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Microfluidic Membrane Filtration Systems to Study Biofouling

Ishita Biswas, Aloke Kumar and Mohtada Sadrzadeh

Abstract

In wastewater treatment, the membrane functions as a semipermeable barrier that restricts transport of undesired particulates. A major problem related to membrane filtration processes is fouling of membranes by colloidal particles, organic matter, and biomaterials. Among the various types of fouling, biofouling is one of the most severe, as it is a dynamic process. Even a few surviving cells that adhere to the membrane surface multiply exponentially at the expense of biodegradable substances in the feed solution. To analyze the mechanism of biofouling, membrane cell is typically considered as a black-box, where only the input and the output can be measured and put into use for analysis. Microfluidic devices are being used to study and understand the nature, properties, and evolution of biofouling. A primary advantage of a microfluidic membrane is the ability to conduct real-time observations of biofilm. This chapter presents an overview of the biofouling in membrane processes and different fabrication technique of microfluidic membrane systems.

Keywords: biofilm, biofouling, microfabrication, microfiltration membrane, microfluidics

1. Introduction

1.1. Membrane biofouling

Pressure-driven membrane processes can be used to filter a wide range of small materials, ranging from monovalent ions and dissolved organic matter to biological substances. They have become very popular for treating sea and waste water. However, they face the problem

of fouling on a continuous basis. Fouling is the unwanted accumulation of substances on the membrane surface. There are five types of fouling including scaling (by divalent ions), heavy metal fouling, organic fouling, colloidal fouling, and biofouling [1, 2]. Among these fouling types, biofouling is the most severe since it is a dynamic process and is also the most confronted one, and can contribute as much as 45% of the total fouling [1].

Biofouling due to biofilms (matrix-encapsulated bacterial colonies) and colloidal materials act as the main components of membrane fouling [2]. Moreover, biofilms have a significant impact on the membranes used for different types of water filtration such as brackish and seawater. Once a cell is attached to the membrane surface, it decreases membrane permeability by forming a gel layer [3]. Biological substances always remain in the membrane. Even if 99.9% of these materials are removed by pre-treatment, the remaining 0.1% can grow exponentially by using biodegradable substances in the feed (waste) water [4].

1.2. Biofouling due to bacterial colonization

Biofouling occurs due to the adsorption of the biological cells on a membrane surface [5]. Biological organisms are usually identified by their length scale. Microorganisms, which lie within very small length scales (1–200 μm), include bacteria, fungi, and algae. Furthermore, length scale >200 μm is referred to macro-organisms such as larvae, barnacles, hydroids, tubeworms, mussels, and bivalves [1]. Bacteria are a common biofouling agent and are found extensively in nature. Bacterial colonization of a surface is an extremely complex process, where several phenomena can take place at multiple length and time scales [6–8]. Colonization on the surface starts with adhesion of bacteria to a solid-liquid interface. The interaction of bacteria with the surface leads to the formation of extracellular polymeric substance (EPS), where bacterial cells are embedded in a matrix. These matrix-encapsulated, surface-associated bacterial communities are referred to as a biofilm [9, 10]. EPS, the binding material of biofilms, is composed of long-chain biomolecules such as polysaccharides, nucleic acids, protein, DNA and lipids [11–14]. Biofilms can play an important role in chronic infections [1]. Moreover, they are prevalent in industrial and shipping environment, causing significant problems related to environmental impacts and health risks [15].

2. Background

2.1. Biofouling due to biofilm on membrane

Biofilm is one of the most challenging issues in membrane technology [16–18]. The adsorption of bacteria cell on the membrane surface depends on membrane properties such as membrane materials, hydrophobicity, and roughness [19]. The adhesive nature of EPS is considered as the most severe problem in membrane biofouling [20, 21]. Biofilm on the membrane surface reduces the permeate flux and salt rejection [22–24]. In membrane technology, the flux and salt rejection are the two primary criteria for characterizing of membrane performance. The more the flux and salt rejection, better the membrane performance is. The volume flux (J) of

porous membrane is usually calculated by Hagen-Poseuille equation where the pores are assumed to have the same radius,

$$J = \frac{\varepsilon\, r^2}{8\eta\tau}\frac{\Delta P}{\Delta x} \tag{1}$$

Where, Δx is the membrane thickness, ΔP is pressure difference across the membrane, η is the viscosity, τ is tortuosity, r is the radius of the pore, and ε is the porosity of the membrane. Porosity can be calculated by,

$$\varepsilon = \frac{n_p\, \pi r^2}{A_m} \tag{2}$$

A_m is the membrane surface area and n_p is the number of pores. Tortuosity is defined by:

$$\tau = \frac{(2-\varepsilon)^2}{\varepsilon} \tag{3}$$

Matin et al. Provided a list of typical bacteria species that can cause biofilm formation on the membrane surface as well as a reduction in flux decline and salt rejection due to the formation of biofilm on the membrane surface [25]. They observed that, without bacterial adhesion, the membrane was able to reject (R) 98.2% salt. The rejection was decreased by 4.6% because of the biofouling on the membrane.

