



Contents lists available at ScienceDirect

Journal of Materials Science & Technology

journal homepage: www.jmst.org

On-Chip Fabrication of Carbon Nanoparticle–Chitosan Composite Membrane

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ARTICLE INFO

Article history:

Received 28 January 2015

Received in revised form

17 March 2015

Accepted 20 March 2015

Available online

Key words:

On-chip fabrication

Composite membrane

Carbon nanoparticle

Chitosan

Interfacial deprotonation

Mixed matrix membrane

The on-chip fabrication of a carbon nanoparticle–chitosan composite membrane (i.e. a sorbent membrane or a mixed matrix membrane) using laminar flow-based interfacial deprotonation technology was presented in this paper. In addition, the effects of carbon nanoparticles and reactant flow rates on membrane formation were investigated. Finally, the permeability and adsorption capacities of the membrane were discussed. During fabrication, an acidic chitosan solution and a basic buffer solution that contained carbon nanoparticles were introduced into a microchannel. At the flow interface, a freestanding composite membrane with embedded carbon nanoparticles was formed due to the deprotonation of the chitosan molecules. The membrane growth gradually stopped with time from upstream to downstream and the thickness of the membrane increased rapidly and then slowly along the reactant flow direction. The formation of the membrane was divided into two stages. The average growth rate in the first stage was significantly larger than the average growth rate in the second stage. Carbon nanoparticles in the basic solution acted as nucleating agents and made the membrane formation much easier. As the flow rate of the chitosan solution increased, the averaged membrane thickness and the membrane hydraulic permeability initially increased and then decreased. Because of the addition of carbon nanoparticles, the formed membrane had adsorption abilities. The carbon nanoparticle–chitosan composite membrane that was fabricated in this study could be employed for simultaneous adsorption and dialysis in microdevices in the future.

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1. Introduction

The rapid development of microfabrication and microfluidic techniques in the last two decades has not only facilitated improvements in biological and chemical analysis devices^[1–4] but also made it possible to miniaturize therapeutic devices, such as artificial livers^[5], kidneys^[6], hearts^[7], and lungs^[8] (or to develop functional tissues and organs on chips^[9]). In artificial liver and kidney devices, the membrane is a core to remove toxins from the patient's blood. Consequently, the key to miniaturization of these medical devices is the integration of high performance membranes into microdevices.

Several approaches are useful for membrane integration. The most straightforward method is the direct incorporation of a membrane into a microfluidic device^[10–16]. Although this method offers a wide variety of membrane choices, it has several limitations, including inefficient sealing between layers, which can cause leaks, and chemical compatibility issues^[17,18]. The preparation of membranes during the microchip fabrication process is also a good method for membrane integration when based on techniques such as chemical etching and thin metallic film deposition^[19–21]. The main drawbacks of this method are the complexity of the microfabrication process and the high cost^[18]. In addition to the sandwich-type integration and the direct membrane preparation in the chip fabrication process, the *in situ* method is receiving greater interest. In this method, the membrane can be prepared by using emulsion photopolymerization^[17,22,23], laser-induced phase separation polymerization^[24,25], and laminar flow-based interfacial polymerization techniques^[26–29]. Among these techniques, the first two allow researchers to form membranes with desired

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thicknesses and at a desired location. The later technique offers an attractive way to obtain new functionalities. However, the main disadvantage of these techniques is the limited choice of materials^[18].

In the last few years, although the integration of membranes into microfluidic devices has achieved a great success in different fields, such as concentration^[23,30], filtration^[15,31], extraction^[18], and microdialysis^[12,16,25], many efforts should be devoted to artificial liver and kidney microdevices. For these devices, the first important issue that should be considered is the biocompatibility of the membranes, which refers to the choice of the membrane materials. In the literature, most membranes that are integrated in microdevices are made of non-biomaterials that could be toxic in subsequent biological applications^[26]. Although the chemical modification of these membranes can improve the biocompatibility to some extent, it makes the microfabrication process more complicated^[18]. Recently, the use of a promising biomaterial, chitosan, was proposed for *in situ* microfabrication of porous membranes, which greatly expanded the function and application of membranes in microfluidic devices^[26,32]. The second most important issue is the area of the membranes. In biological and chemical analysis fields, to precisely control and manipulate fluids at the micro, nano and pico liter scales, the area of the integrated membranes is often small. However, these membranes are far from reaching the requirements for application in therapeutic devices. To solve this problem, membranes with adsorption properties may be fabricated on chips to reduce the membrane usage. In the current blood purification systems, adsorption technology has been applied to replace membrane technology partially or completely. The use of adsorption technology not only contributes to the increase in the detoxification efficiency but also removes some toxins that cannot be removed by dialysis. More importantly, adsorption technology may reduce the use of dialysate and membranes, which would make the treatment device portable^[33,34].

