Journal of Membrane Science 369 (2011) 560–568

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Capturing and recovering of *Cryptosporidium parvum* oocysts with polymeric micro-fabricated filter

Majid Ebrahimi Warkiani, Longqing Chen, Chao-Ping Lou, Hao-Bing Liu, Rui Zhang, Hai-Qing Gong*

BioMEMS Laboratory, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

ARTICLE INFO

Article history:
Received 9 September 2010
Received in revised form 3 November 2010
Accepted 21 December 2010
Available online 31 December 2010

Keywords:
Micro-fabricated filter
Microfiltration
Cryptosporidium parvum oocysts
High flux
Polymers

ABSTRACT

A novel High-Aspect-Ratio (HAR) polymeric micro-filter was micro-fabricated and validated for concentration and recovery of *Cryptosporidium parvum* (*C. parvum*) oocysts from tap water. Perfectly ordered pores of the micro-filter were achieved by a high yield and cost effective dissolving mold technique. Obtained filter has micro structural features such as high pore density, uniform pore size, smooth surface, and straight pore path. Sample loading and back-flushing using the filter resulted in nearly 100% recovery of the *C. parvum* oocysts spiked. The micro-fabricated filter showed improved performance than current commercial filters in sample throughput, recovery ratio, and reusability. This research demonstrated the potential application of the micro-fabricated filter in monitoring *C. parvum* oocysts contamination in tap water supply system.

1. Introduction

Life-threatening diseases caused by *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 [1]. However, *C. parvum* oocysts 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 large volume of drinking water into smaller volume is critical for accurate detection and quantification of *C. parvum* oocysts from drinking water. Filtration based concentration techniques have been widely used to recover *C. parvum* oocysts into small volume for downstream analysis. Fig. 1(a)–(d) shows SEM photos of some widely used membranes for this purpose. Because of their micro-structural defects like tortuous pore path (Fig. 1(b) and (c)), low pore density (Fig. 1(d)), high coefficient of variation [2] (CV > 20%), and overlapped pores (Fig. 1(a) and (d)) in commercial micro-filters, they are relatively low in sample throughput and cell recovery rate, which lead to prolonged processing time and compromised accuracy for detection of *C. parvum* oocysts.

We developed micro-fabricated membranes (Fig. 1(e) and (f)) that contain pores with uniform size and shapes have the capability to overcome these micro-structural defects. 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% capturing of *C. parvum* oocysts, which has a spherical shape with diameter of 3–6 μm, micro-fabricated filter with pore size of 2–3 μm can be used, while commercial filters use average pore size around 1 μm (e.g. Envirochek HV). This ability to used bigger pore size for the same capture ratio significantly increases the throughput of the micro-fabricated filter in comparison to the commercial ones. Likewise, narrow pore size distribution of such filters allows the small and unwanted particle (<pore size) pass from the filter. This will help to have a lower turbidity level in eluent during back-flushing step, which is crucial for detection limit of bio-sensors. This property also helps to have lower deposition rate of small particles on the membrane surface. In addition, a straight instead of a tortuous pore path helps preventing accumulation of particles inside the pores (see Fig. 2) [3].

Nowadays, great efforts are being deployed to produce micro-filters with different methods, but existing micro-fabrication techniques for micro-filters have some difficulties and feature limitations. Kuiper et al. [4] developed an inorganic microfiltration membrane with a pore size down to 0.1 μm using laser interference lithography and silicon micro machining technology. Pores with uniform diameter and smooth surface allow the filtration membrane to have low transmembrane pressures and large flux.

* Corresponding author. Tel.: +65 67904279; fax: +65 67904279.
E-mail address: mhqgong@ntu.edu.sg (H.-Q. Gong).