Biofilm is a complex structure due to the viscoelastic nature of EPS that can lead to the formation of memory effect in a material [10, 26]. Rheological measurement of the biofouling layer on the membrane surface is required to understand the EPS nature. Patsios et al. [27] performed some rheological measurements of the biofouling layer on the membrane. They obtained nonlinear behavior of shear stress and strain of the EPS. They claimed that EPS shows more elastic nature than viscous on the membrane surface. The storage modulus G´, the elastic part, was higher than the loss modulus G´´ that is the viscous component. [25].

2.2. Microfluidic approach in biofouling study

In wastewater treatment, microfiltration membranes with the pore sizes lying between 0.1 and 10 μm are used to remove bacteria. Membranes are usually part of an opaque setup, where only the input and the output can be measured. Advancements in micro-/nano-technologies, for example, microfluidic devices can be employed to study membrane processes at the pore-scale. An example of this is the use of microfluidic-based membrane mimics, which are being used to explore a wide variety of membrane related issues, including biofouling. An essential advantage of microfluidic membrane mimics in studying biofouling is that they make real-time microscopy of biofouling possible. **Figure 1** shows a basic schematic difference between membrane filtration mode and microfluidic approach. The pillars are shown in **Figure 1b** are solid in structures and usually made of polydimethylsiloxane (PDMS). The gap between the pillars is considered as the pore. The coverslip is used to seal the device.
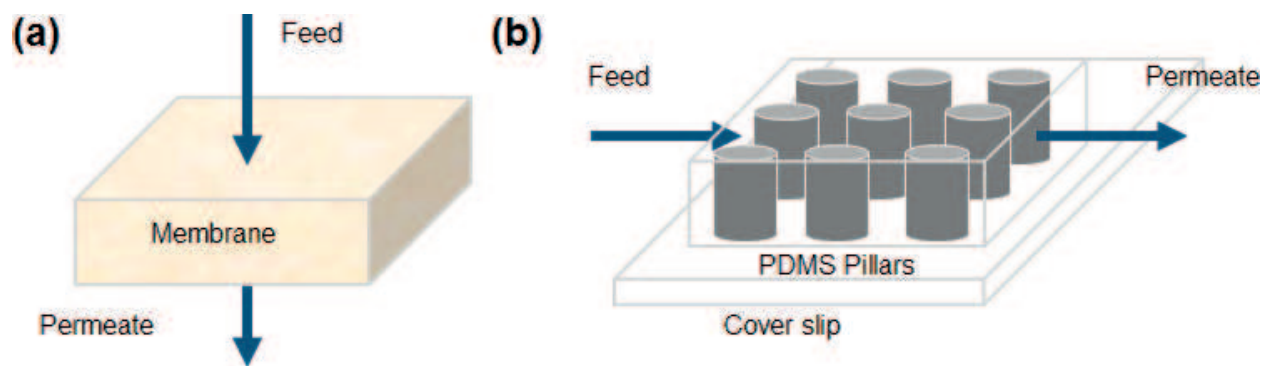
Figure 1. Schematics of (a) membrane filtration where feed is wastewater and permeate is the clean water and (b) microfluidic filtration mode.

Design and fabrication are the initial steps to work with microfluidic devices. Different types of fabrication techniques include photolithography, electron lithography, hot embossing and injection molding, etc. Photolithography is a common technique when feature sizes larger than 1 μm are desired. The nanoscale feature can also be fabricated by e-beam lithography where the minimum resolution could go down to 10 nm [28, 29].

2.3. Bacterial streamer due to biofouling

The impact of hydrodynamic flow on biofilms is the large time-dependent deformations that can result in nonlinear phenomena. An example of such phenomena is the bacterial streamer. Streamers form in flowing water and attach to the surface by the upstream "head" while the downstream "tail" can oscillate [6, 10, 30–32]. Streamers in a microfluidic system are typically tethered at one end to the pillar walls while the rest of the body is suspended in the downstream direction. Their filamentous structure can extend significantly with the flow [6, 33, 34]. Drescher et al. [35] revealed that streamers can cause a sudden and rapid clog in the fluid flow system in comparison with the biofilm attached to the surface. Surface hugging biofilms have a very modest effect on the flow rate whereas; streamers can drastically decrease the flow rate in a very short period [31].

Rusconi et al. [36] reported streamer formation in the microfluidic channel under laminar flow conditions. They observed formation of a single streamer in the middle of the channel connecting the inner corners of the channel. They also claimed that secondary flows in the curved edge of the channel were responsible for the location of the streamer, which was located at the mid-plane. They further investigated the streamer formation behavior by changing the radius of the curvature of a zigzag microchannel and discovered that streamer formation depends on the geometric angle of microchannel [37].