There are two main methods for making adsorptive membranes on microchips. One is to chemically modify membranes^[28]. However, this method may cause biological and chemical compatibility issues. In some cases, one could use the adsorption ability of the membranes themselves; however, this type of on-chip membrane has rarely been reported. The second method is to embed adsorbents into the membranes during the microfabrication process. In the late 1970s, sorbent membranes were developed for heavy blood purification systems^[35,36]; however, due to manufacturing difficulties and a lack of high purity adsorbents, these membranes were not widely used^[37,38]. Recently, sorbent membranes have attracted attention again with the development of science and technology^[39-42]. This approach can be used to make versatile membranes by embedding various specific or non-specific adsorbents. However, to the best of our knowledge, no such sorbent membranes integrated into microdevices have been reported.

Therefore, in this study, we report for the first time the on-chip fabrication of a carbon nanoparticle–chitosan composite membrane (i.e., a sorbent membrane or a mixed matrix membrane), study the membrane formation under the effects of carbon nanoparticles and reactant flow rates, and discuss the feasibility of using composite membranes for dialysis and adsorption. Compared to the previous work in the literature^[26], this study is significant because it aims to embed carbon nanoparticles into chitosan to create an on-chip composite membrane that is able to perform adsorption and dialysis dual functions. More importantly, we propose an approach for exploiting membranes for versatile uses and for application in microfluidic chips. It should be noted that the approach developed here is significantly different from the traditional method used by Huang et al.^[43] because the traditional method is suitable only for the macroscale membrane formation, not for the microscale membrane formation. This study is significant for the development of artificial liver and kidney microdevices.

2. Materials and Methods

2.1. Materials

Chitosan (average molecular weight 600,000 g/mol, powder), creatinine (white crystal, ≥99.0%), sodium chloride (NaCl, colorless crystal, ≥99.5%), potassium chloride (KCl, white powder, ≥99.5%), sodium hydroxide (NaOH, tablets, ≥96.0%), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O, colorless crystal, ≥99.0%), potassium dihydrogen phosphate (KH₂PO₄, colorless crystal, ≥99.5%), sodium dodecylbenzene sulfonate (NaDDBS), and hydrochloric acid (HCl, 36%–38%, solution) were obtained from the Sinopharm Chemical Reagent Co. Ltd, Shanghai, China. Syringes (1, 5, 50 mL) were obtained from the Kaile Infusion Sets Factory (Shanghai, China). Carbon nanoparticles (30 nm, 99.5%), fluorescein (MW 332.31, 90%, λ_{ex} = 490 nm, λ_{em} = 514 nm), and fluorescent quantum dots (8 ± 5 nm diameter, λ_{em} = 585 nm, 0.05 μmol/L) were purchased from Aladdin Industrial Corporation (Shanghai, China). The polydimethylsiloxane (PDMS) Kits (Sylgard® 184 silicone elastomer base and curing agent) were purchased from Dow Corning, Midland (Michigan, USA). The creatinine reagent (OSR6178) was obtained from Beckman Coulter Inc. (California, USA). The fine bore polythene tubing (0.38 mm ID/1.09 mm OD) was obtained from Smiths Medical International Ltd (Kent, UK). Syringe pumps (NE-1000) were purchased from New Era Pump System Inc. (Farmingdale, NY, USA) and the biopsy punch (BP-10F, 1.0 mm) was purchased from Kai Industries Co. Ltd (Seki, Japan).

2.2. Microchip fabrication

The PDMS chip was fabricated according to the standard rapid prototyping protocol as previously reported^[44]. Briefly, the master composed of SU-8 2050 photoresist (Microchem, Newton, MA, USA) was made using the photolithography technique. Then, a 10:1 mixture of elastomer base and curing agent was cast and cured over the master at 80 °C for 2 h to form the upper and lower PDMS layers. After 4 holes were punched on the upper PDMS layer, two layers were treated with oxygen plasma^[14] and irreversibly sealed. The X- or H-shaped microchannel (~500 μm wide, 5 mm long, ~50 μm high) was fabricated for membrane integration.

2.3. Reagent preparation

The 0.5% chitosan solution (w/v) (pH = 5) was prepared as follows^[26]: (1) the chitosan powder was dissolved in deionized water and HCl was added drop by drop to achieve a pH of approximately 3; (2) the chitosan solution was filtered after overnight mixing; and (3) a NaOH solution was added to the chitosan solution to adjust the pH to 5. The buffer solution (pH = 10) containing 0.02% carbon nanoparticles was prepared by adding a NaOH solution drop by drop to phosphate-buffered saline (PBS) (10 mmol/L phosphate buffer, 2.7 mmol/L KCl and 137 mmol/L NaCl, pH 7.4). Next, 0.028% of surfactant sodium dodecylbenzene sulfonate (NaDDBS) was added to help suspend the carbon nanoparticles^[45,46]. Then, carbon nanoparticles were added to the buffer solution by using the ultrasonic cleaner (KQ-50B, 50 W, Kunshan Ultrasonic Instrument Factory, Jiangsu, China) to treat the mixture for 60 min to ensure that the carbon nanoparticles were distributed evenly. Finally, the solution was filtered for use before the experiment to remove the large-grained carbon nanoparticles.

2.4. Membrane formation

In the experiments (Fig. 1), the chitosan and buffer solutions were pumped into the microchip from the two inlets, A and B, respectively. The flow ratio of the chitosan solution to the buffer solution

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