0376-7388/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2010.12.038

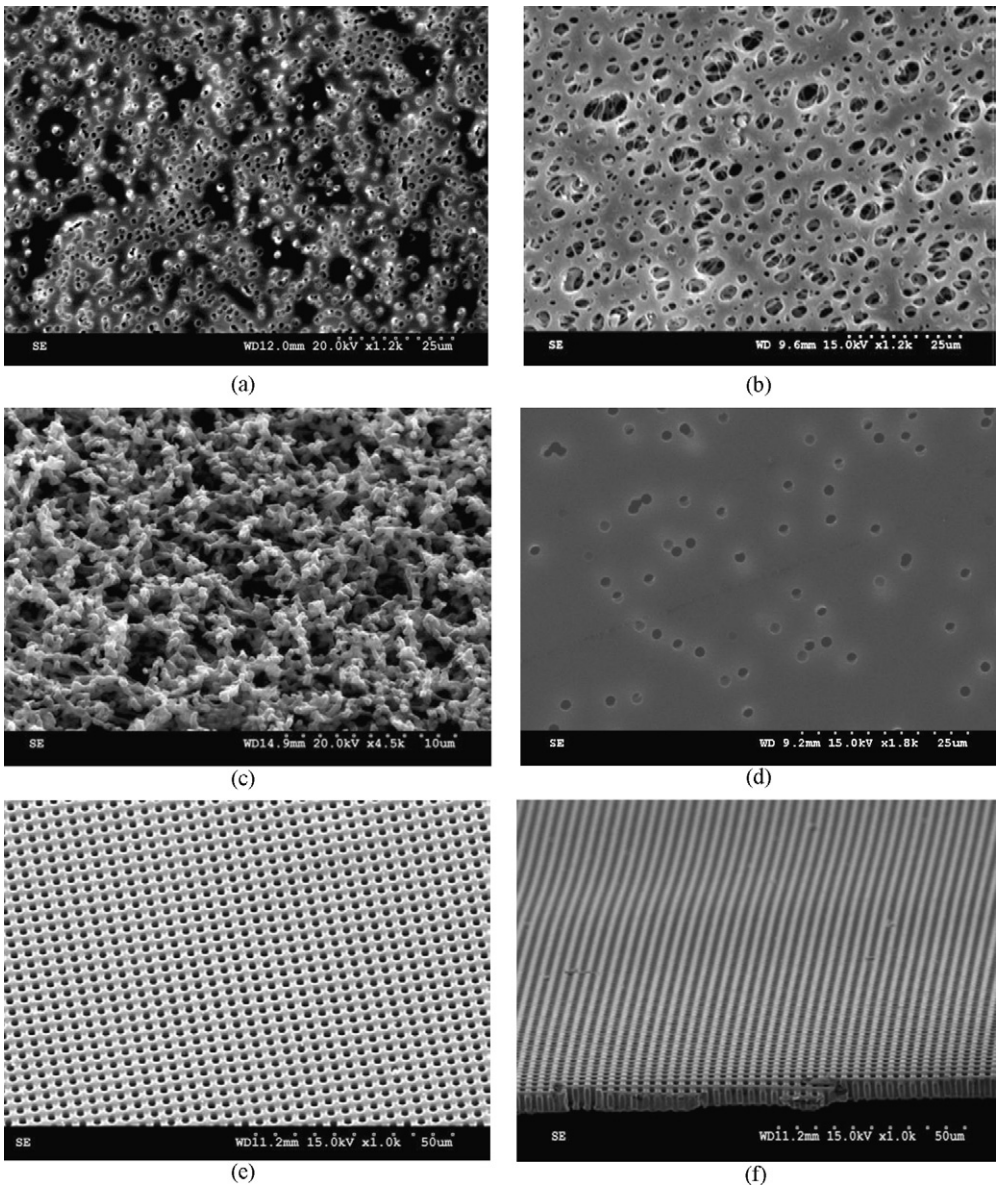

Fig. 1. SEM images of commercial filters and our micro-fabricated filter for concentration of *C. parvum* oocysts (a) Envirochek high volume (EC-HV) membrane filter, (b) Envirochek (EC) standard membrane filter, (c) mixed cellulose esters membrane filter, (d) track-etched polycarbonate membrane filter, (e) HAR polymeric micro-fabricated membrane, and (f) cross-section view of HAR polymeric micro-fabricated.

However, the small thickness of the silicon nitride film (<1 μm) allows only low working pressure (<2 bar), and the lack of choice of membrane material (limited to nitride) also limits the application of this type of filtration membrane [4,5].

Masuda and his co-workers also investigated the feasibility of using an anodic porous alumina template for fabrication of through-hole polymeric membrane [6]. By employing this technique, they could fabricate polymeric membrane with a hole-diameter down to hundreds of nanometers. The major difficulty in this method is in demoulding step because peeling off the membrane from the mold often results in membrane damage and failure. Also, the choices of pore size and pore density are restricted due to the use of alumina templates.

In recent years, some other methods have been also proposed to produce membranes with uniform pores like phase separation micromolding [7], excimer laser [8] and aperture array lithography [9]. However, because of some obstacles like high cost, difficulty in demoulding, formation of debris and ridges (i.e. laser method), and membrane folding, these methods could not be considered as ideal and rapid processes for mass production of microfiltration membranes.

In this study, we use a 'dissolving mold technique' for fabrication of a High-Aspect-Ratio (HAR) polymeric through-hole filter membrane with high pore density, uniform pore size and smooth surface for monitoring *C. parvum* oocysts contamination in tap water supply system. We chose 2 μm pore size for the micro-fabricated filter in order to have higher flux and equivalent or even better oocyst capturing ability in comparison to Cellulose filter (1.2 μm) or Envirochek HV (1 μm) membrane. The dissolving mold technique solves thoroughly the demoulding problem of embossed or replicated membrane filters that are associated with the existing fabrication methods and therefore, through-hole membranes with large area and thickness can be easily made. In addition, by bonding the membrane, firstly, to a metal support grid before dissolving the pillar mold, the folding (curling) problem, which happens during release step, is eliminated. Our tests showed improved performance in sample throughput and recovery of *C. parvum* oocysts than existing commercial micro-filters. In addition, the dissolving mold tech-

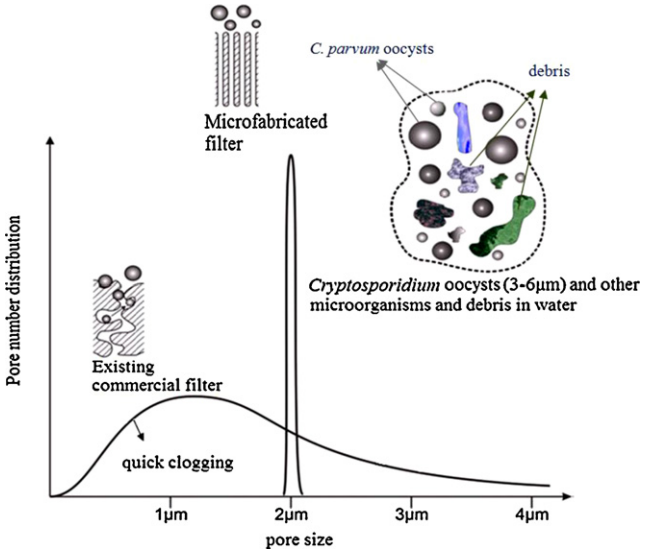

Fig. 2. Schematic of pore size distribution in the commercial filters and the micro-fabricated filters.

nique is a low cost and high yield process, which can be used for mass production purpose.

2. Experimental

2.1. Design and fabrication of polymeric micro-filter

The micro-fabrication process of the membrane is illustrated in Fig. 3. A silicon wafer was first cleaned in piranha solution and then a thin layer of photoresist was coated on the wafer surface. Photolithography was carried out on a mask aligner and a resist dot array with dot size of 2 μm and space of 2 μm was patterned on the silicon wafer. The patterned resist was used as an etching mask and silicon etching was then conducted on an STS deep reactive ion etching (DRIE) system to form high aspect ratio micro-pillars with about 2 μm wide and 20 μm high (Fig. 3(a)–(c)). After removing the resist and processing a passivation treatment, the silicon pillar mold was employed as a master mold for the fabrication of the PDMS (polydimethylsiloxane) interim blind-hole mold (Fig. 3(d) and (e)). Then a dissolvable polymer pillar mold was replicated from the PDMS interim blind-hole mold (Fig. 3(f)). Polymer through-hole membrane can be fabricated using the dissolvable polymer pillar mold with a UV embossing method. As shown in Fig. 3(g)–(j), a UV curable resin (e.g. SU-8) was dispensed onto the polymer pillar mold. Subsequently, a glass plate was pressed onto the uncured resin to spread it between the pillars. The assembly was exposed under UV light for 120 s. After exposure and removing of the glass plate, oxygen plasma dry etching was carried out to remove the residual layer on the top of the pillars. To strengthen the polymeric membrane and to avoid curling of the membrane during subsequent processes and handling, a piece of steel mesh was bonded to the membrane by UV curable glue at the marginal area. Afterward, the entire membrane was immersed into DI water (60 °C over night) to dissolve the polymer pillar mold. Finally, another piece of steel mesh was 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(k)). The material of dissolvable polymer pillar mold should be curable (e.g. UV curable, hot curable, etc.) and also dissolvable in a solvent such as water or acetone. Polyvinyl alcohol (PVA), polyimide (PI), polymethyl methacrylate (PMMA) and photoresist can be used for this purpose. In the present work, PVA was used because of its good mechanical properties and dissolvability in water. The micro-fabricated membrane is shown in Fig. 1(e) and (f).