Valiei et al. [6] observed streamers through the height of the channel with 50 × 8 array of micro-pillars and mentioned it as a 'web' of the streamers. They claimed that flow rate has a significant impact on the number of streamer formation. A higher number of streamer formations was reported in the middle of channel height. **Figure 2** shows the formation of bacterial streamers in a microfluidic device with an array of micropillars. The white arrow indicates the
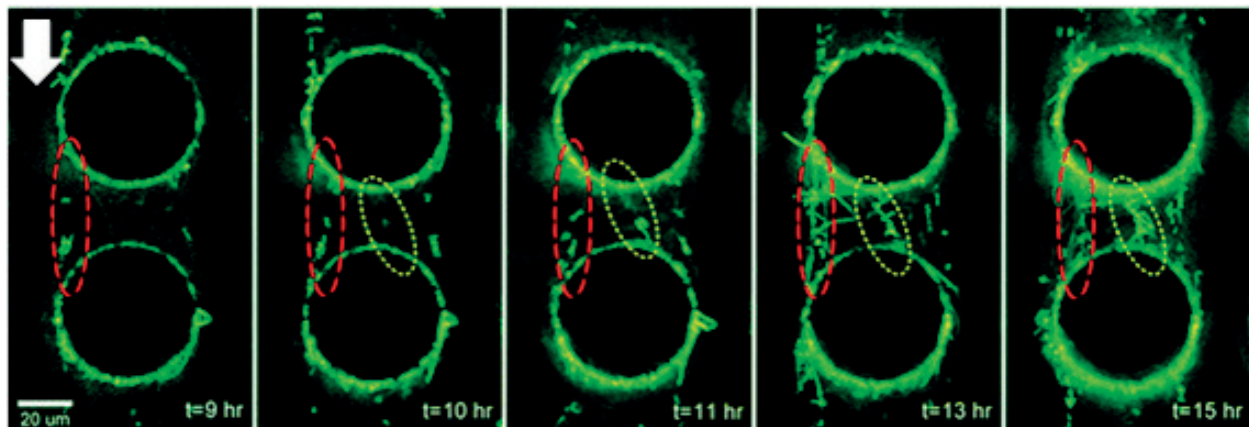
Figure 2. Streamer formation in a microfluidic channel. Figures are reproduced with permission from Ref [6].

flow direction, and the red and yellow ellipses show the formed streamers attached between two pillars. As can be seen, the thickness of the streamers increased with the increase of time of the streamer. Fluorescence microscopy was used to capture the image where only bacteria cells are visible (green). The fluid media and the EPS appear dark.

Marty et al. [33, 34] studied the effect of different pore sizes and filtration modes on the lengths of streamers that formed in a microfluidic membrane mimic system. They fabricated a microfluidic device with 25 straight, interconnected and staggered PDMS pillars to observe the nature of biofouling in a membrane mimic. The width and height of pillars were 10 and 50 μm respectively, and the mimic membrane pore size was 10 μm. They found that flow configuration and presence of tortuosity in a microchannel has a significant impact on streamer formation.

3. Basic overview of fabrication techniques

3.1. Membrane fabrication

Membrane process is an emerging separation technology. The membrane itself is the heart of a membrane process. It can be classified as polymeric and inorganic, porous and dense, isotropic and anisotropic, hydrophilic and hydrophobic, etc. **Figure 3** gives an overview of types and preparation process of the polymeric membranes. Phase inversion (phase separation) and track etching are the most widely used techniques for the preparation of porous membranes [38].

In phase inversion process method, the polymer is transformed in a controlled manner from liquid to solid state by changing the thermodynamic state of the polymer, solvent and the solution [38, 39]. Symmetric porous phase inversion membranes are made by using water vapor as the coagulant. For making asymmetric membranes by phase inversion temperature increase and a liquid nonsolvent is used to precipitate the polymer (**Figure 3**). In track etching method, a high energy particle radiation is applied to the polymeric film, to damage the polymeric matrix and create tracks. By etching the polymeric material along the track uniform cylindrical
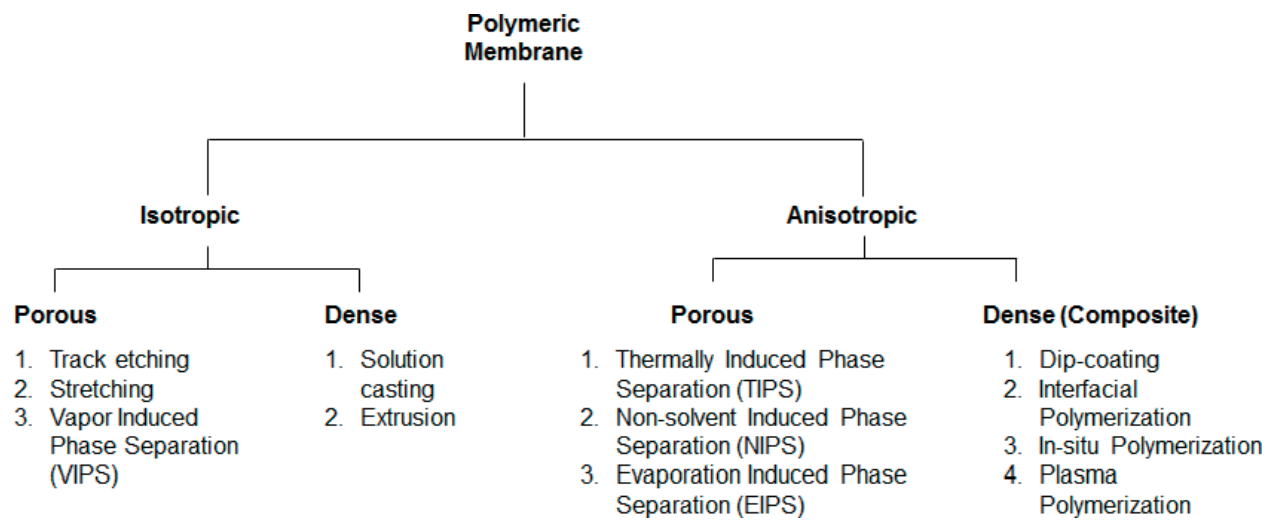
Figure 3. Preparation methods of polymeric membrane.

