shapes have been explained. From a theoretical point of view it is apparent that the porosity has a major role in the flow resistance of a microfilter. Membrane pore geometry (i.e., circular or slotted) also plays a big role in membrane fouling during microfiltration of micro-size particles (i.e., shown in Chapter 8). Prevention of the Vena Contracta effect is another issue in the optimization of membrane performance. It is the point in a fluid stream where the diameter of the stream is the least (see Fig. 10.1), such as in the case for a stream coming out of an orifice or small micro-holes (such as microfilters) [184,185].

Therefore, after an orifice or a micro pore, the streamlines that converged when entering the pore will only gradually diverge after exiting the pore. The place where the flow diameter is minimal is called the Vena Contracta (i.e., highest velocity and lowest pressure). During microfiltration using microfilters with a short length, flow instabilities can occur at the exit points that normally cause transmembrane pressure (TMP) increases.

Figure 10.1: Schematic representation of the Vena Contracta effect. The effective flow diameter is decreased after passing an orifice.

Figure 10.2: Schematic representation of the tapered pore design [184].

This can also have a direct effect on the rate of membrane fouling during filtration. It is known that this effect can be reduced by using jet-shaped (tapered) pores. This shape helps the stream lines to follow the pore walls such that the effective flow through the pore is increased (see Fig 10.2). To make a membrane with such pore geometry, some preliminary experiments were carried out, but future research needs to be done for this purpose.

10.3.4.1 Fabrication of polymeric filters with tapered pores using dissolving mold technique

In Chapter 3 the novel “*dissolving mold technique*” for fabrication of polymeric membranes was explained. The key elements that control the size and shape of the final pore are the size and shape of the silicon pillar mold [186], because its geometry replicated on the dissolvable pillar mold via the PDMS mold. In order to fabricate a membrane with tapered pores using this technique, MEMS techniques were employed to design and construct a conical-pillar mold on silicon using the DIRE process. Dry-etching of micro/nano-structures requires precise control of the etch profile. In order to control the etch profile of micro-pillars (i.e., to achieve conical shape), we increased the oxygen content and decreased the temperature simultaneously. Figure 10.3 shows an SEM image of a conical-shape pillar mold made with this technique. This can be used as a master mold for fabrication of tapered pore micro/nano filters using the *dissolving mold technique*.

Figure 10.3: SEM image of a silicon micro-pillar mold with a conical shape.

10.3.4.2 Fabrication of polymeric filters with tapered pores using the direct lithography technique

In another approach for fabrication of polymeric micro/nano filters with a conical shape, contact-mask lithography technique was used to perform direct patterning on a SU-8 film. The approach to obtain the tapered pores consists of the addition of a small amount of TINUVIN (384-2, Ciba®) as a UV-absorber to the photoresist that induces a UV light intensity gradient in the cross-section of the polymeric film. The amount of UV absorber to be added has to be determined for this purpose [184,185]. It has been reported that adding a small amount of UV-absorber ($\approx 1\text{--}4$ wt %) to the photoresist will result in a 20-30% reduction in the transmission spectrum [185]. For calculating precise values we can also employ the Lambert-Beer equation for absorption [184]. In our experiments (for a film with 10 μm thickness) we found that addition of 4 wt% TINUVIN to the SU-8 photoresist (2010, MicroChem) will result in an intensity pattern with $\theta \approx 10^\circ$ during lithography using an exposure dose of 120 mJ/cm^2 . Figure

10.4 shows an SEM image of a polymeric microfilter with a pore size of 3 μm at the inlet and 5 μm at the outlet made with direct lithography technique.

Figure 10.4: SEM image of a membrane with tapered pores. Inlet diameter is 3 μm and outlet diameter is 5 μm .

10.3.5 Other strategies to reduce fouling

Various strategies can be used to enhance the performance of micro-fabricated filters while retarding fouling. Among them, surface modification using plasma and chemical etching methods has been investigated in this study. Other methods such as quick back-pulsing, air sparging, electrofiltration and chemical treatment (i.e., explained in Chapter 2) can be employed to boost the throughput of these filters and enhance their productivity.

10.3.6 Fouling investigation

Micro-fabricated filters have been used in this study as a new tool for investigation of membrane fouling at the pore scale (Chapter 8). Latex particles of different sizes were employed as model foulants to study the mechanism of particle deposition. Additional

research can be done using other foulants such as BSA, yeast, bacteria, and algae to model the mechanism of fouling (and biofouling) using these new filters. Appropriate numerical studies can be also carried out to validate the experimental results and open new pathways for future research. In addition, transparent polymeric micro-fabricated filters made from SU-8 can be good option for DOTM applications. The preliminary tests with the polymeric micro-fabricated filters with different pore geometries showed promising results. Future attempts using these filters and DOTM setup can be directed toward biofouling and its effect on the critical flux. In situ observation of cake formation by the microorganisms on a membrane surface at and above the critical flux as well as observation of the removal of the cake (i.e., biofilm) in response to reduced flux changes can be a great interest [187].

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