SELF-INTEGRATION OF ION TRANSPORT TUNABLE NANOPOROUS MICROPLUGS IN A MICROFLUIDIC CHIP FOR ELECTROKINETIC BIO-SAMPLE CONCENTRATION

Minseok Kim[1], Taesung Kim[1]

[1]*Ulsan National Institute of Science and Technology (UNIST), Republic of Korea*

ABSTRACT

We describe simple and robust methods that can be used for microfabricating nanoporous materials as leakage-tight membranes in a microfluidic channel network. The methods consist of a common self-integration process and individual solidification processes such as solvent evaporation, UV-curing, and temperature treatment. We demonstrate that the fabricated membranes can be used for electrokinetic, nanofluidic pre-concentration of bio-samples such as proteins, cells, and nanobeads on either the anodic or cathodic side of the membranes. In addition, we not only compare the physicochemical properties of the membranes but also characterize biocompatibility and robustness of the membranes. The methods are versatile for many nanoporous precursor materials, and it is easy to control the location and dimension of the membranes. Hence, the methods developed in this work and the characterized properties of the membranes could be widely employed for further applications of nanoporous membranes in microfluidic systems.

KEYWORDS

Microfluidics, nanoporous membranes, electrokinetics, pre-concentration

INTRODUCTION

Nanoporous membranes integrated in a microfluidic channel network allow a wide range of bio-/chemical applications such as generating stable concentration gradients, identifying human genomic sequences, amplifying initial sample concentrations, and filtering charged ions or non-target molecules in a controllable manner [1, 2]. To date, many attempts were made to microfabricate various nanoporous materials in the microfluidic device but the methods developed seem to have some drawbacks. In this work, we describe a simple but robust method to microfabricate various nanoporous materials as microplugs in a microfluidic channel network and demonstrate that the proposed method can be utilized to electrokinetic, nanofluidic concentration of bio-samples such as proteins, cells and nanobeads in both the anodic and the cathodic side of the microchannels.

EXPERIMENT

Fig. 1 illustrates the microfluidic device that consists of a shallow channel connecting two deep microfluidic channels. When a solution containing nanoporous materials approaches to the channel junction along the shallow channel, it keeps flowing only along the shallow channel and does not enter the deep microfluidic channels because of surface tension as depicted in Fig. 1(B) [3]. As result, it is possible to selectively fill the shallow channel with various solutions that can be solidified by using light, evaporation or heat. This fabrication method is simple because the microplugs are self-integrated, free from fluid leakage because an additional solution is provided to make gaps tightly sealed during the solidification process, and applicable for various nanoporous materials.

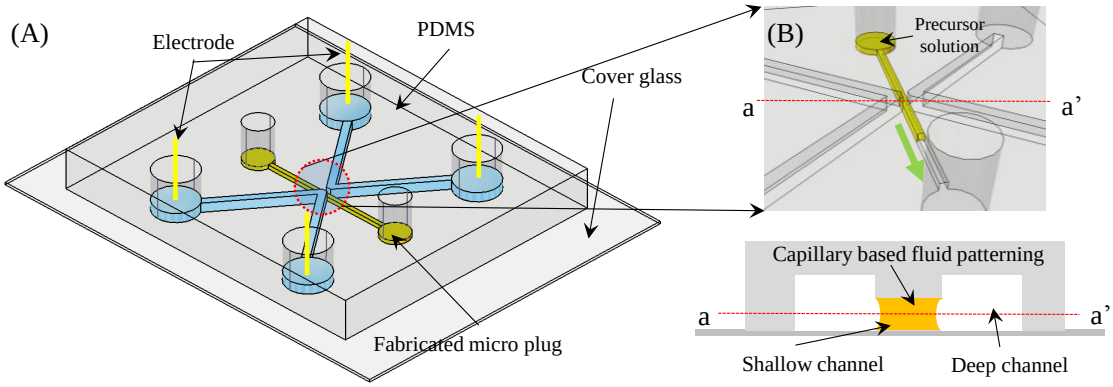

Figure 1. (A) Schematic representation of a microfluidic bio-sample concentrator that is integrated with nanoporous materials. (B) Capillary force-based filling of the precursor solution using a dual-depth microchannel network enables precise fabrication of the nanoporous materials in the microchannel.

In this work, for instance, we fabricated four different kinds of the nanoporous microplug in the microchannel in order to pre-concentrate bio-samples at a desired side of the microchannel: Nafion, cation selective poly-hydrocyethyl methacrylate (HEMA), anion selective HEMA, and agarose gel. As shown in Fig. 2, each nanoporous material has different solidification mechanism and different physical and electrokinetic properties that are well summarized in Table 1. First, the Nafion precursor solution was introduced along the shallow channel (Fig.