pores can be obtained. Dense membranes (symmetric and asymmetric) are mainly synthesized by solution casting and interfacial polymerization of two monomers on a substrate. A detailed explanation of membrane preparation techniques is available in the literature [38].

Membrane process	Polymer used in the fabrication process	Fabrication technique	Pore size	Pressure range (bar)	Flux range (l.m^{-2}.h^{-1}.bar^{-1})	Application
Microfiltration (MF)	Polyvinylidene fluoride (PVDF), poly (tetraflurethylene) (PTFE), polypropylene (PP), Polyethylene (PE), polyethersulfone (PES)	Phase inversion, stretching track etching	Porous 10^{-1}–10 μm	0.1–2.0	>50	Separation of macromolecular to cellular size particles (Bacteria/ fat and some proteins)
Ultrafiltration (UF)	Polyacrylonitrile (PAN), polysulfone (PS), poly (phthazine ether sulfone ketone) (PPESK), poly (vinyl butyral) PVDF PES	Phase inversion, solution wet-spinning	Porous 10^{-2}–10^{-1}μm	1.0–5.0	10–50	Separation of molecular to macromolecular size particles (all proteins)
Nanofiltration (NF)	Polyamides, polysulfones, polyols, polyphenols	Interfacial polymerization, layer-by-layer deposition Phase inversion	Porous 10^{-3}–10^{-2}μm	5.0–20	1.4–12	Separation of Ionic molecular size particles (Lactose)
Reverse osmosis (RO)	Cellulose acetate/ triacetate aromatic polyamide, polypiperzine, polybenziimidazoline	Phase inversion Solution casting	Dense/ Porous 10^{-4}–10^{-3}μm	10–100	0.05–1.4	Separation of ions (all minerals)

Table 1. Summary of different types of pressure-driven membrane processes [38–41].
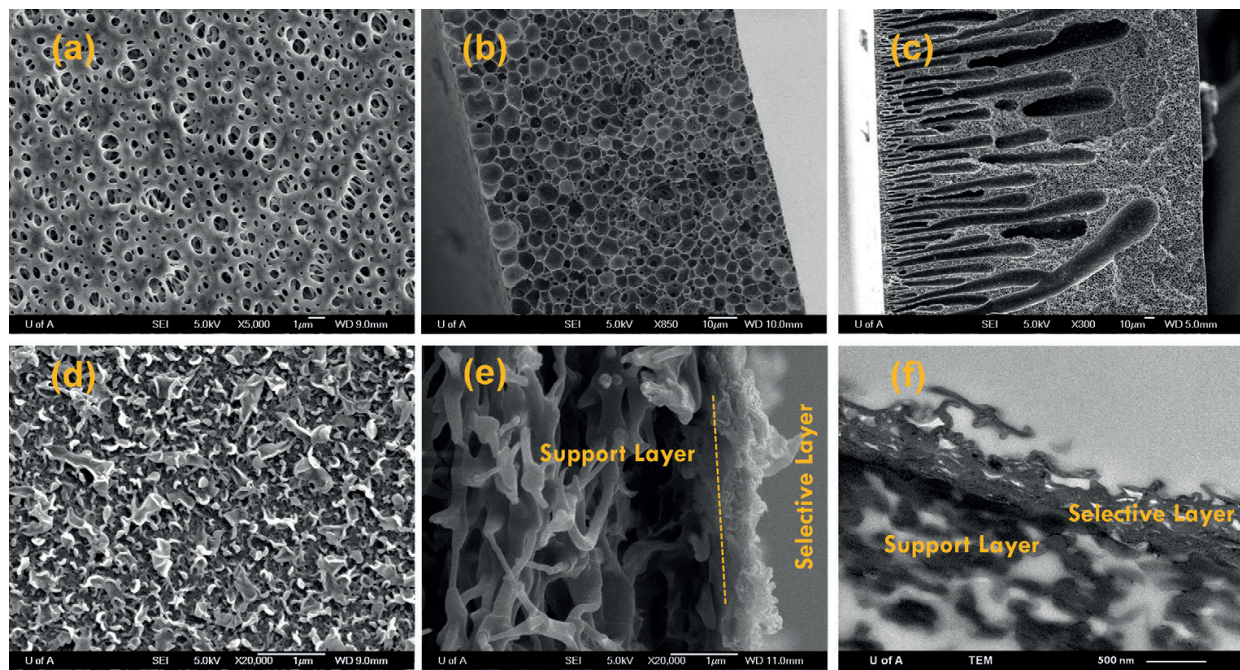
Figure 4. (a) Surface SEM image of (a) a phase inversion porous membrane, (b) cross-sectional SEM image of an isotropic phase inversion membrane, (c) cross-sectional SEM image of an anisotropic phase inversion membrane, (d) surface SEM image of thin film composite (TFC) dense membranes, (e) cross-sectional SEM image of a TFC membrane, and (f) cross-sectional SEM image of a TFC membrane.