2.2. Filter holder for filtration and back-flush

In order to test and compare the result of our micro-fabricated filter with commercial filters, a filter holder was designed and fabricated from PMMA (Polymethyl methacrylate) by laser cutting and thermal bonding technique. It comprises a base for sample flow in and eluent flow out, a cap for flow out of the filtered sample and for intake of eluent, a gasket for leakage prevention and also an upper and a lower perforated support for keeping commercial filters during the test firmly. The major parts of this holder are depicted schematically in Fig. 4.

2.3. Turbidity condition of the tap water

For all the tests involving to tap water, the turbidity of the water influences the results of the flow rate, flux and also the oocyst capture and collection with filters. Turbidity values of water samples from 3 taps in our lab were measured (Oakton® T-100) hourly through a whole day. The turbidity falls into a range of 0.2–0.7 NTU. In our test, water from a same tap with turbidity around 0.36 NTU was used in order to test and evaluate different filters.

2.4. Materials and methods

2.4.1. 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 micro-fabricated filters. Purified oocysts suspension was supplied in deionized water and stored at 4 °C. The antibody reagent consists of the fluorescein-labeled mouse monoclonal antibodies targeted against the oocysts outer wall epitopes or antigenic sites. All the reagents were utilized from the Crypt-a-Glo G/C Direct Comprehensive kit (Waterborne Inc., New Orleans, LA, USA).

2.4.2. Water samples

Tap water samples were obtained on the day of testing from the laboratory faucet, which was flushed for 5 min before sample collection to reach relatively stable turbidity. In order to simulate the condition of impurities in water during filtration and recovery of *C. parvum* oocysts from a large amount of tap water while avoiding large quantity of bio-hazard waste, water samples with condensed impurities were prepared by filtering 10 L tap water through 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.

3. Results and discussion

3.1. Membrane morphology

In present work, we fabricated over 80 filters with membrane diameter of 25 mm or 47 mm. The pore density of fabricated membranes varied between 2.5×10^7 pores/cm^2 and 0.21×10^7 pores/cm^2. Fig. 5 shows SEM photos of 2 μm micro-filters with different pore densities other than in Fig. 1(e): (a) 2.5×10^7 pores/cm^2, (b) 0.81×10^7 pores/cm^2, (c) 0.4×10^7 pores/cm^2, and (d) 0.21×10^7 pores/cm^2. This figure indicates that the HAR micro-fabricated membrane comprises narrow pore size distribution and also smooth surface, which are independent of pore density.