A summary of different types of pressure-driven membrane processes with their fabrication technique, separation principle, pore morphology, pressure and flux ranges are given in the **Table 1**. Scanning electron microscopy (SEM) images of different types of membranes are presented in **Figure 4**.

3.2. Microfluidic device fabrication

There are many types of fabrication techniques available for making micro/nano devices such as photolithography, etching, soft lithography, hot embossing, injection molding, E-beam lithography, and micro-stereolithography. Photolithography and etching are two popular fabrication techniques. Soft lithography is a well-known method for microfabrication. McDonald et al. [42] fabricated microfluidic system with PDMS by a soft lithography technique to make 20–100 μm microfluidic structure. This technique has also worked well on hydrogel polymers (calcium alginate) to fabricate microfluidic network of 100 μm wide and 200 μm deep and 25 × 25 μm cross-section [43]. A complex structure with feature sizes larger than 20 μm can be achieved by using rapid prototyping [44]. The fabrication of 500–2000 μm diameters and 200–1000 μm height cylindrical columns [45] is possible by hot embossing technique. A schematic diagram of a microfluidic device is shown in **Figure 5**. This device is used to observe the biofilm behavior and the change of hydrodynamics of the fluid flow through the channel [6]. The chip has one inlet and one outlet and is made by traditional photolithography using polydimethylsiloxane (PDMS).
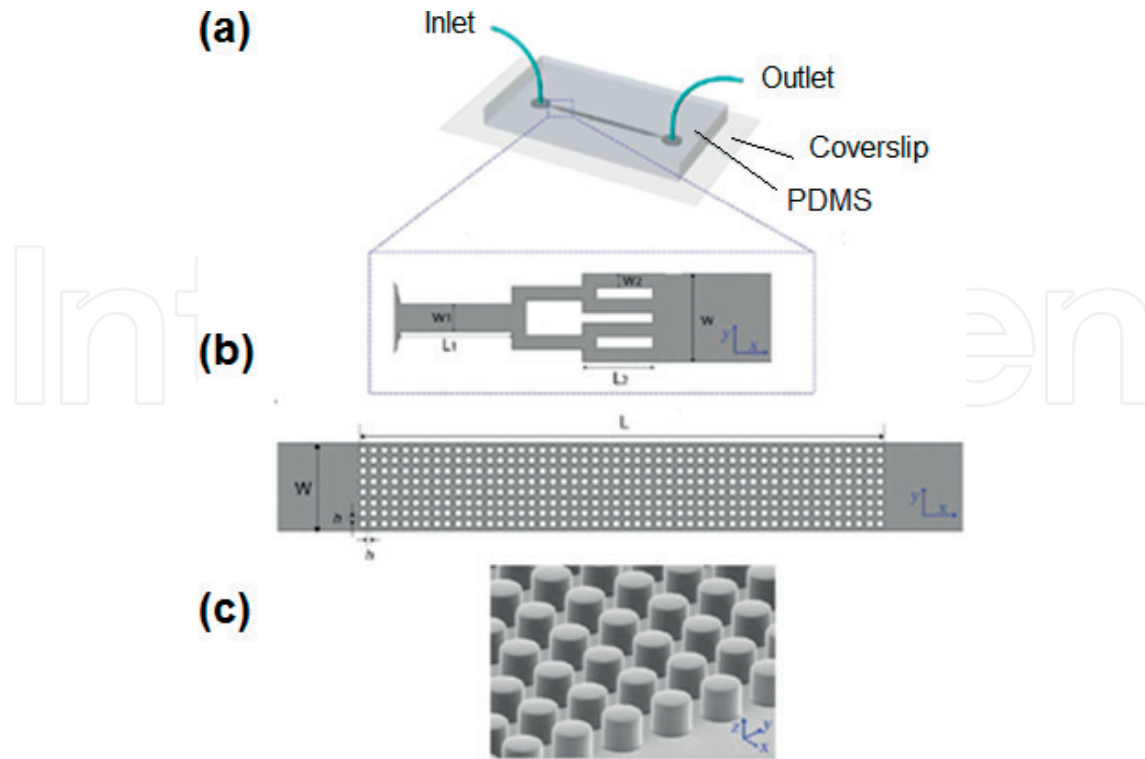
Figure 5. (a) Schematic of an microfluidic chip, (b) top view of the micropillar array, and (c) SEM image of micropillars of the equal diameter of 50 μm. Figures are taken with permission from Ref. [6].