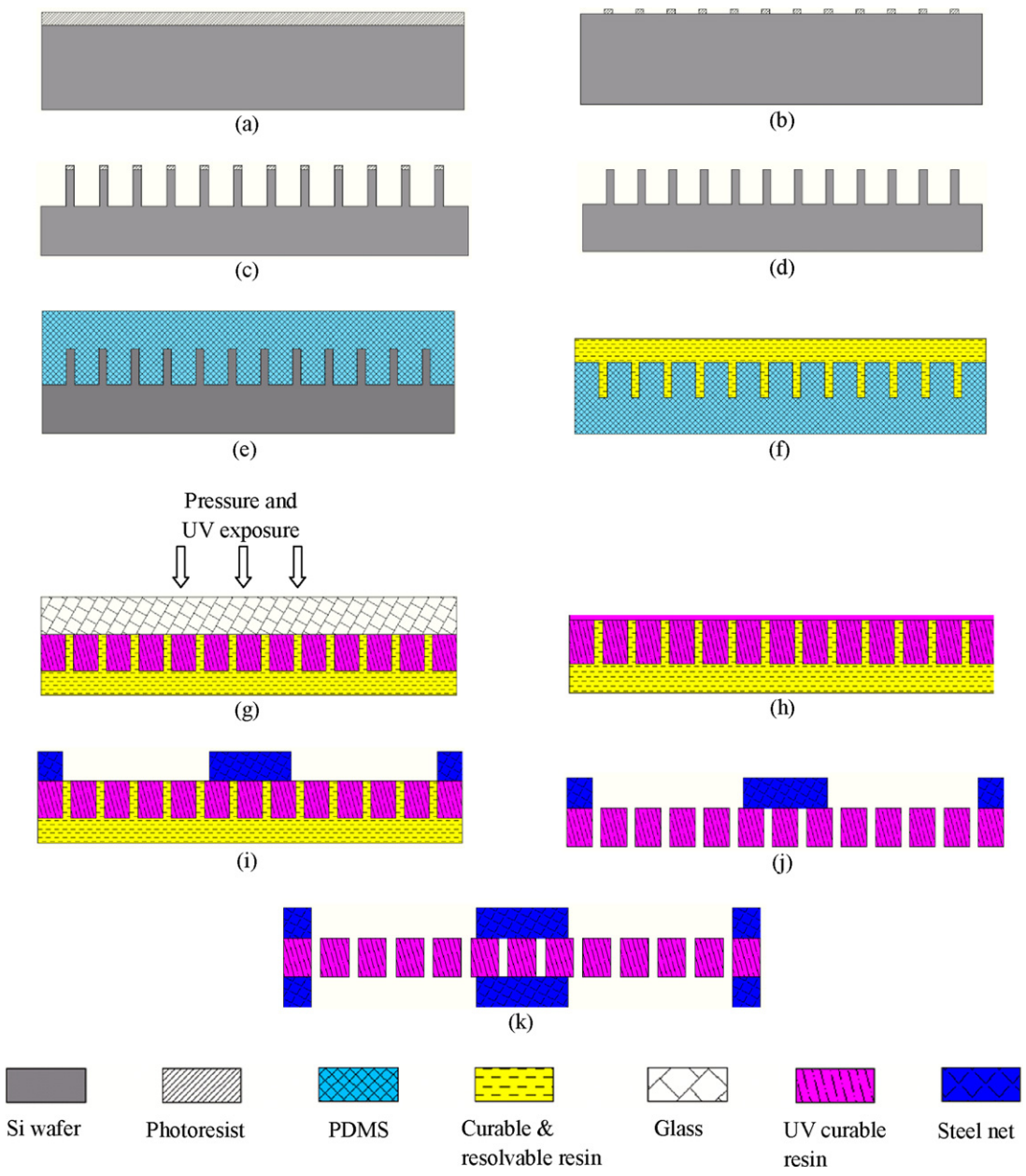

Fig. 3. Process flow for the fabrication of HAR polymeric micro-filter, (a) spin-coating photoresist, (b) photolithography, (c) DRIE, (d) removing photoresist pattern, (e) dispense PDMS on the Si pillar mold, (f) dispensed PVA solution on the PDMS interim blind-hole mold, (g) dispense UV curable resin on the polymer pillar mold, press the resin with the glass plate, and expose the assembly to UV, (h) remove the residual layer by oxygen plasma etching, (i) bond a support mesh to the cured SU-8 on the dissolvable pillar mold, (j) dissolve the polymer pillar mold to obtain the through-hole membrane bonded to the support mesh, and (k) the final micro-filter with support meshes on the both sides.

3.2. Pore size distribution and membrane strength

The UV lithographic technique is a precise process and many shape, size and distribution can be obtained by this technique. In micro-fabricated polymeric membranes, the 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 polymeric filter are 2 μm and 80 nm, respectively. The corresponding coefficient of variation (CV = σ/M) for this membrane is 4%, which is much lower than commercial micro-filters (e.g. track-etched membranes) with CV around 20% [2]. The histogram of analyzed samples is illustrated schematically in Fig. 6.

The strength of the filter is determined by the strength of the membrane and its support together [4]. Finite element simulation (i.e. by ANSYS software) was carried out in this work to investigate the stress distribution and maximum load in the polymeric micro-fabricated filters. Fig. 7 shows SEM image of a micro-fabricated membrane with a support mesh and fulcrum points where the membrane placed on them.

Fig. 8 shows that the largest stress is located at the corners of membrane (i.e. fulcrum points) where it is placed on the steel mesh, and it is approximately proportional to the applied pressure. By choosing following parameters for our micro-fabricated membrane, the FEM analysis shows that membrane can withstand up to

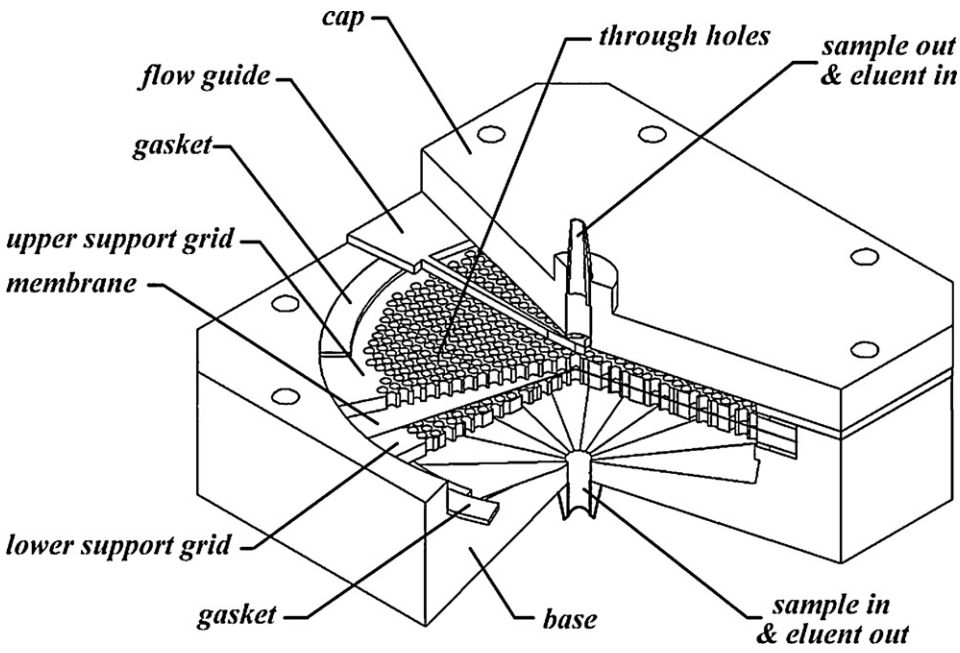

Fig. 4. A cut-away view of the filter holder.

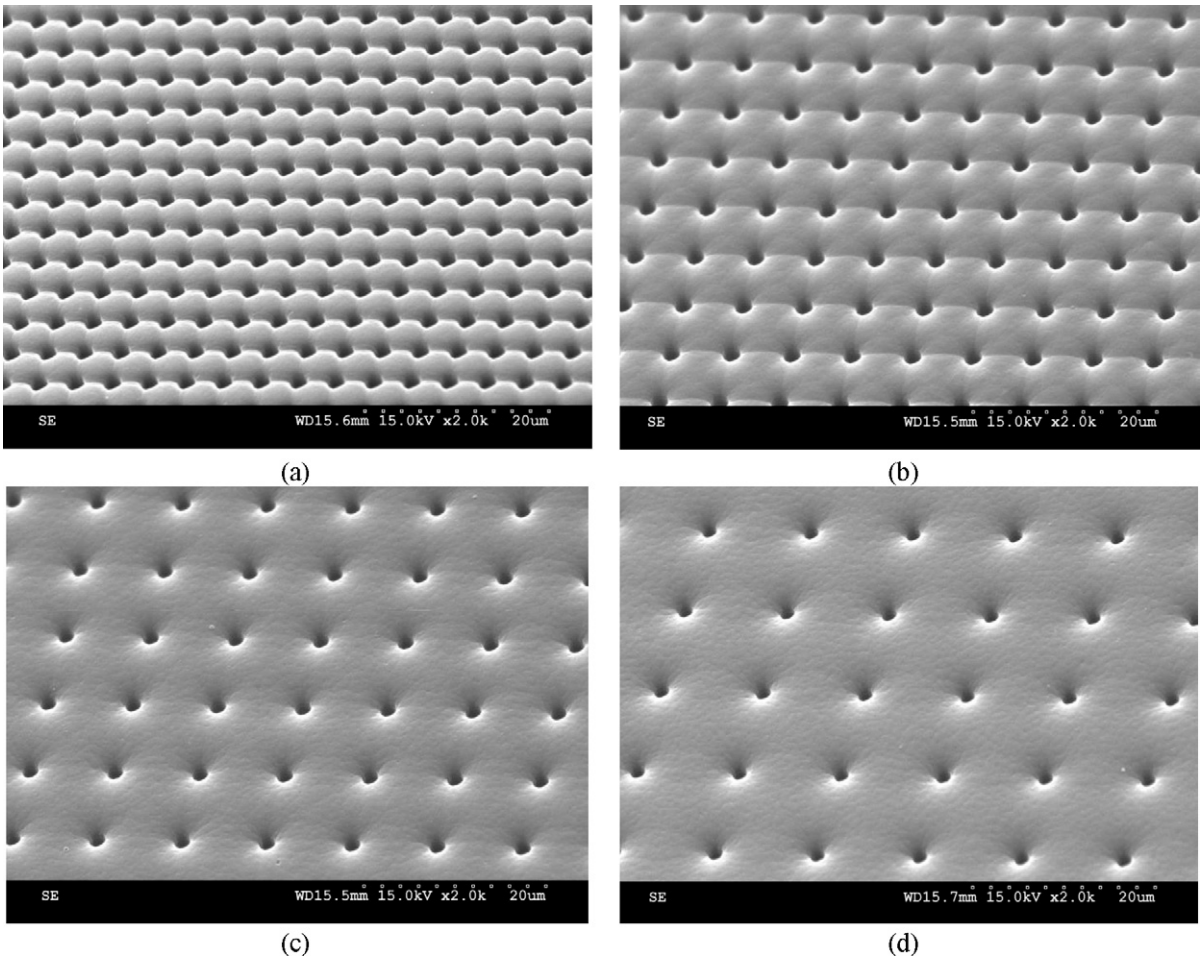

Fig. 5. SEM photos of HAR micro-fabricated filters with four different pore densities: (a) 2.5×10^7 pores/cm^2, (b) 0.81×10^7 pores/cm^2, (c) 0.4×10^7 pores/cm^2, and (d) 0.21×10^7 pores/cm^2.

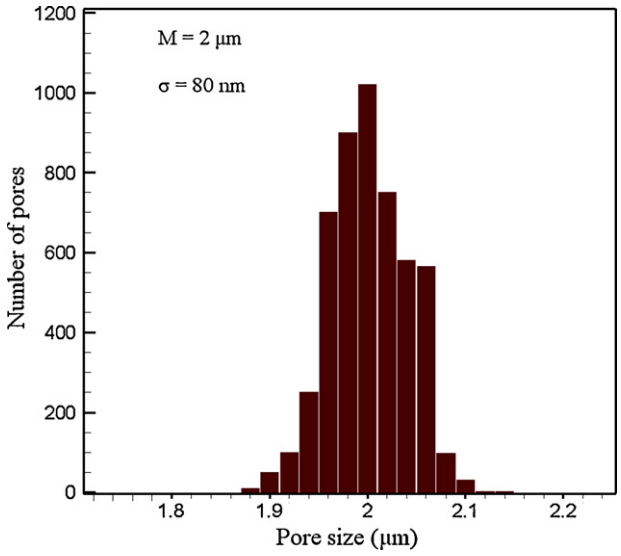

Fig. 6. Pore size distribution of a 2 μm microfabricated membrane filter.

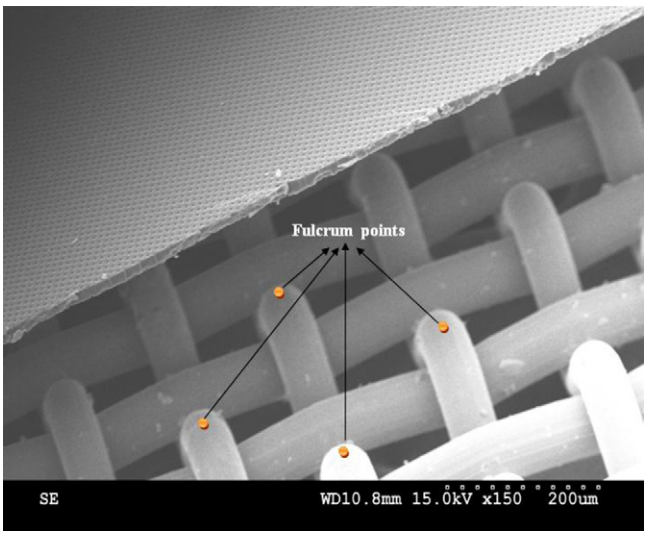

Fig. 7. SEM photo of a membrane on mesh and fulcrum points where the membrane placed.

3 bar pressure during the filtration:

$$\begin{aligned}\text{Membrane thickness} &= 20\,\text{mm, aperture of support mesh}\\ &= 200\,\text{mm, Young's modulus}\,(E)\,\text{of SU}-8\\ &= 2\,\text{GPa, yield strength of SU}-8\,(\text{syield})\\ &= 60\,\text{MPa}\,[10],\ \text{and porosity}\,(\text{K}) = 25\%.\end{aligned}$$

For a specified pressure, the deflection of perforated membrane is around 7% larger than for non-perforated membranes. In addition, membrane with higher porosity deflects more than membranes with lower porosity. The aperture size of support grid has a significant influence on the membrane strength. On one hand, the smaller the aperture the higher the membrane strength; On the other hand, it was reported that the openings should be large enough to avoid any effect on hydraulic resistance [9].

3.3. *Integrity test and recovery evaluation with spherical mono-sized microbeads*

In order for a membrane process to be an effective barrier against pathogens, the filtration system must be integral or free of any leaks or defects resulting in an integrity breach [11]. Direct integrity testing represents the most accurate means of assessing the integrity of a membrane filtration system which can be done by a marker-based test. Marker-based tests employ either a spiked