4. Membranes in microfluidic devices

4.1. Direct incorporation of membranes into microfluidic devices

The commercial membrane can be incorporated into the microfluidic devices directly. The membrane can be fabricated as per the requirement by following the traditional membrane fabrication techniques described above and then bonded to the microfluidic chip. Russo et al. [46] directly incorporated polymeric membrane into silicon-based lab-on-chip device. Silicon substrate coated with a thin nitride film was used to serve as a support structure for the track-etched membrane. Patterning was conducted by UV exposure through chrome glass mask and CF_4 reactive ion etching to transfer the pattern to the nitride layer. The process was repeated on the other side of the wafer by using the second mask with pores on it. The membrane was finally incorporated into the PDMS device.

The membrane can be placed between two microfluidic chips and make a sandwiched structure. This is also another way of using a membrane directly in the microfluidic devices. By using this technique, a three-dimensional microfluidic network was designed by Ismagilov et al. [47] to investigate the interactions of chemical and biochemical reagents. They used a polycarbonate membrane between two PDMS microfluidic devices to make the sandwiched structure.

Membrane integrated with microfluidic device plays an essential role in the medical application [48–51]. To study the complex phenomena inside the vascular system different types

of membrane with the microfluidic devices are used. A microfluidic device was fabricated by sandwiching polyester membrane between microfluidic chips and used to study the interaction of cancer cells with a vascular endothelium and to prevent the metastatic disease [49]. A membrane with microfluidic device was also used to demonstrate the lungs injury [50] by toxic substances [51]. Huh et al. made a microfluidic airway system with an approximate diameter of respiratory bronchioles (narrowest airways of the lungs) to explore the cellular-level lung injury [50]. To make a sandwich structure of a membrane in a microfluidic device, bonding of the membrane and the device is a critical issue to deal with the leakage. PDMS mortar film, which is made by mixing PDMS and toluene, can be used to effectively make the bond [52, 53]. Young et al. [52] fabricated such kind of devices to measure the biomolecule permeability across the porous membrane. PDMS prepolymer was cured, and 3 mm diameter holes were punched through the cured PDMS. PDMS mortar layer was then generated on a glass support, and the PDMS substrate was placed on the support so that the holes were not in contact with the mortar layer. On the other side, the membrane was pressed down into the mortar layer. Finally, the membrane was placed between two substrates and bonded with PDMS mortar layer. Using an additional PDMS separator with the membrane can be another way to prevent the leakage [54].

4.2. Membrane fabrication as a part of the microfluidic device fabrication

The membrane can be fabricated as a part of a microfabrication process instead of using the traditional membrane fabrication technique. Karnik et al. fabricated a composite membrane of copper, aluminum, spin-on-glass (SOG), and palladium for the water gas shift reaction experiment [55]. Silicon nitride was deposited on both sides of silicon wafers by chemical vapor deposition process. A thin layer of aluminum acted as an adhesive layer of the palladium. Photolithography and wet etching were used to pattern holes on the copper-aluminum layer and to obtain a microchannel. Ookawara et al. [56] fabricated a microchannel as a microseparator for oil and water separation. They made 10 mm curved radius and 112 μm width slits on 80 μm thick SUB308 plates by photolithography. A stack was made by putting the plates with and without slits in turn and diffusion bonded to make microchannel feature. Heyderman et al. [28] fabricated nanopore membrane chip by combining the techniques of hot embossing and photolithography. Silicon (Si) master mold with nanopore arrays was fabricated by using electron beam lithography, and the pores were replicated on PMMA by a hot embossing technique. Various etching processes were used to transfer the pores on Si_3N_4 to fabricate the final nanopore membrane. Though they used PMMA resist with chromium, Si, and silicon nitride (Si_3N_4), the final membrane they obtained was made of Si_3N_4. The pores diameter varied from 100 nm to 450 nm of Si_3N_4 nanopore membrane. To analyze and separate the biological cell Dong et al. fabricated micromachined separator with soft magnetic micropillar arrays that could act as a membrane to observe the performance of the cell separation [57]. A membrane with embedded channel was used to study the hydrodynamic behavior and the fouling formation on the membrane during filtration of synthetic wastewater made of polystyrene particle [58]. They used square-shaped silica capillaries to template the membrane. Polyvinylpyrrolidone (PVP) and N-methylpyrrolidone (NMP) were used as polymer and solvent for membrane preparation, respectively. The silica capillaries were glued to a glass plate and the polymer solution was cast on the glass plate at room temperature. The structured membrane was then kept in a vapor bath and tap water bath for coagulation and

phase separation. After making the final structured membrane the silica capillary is placed in the channel of the membrane. The membrane was then placed between two lamination sheets to seal the chip.