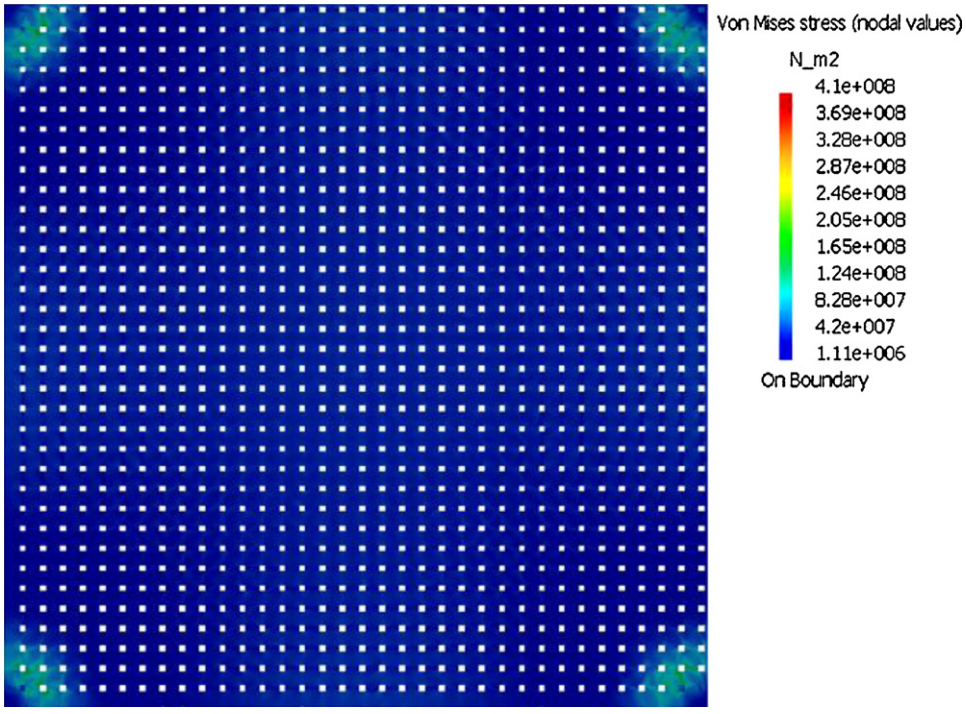

Fig. 8. FEM simulation of the stress distributions of perforated membranes, only stress distribution for 200 × 200 μm area is shown here.

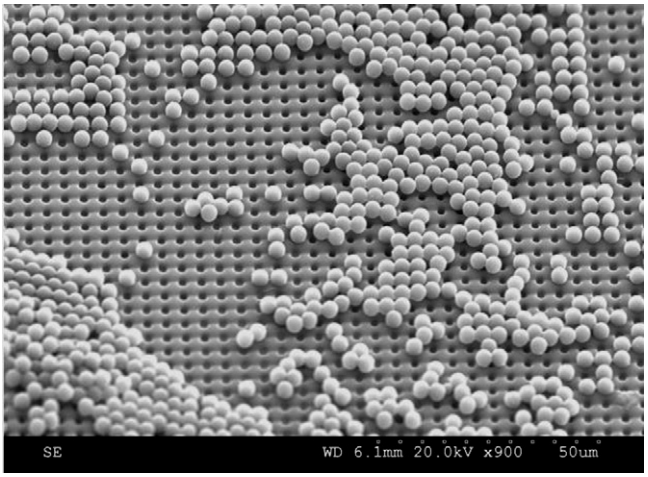

Fig. 9. SEM photo of a HAR micro-fabricated filter with captured microbeads on the surface.

particulate or molecular marker to verify membrane integrity by directly assessing the removal of the marker. The recommended surrogate for *C. parvum* oocysts must have an effective size of 3 μm or smaller [11]. For this purpose, we used spherical monosized polymer particles (Microbeads® AS, 3 μm) for integrity test of our micro-fabricated filter. 0.1 ml of cross-linked polystyrene particles were added to 50 ml distilled water and mixed together completely. The sample was forced to pass a micro-fabricated filter (i.e. by a dead-end filtration setup under 1.5 bar pressure) and then the collected liquid was forced to pass through an Anopore® Inorganic Membrane (Anodisc) with nominal pore size of 0.2 μm to capture any microbeads that may have leaked. Fig. 9 shows the surface of polymeric micro-fabricated membrane which fully captured the spherical microbeads. The surface of the 0.2 μm membrane was fully observed under a microscope and it was confirmed that no beads have been passed through the micro-filter. In order to check the recovery rate, the polymeric micro-fabricated filter was back-flushed with pure water to remove the beads' layer from the surface. The following optical observation of the filter membrane showed that more than 99% of microbeads were recovered.

3.4. Capturing evaluation with heat inactivated C. parvum oocysts

The capturing capability of our HAR micro-fabricated filter was also verified by a two step oocysts filtration method. In the first step, 10 ml of sample, which was pure water spiked with heat inactivated *C. parvum* oocysts, was filtered using a HAR polymeric micro-fabricated filter. Then the filtered water goes to a subsequent filtration step using Anodisc filter to capture any oocyst that may pass through the micro-fabricated filter. *C.parvum* oocysts attached to both two filters were observed under a fluorescence microscope by FITC (fluorescence iso-thiocyanate) technique (Waterborne. Inc., New Orleans, LA, Cat no. A400FLK). It was observed that all oocysts were captured by the HAR micro-fabricated filter and no oocyst was found on the second filter (i.e. the Anodisc filter).

3.5. Recovery evaluation and comparison with commercial filters

The recovery performance of the HAR micro-fabricated filter was also evaluated by comparing with two different types of commercially available filters, which were Millipore multi cellulose acetate membrane (pore size of 1.2 μm, Cat No: RAWP01300) and US EPA recommended Envirochek filter membrane (pore size of 1 μm, Cat No: 121110) which is now serving as the golden standard in concentration *C. parvum* oocysts from the environmental water sample. For the recovery evaluation test, three similar samples of *C. parvum* oocysts were prepared by labeling 100,000 viable *C. parvum* oocysts with fluorescent tagged antibodies and spiking into 10 ml of formerly prepared water sample containing condensed impurities from tap water. The turbidity of each sample was determined using Oakton® T-100 Turbidity meter (Cole-Parmer, CO, USA). The pH of each was measured using a Corning 140 pH meter (Corning, Essex, UK). Membranes were wetted by immersion in ethanol (70%) for 15 min, and then flushed with sterile distilled water before mounting on the membrane support. Then, each membrane was loaded onto the fabricated holder (see Section 2.2) and samples were passed through the micro-filters by a dead-end filtration test rig under 1.5 bar pressure. Then *C. parvum* oocysts were recovered from the filters either by lateral swing, which involved immersing and swinging the filter in water for 5 min, or by back-flush using a 10 ml back-flush buffer solution containing 1% sodium polyphosphate (NaPP) and 0.1% Tween 80. *C. parvum* oocysts attached to the filters before and after the recovery were visualized by staining the membranes with FITC technique followed by observation under the fluorescence microscope. The obtained microscopic pictures are depicted in Fig. 10.

It can be seen that some *C. parvum* oocysts still adheres to the other two types of commercial filters after back-flush with appropriate buffer while nearly all oocysts were recovered from the polymeric micro-fabricated filter. Unique features of the HAR micro-fabricated filter like the smooth surface, straight pore path and uniform pore-size greatly reduce the oocyst adhesion to the filter surface and enable us to achieve a very high recovery rate (95–99%) of *C. parvum* oocysts when applying back-flush or lateral swing. Additionally, the filtered water from micro-fabricated membranes went to a subsequent filtration step using Anodisc membrane to capture any oocyst that may have passed through the filter. Microscopic inspection of Anodisc filter reveals that no oocyst passed from the polymeric micro-fabricated membrane during filtration of viable *C. parvum* oocysts.

For the case of micro-fabricated membrane, the reproducibility of enrichment and recovery was determined for a variety of *C. parvum* oocysts concentrations (i.e. 10^6, 10^5, 10^4 *C. parvum* oocysts), and it was realized that the recovery was $97 \pm 2\%$ *C. parvum* oocysts according to three individual concentrations enriched in triplicate experiments. Furthermore, from the images of the HAR micro-fabricated filter after back-flush we can observe that the filter surface is clean and had been nearly restored to its original status before sample loading, which indicate high reusability of the filter.

3.6. Flow rate

Fig. 11 shows the flow rate of three commercial micro-filters and the micro-fabricated filter under a constant pressure condition using tap water. The operating pressure was 1 bar and the turbidity was around 0.36 NTU. The results indicate that the HAR micro-fabricated filter has much higher flow rate in comparison to other filters. The Envirochek filter presents a higher flow rate in first 10 min of filtration because its porosity is higher than the micro-fabricated filter, but its flow rate gradually decayed and stopped within 40 min when the flow rate had decreased to a limit of 1 ml/min. By increasing the porosity and decreasing the thickness of micro-fabricated membrane, flow rate of about 2000 ml/min/cm^2 has been achieved from our filtration testes.

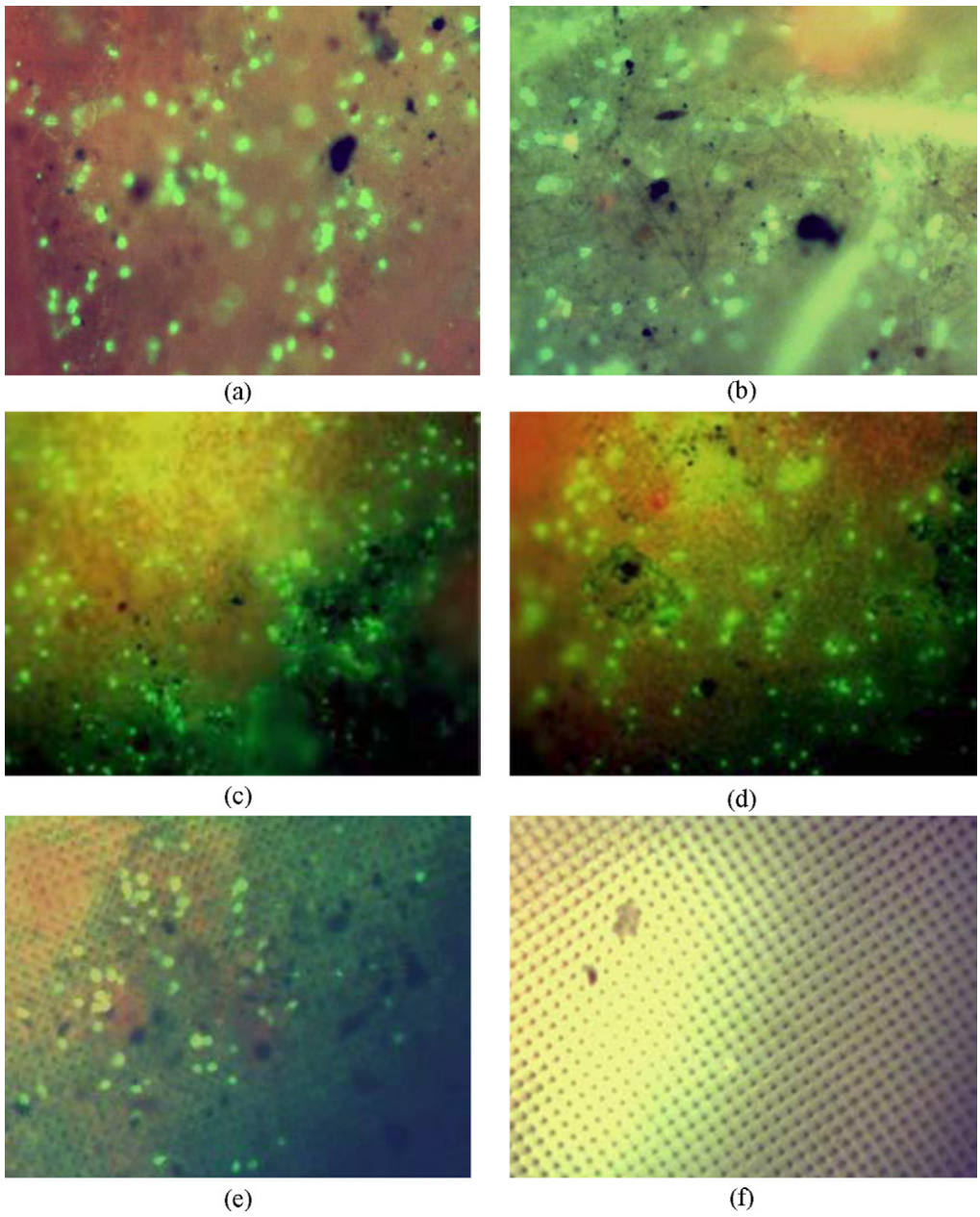

Fig. 10. Fluorescence microscopic images showing the surfaces of micro-filters after filtration of *C. parvum* oocysts spiked in concentrated tap water (Left) and after back-flushing (Right), using a Cellulose membrane filter (a and b), an Envirochek filter (c and d), and a HAR micro-fabricated filter (e and f).