4.3. Microfluidic membrane mimic

The microfluidic membrane mimic can be defined as a part of the device with pillars or curvature or any designed structured and the tiny gap between the structures that serve as a porous membrane. The design of the microseparator can be changed to mimic the different pore sizes and shapes for the membrane study in a microfluidic device. For instance, Hassanpourfard et al. [59] designed and developed a detailed fabrication protocol for making microfluidic device that mimics the porous media to study the biofilm formation. Bacchin et al. [60] used different shapes of PDMS microseparator to ensure the uniform flow of the suspension over the width of the filtering part and to study the fouling. Derekx et al. [61] investigated the fouling behavior in a PDMS microfluidic mimic membrane by
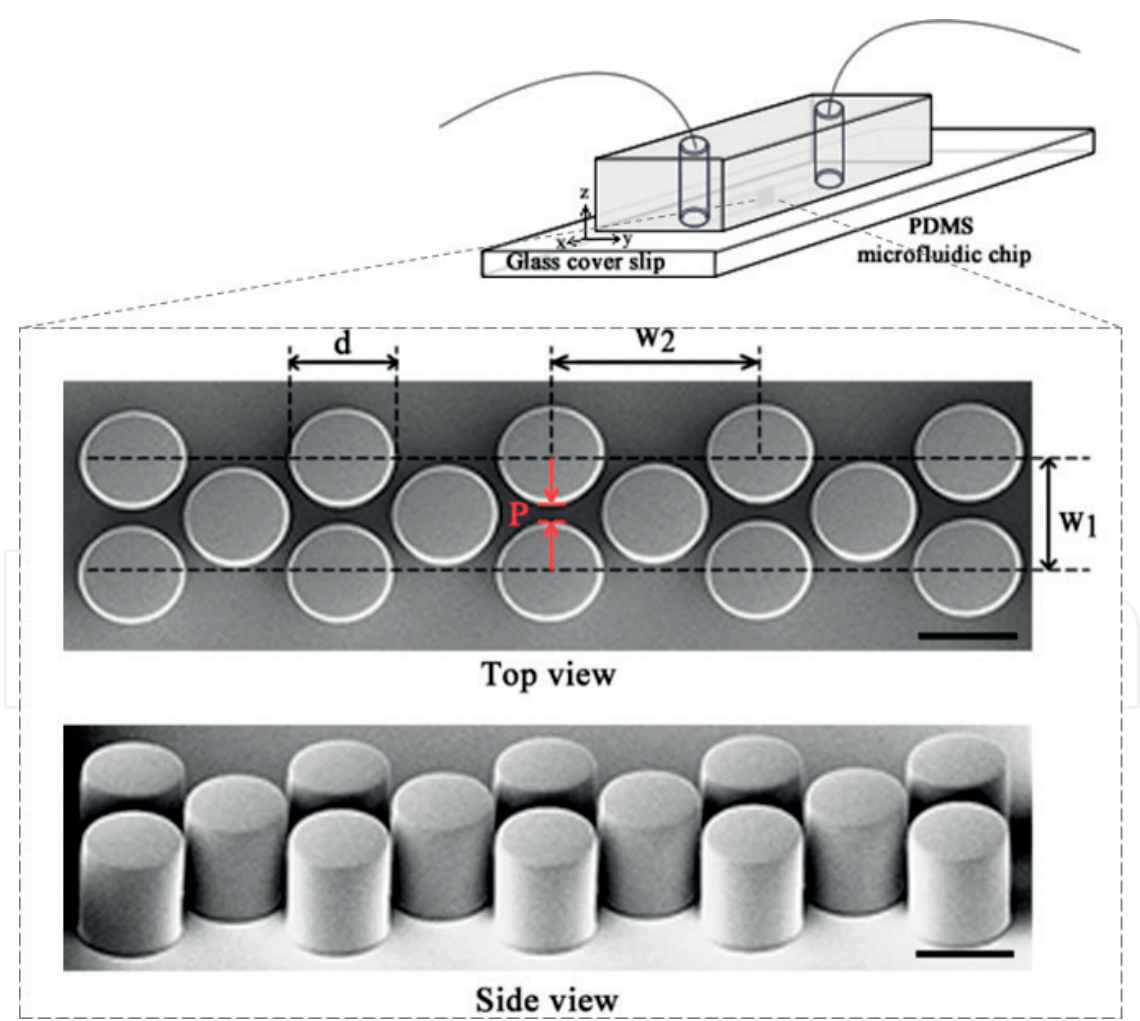

Figure 6. Schematic of microfluidic device with the mimic membrane structure [30]. The dimensions are d = 50 μm, w_1 = 60 μm, w_2 = 104 μm and P = 10 μm. The scale bar is 50 μm.

the experiment and computer simulation. The research on microfluidic membrane mimic has been mainly focused on fouling phenomena in porous media. For instance, Marty et al. [34] fabricated microfluidic devices with straight, interconnected and staggered channels to observe the biofouling nature in the microfluidic device due to biofilm. They studied the

Different materials and polymers used in the fabrication process	Membrane pore size	Fabrication technique of membrane	Incorporate membrane in microfluidic device	Different types of applications
Cellulose acetate [46]	MWCO: 350 Da [46]	Casting	Direct casting and sandwich the membrane in between the microfluidic devices	1. Biological analysis
Polyetherimide (PEI), Polyvinylpyrrolidone (PVP), and N-methylpyrrolidone (NMP) [58]	3–8 μm [58]			2. Investigate chemical or biochemical interaction
				3. Medical Application
				4. Fouling characterization
Polycarbonate [47, 53]	0.1–1 μm vertical pore 10 μm thick [47]	Commercial membrane		
Polyester [49, 50, 53]	400 nm [49, 50] 10 μm thick and 3 μm and 20 μm pore [53]			
Polyamide [54]	RO: MWCO: 200DA [54]			
Polyethylene terephthalate (PET) [48]	8 μm [48]	Track etching		
Cyclopore polycarbonate regular and thin clear, nuclepore polycarbonate [52]	1 μm [52]			
PDMS [51]	10 μm thick and 10 μm effective diameter [51]	Soft lithography		
Copper, aluminum and palladium [55]	60, 200 and 500 nm [55]	Composite membrane and MEMS fabrication	Membrane fabrication as a part of microfluidics device fabrication	1. Oil-water separator
				2. Magnetic micro separator
PMMA, Si3N4, Si, Si3N4 and Cr [28]*	Micro-slit, 112 μm [28]	Hot embossing & photolithography		3. Fouling analysis
				4. Biofouling study
SUS304 Plate[56]	500, 330, 140 nm [56]			
PDMS [33, 34, 60]	width: 10 μm, Length: 200 μm or 170 μm and Depth: 50 μm [33, 34] Constriction: 20 μm smallest width: 50 μm [60]	Soft lithography		
Cellulose ester [61]	5 μm [61]	Commercial membrane		

Table 2. Summary of different types of microfluidic membrane device fabrication.

effect of different pore sizes and dead-end and pseudo cross-flow filtration modes on the biofouling during filtration. In subsequent work, they also reported that pore tortuosity and secondary flows have a significant impact on biofouling formation in the mimic system [33]. In the pseudo filtration mode, they did not work on the effect of pressure difference on the biofouling formation during filtration.

Biswas et al. [30] designed a microfluidic membrane mimic by using photolithography technique to investigate the biofouling under different flow condition. The minimum pore size considered was 10 μm and the micropillars were distributed in a staggered pattern. **Figure 6** shows the schematic of their microfluidic device with the mimic membrane structure [30]. Transparent PDMS microsystem is used to mimic the membrane to study the bacteria transfer in the porous interface. The diameter and depth (in z-direction) of the micropillars are 50 μm. Their primary focus was to study the deformation mechanism of bacterial streamer that occur at the downstream location of the membrane during filtration process. They did not focus on the effect of pressure on the biofouling formation. **Table 2** shows a summary of different microfluidic membrane fabrication techniques with pore information and their application.

5. Conclusion

Membrane processes have been widely used in various industries for water and gas treatment. Pressure-driven membrane processes for water treatment are typically categorized by their rejection ability into MF, UF, NF, and RO. Biofouling on the membrane surface is the most severe fouling among all fouling phenomena including colloidal fouling, scaling, and organic material fouling. The dynamic behavior and viscoelastic nature of biofouling make it more complicated. Hence, it is very important to observe the real-time phenomenon that is occurring during biofouling. Microfluidic devices have therefore become essential tools to study the biological growth in a flow regime. Integrating membranes with microfluidic devices has become very popular over the past decade. There are several ways to incorporate membrane into the microfluidic device. The commercial membrane can be bonded to the device directly, or the membrane can be fabricated as a part of a fabrication process. Microfluidic devices equipped with membranes have been widely used in the medical application to study the complex permeability of macromolecular, drug or other protein. Such devices have recently used to study the fouling phenomenon in porous media. In this chapter, a thorough literature review was also provided about the microfluidic membrane filtration for biofouling study.

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