3.7. Turbidity of eluent in filtration of tap water

The turbidity of eluent of polymeric micro-fabricated filter was evaluated by comparing with three different types of commercially available micro-filters. In all experiments, 10 l of tap water was passed through the membranes with diameter of 25 mm. Then 5 ml of buffer containing 1% NAPP and 0.1% Tween 20 was back-flushed the membranes with an air pressure of 1.5 bar. Table 1 shows the summary of experiments.

All experiments were performed in triplicate and presented results are the average of the measurements. The obtained results indicate that polymeric micro-fabricated membrane presents much lower turbidity of eluent during the back-flushing step. This is attributed to the smooth surface of the micro-filter and also passage of small particles (i.e. unwanted) through the pores during the filtration process. Inspection of micro-filters using SEM images also revealed that majority of the pores (more than 95%) in the commercial micro-filters clogged during the filtration process while surface

Table 1
Turbidity of eluent for different types of micro-filters.

	Envirochek high volume (HV) filter	Cellulose filter	Track-etched polycarbonate filter	HAR micro-fabricated filter
Turbidity	4.5 NTU	7.5 NTU	5 NTU	1.9 NTU

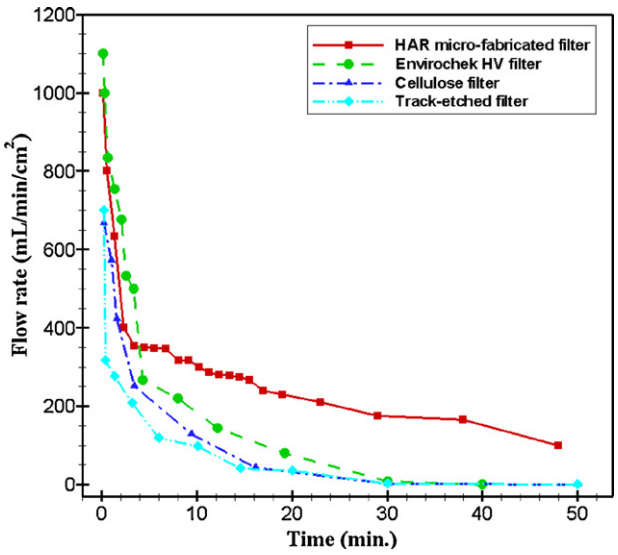

Fig. 11. The flow rate of three commercial membrane filters and micro-fabricated filter for filtration of tap water at a pressure of 1 bar and turbidity of 0.36 NTU.

of micro-fabricated filter was quite clean and just some pores were closed.

4. Conclusions

In this study, we present a micro-fabricated HAR polymeric membrane for capture and recovery of *C. parvum* oocysts from tap water. The fabrication method of the membrane has some advantages over the existing methods. Firstly, it resolves thoroughly the demoulding problem in existing membrane fabrication techniques by dissolving the pillar mold. Secondly, folding (curling) of membrane upon release from the mold is solved by bonding membrane to a support grid before dissolving the pillar mold. The significant properties of the micro-fabricated filter for isolation of *C. parvum* oocysts are as follows:

1. *High sample throughput*: According to the tests and measurements, the flow rate and throughput of the micro-fabricated filter is higher than the commercial filters like track-etched membrane filter for the same purpose of *C. parvum* oocyst capturing. The higher throughput of tap water leads to higher concentration ratio of the oocysts.
2. *High recovery rate*: HAR micro-fabricated filter shows a high recovery rate (i.e. 95–99%) when it was employed for filtration of *C. parvum* oocysts. This valuable property is attributed to its properties like smooth surface, low coefficient of variation and straight pores.
3. *Reusability*: Commercial filters are not designed to be reusable, and they can hardly retain their initial status after the filtration and back-flushing process while in the micro-fabricated filter, it can retain its original condition with a back flush or shaking process.

This study highlighted the potential application of micro-fabricated filer in rapid filtration and recovery of *C. parvum* oocysts for downstream detection. Additionally, this filter can be used in other applications such as air monitoring, blood filtration, cell analysis, biosensors and HPLC sample preparation process.

Acknowledgement

The authors acknowledge the financial support of the Environment & Water Industry Programme Office of Singapore under the project grant MEWR C651/06/149.

References

[1] M. Inoue, S.K. Rai, T. Oda, K. Kimura, M. Nakanishi, H. Hotta, S. Uga, A new filter-eluting solution that facilitates improved recovery of Cryptosporidium oocysts from water, J. Microbiol. Methods 55 (2003) 679–686.
[2] V. Ramachandran, H.S. Fogler, Plugging by hydrodynamic bridging during flow of stable colloidal particles within cylindrical pores, J. Fluid Mech. 385 (1999) 129–156.
[3] C. van Rijn, M. Van der Wekken, W. Nijdam, M. Elwenspoek, Deflection and maximum load of microfiltration membrane sieves made with silicon micromachining, J. Microelectromech. Syst. 6 (1997) 48–54.
[4] S. Kuiper, C.J.M. van Rijn, W. Nijdam, M.C. Elwenspoek, Development and applications of very high flux microfiltration membranes, J. Membr. Sci. 150 (1998) 1–8.
[5] M. Gironès, Inorganic and polymeric microsieves, strategies to reduce fouling, PhD Dissertation, University of Twente, Enschede, The Netherlands, 2000.
[6] T. Yanagishita, K. Nishio, H. Masuda, Polymer through-hole membrane fabricated by nanoimprinting using metal molds with high aspect ratios, J. Vac. Sci. Technol. B: Microelectron. Nanometer Struct. 25 (2007) 35–38.
[7] M. Gironès, I.J. Akbarsyah, W. Nijdam, C.J.M. van Rijn, H.V. Jansen, R.G.H. Lammertink, M. Wessling, Polymeric microsieves produced by phase separation micromolding, J. Membr. Sci. 283 (2006) 411–424.
[8] I. Saxena, A. Agrawal, S.S. Joshi, Fabrication of microfilters using excimer laser micromachining and testing of pressure drop, J. Micromech. Microeng. 19 (2009) 025025.
[9] K. Han, W. Xu, A. Ruiz, P. Ruchhoeft, S. Chellam, Fabrication and characterization of polymeric microfiltration membranes using aperture array lithography, J. Membr. Sci. 249 (2005) 193–206.
[10] H. Becker, C. Gärtner, Polymer microfabrication technologies for microfluidic systems, Anal. Bioanal. Chem. 390 (2008) 89–111.
[11] B. Leonard, Membrane filtration guidance manual, United States, Environmental Protection Agency, office of Water, 2003.