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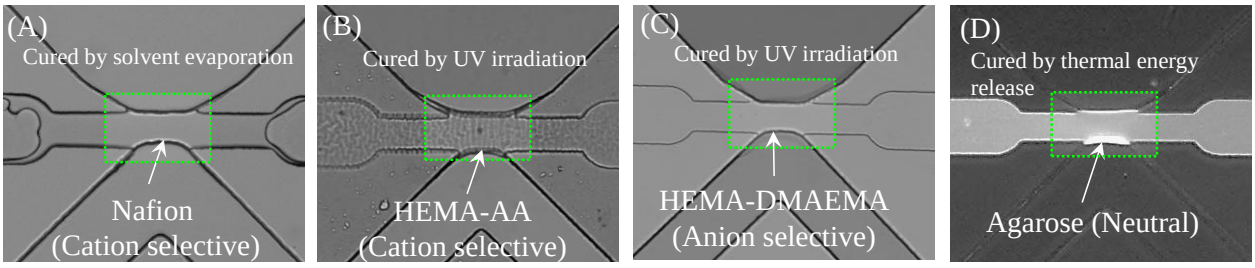

Figure 2. Microfabricated nanoporous microplugs in the shallow channel between two deep microfluidic channels. (A), (B), (C), and (D) show solidified Nafion, HEMA-AA, HEMA-DMAEMA, and agarose, respectively.

2(A)) and then solidified by evaporating the solvents in the precursor solution on 95 ℃ hotplate for 10 min. Since the Nafion solution contacted air on the deep microchannel sides, the evaporation of the solvents initiated at the center of the junction and caused volume contraction of the solution during the solidification process. However, the contraction volume was replenished with extra solution in the shallow channel, resulting in the fabrication of a tight-sealed and robust Nafion membrane that causes no flow leakage during the electrokinetic pre-concentration of biomolecules. Second, poly-HEMA membranes were fabricated in the similar manner as the Nafion (Fig. 2 (B) and (C)). For a cation selective transport membrane (CSTM), the poly-HEMA was mixed with acrylic acid (AA) while for an anion selective transport membrane (ASTM) it was done with 2-(dimethylamino)-ethyl methacrylate (DMAEMA); the detailed mixing ratio and concentration are listed in Table 1. Lastly, agarose hydrogel (1.5 wt. %) was employed to form a hydrogel membrane that has relatively large pore sizes but no permselectivity. An agarose solution that was stored in a water bath at a constant temperature (65 ℃) was introduced to the microfluidic device on a 55 ℃ hotplate so that the agarose solution was kept in a fluidic state for the entire period of the filling process. As soon as the device was removed from the hotplate to a work bench at room temperature (24 ℃), the agarose solution was cooled down and then turned into a solidified hydrogel without any fluid leakage across the deep microchannels. We summarized physicochemical properties of the NPMs in Table 1.

	Nafion	**HEMA-AA (CSP)**	**HEMA-DMAEMA (ASP)**	**Agarose**
Composition	5% Nafion solution	HEMA(5), AA(1),	HEMA (5) DMAEMA (1)	Agarose 1%
Surface charge property	Negative charge	Negative charge	Positive charge	Neutral
Nanopore size	2~5 nm	2~5 nm	2~5 nm	100 nm
Solidification method	Solvent evaporation	Photo curing	Photo curing	Temperature based curing
ICP ability	++	+	–	–
Durability/Stability	++	–	––	–
Tuning/Pre-treatment	–	++	++	++

Table 1. Characterization of electrokinetic and physical properties of the nanoporous materials.

As an application of the nanopores, we generated ion concentration polarization (ICP) phenomena to concentrate a protein using perm-selective nanopores such as Nafion and HEMA (Fig. 3). As the ICP (ion depletion) is induced in the anodic channel (left), a local electric field near the CSTMs has a stiff gradient due to the local conductance change such that the analyte solution makes an electrokinetic balance; the net mobility of the analyte becomes zero. Therefore, the negatively charged biomolecules are continuously delivered by the bulk electroosmosis flow (EOF) from the sample reservoirs and accumulated near the ion depletion region. The quantitative result in Fig. 3(C) shows that Nafion is better than other materials in terms of depletion areas (ICP strength). The self-integration methods for the NPMs in the microfluidic channel can be used a standardized microfluidic platform that can characterize and compare the electrokinetic properties of various membrane materials because it is possible to fabricate membranes with a same dimension. In order to expand on electrokinetic properties of the NPMs, we measured ion depletion areas that seem to be directly proportional to the cation transport flux through the membrane, which can be a good quantitative index to relatively compare the pre-concentration rate of the NPMs. As shown in Fig. 3(B), the Nafion membrane shows the largest depletion area, followed by the HEMA-AA while both the HEMA-DMAEMA and the agarose hydrogel membrane could not produce ion depletion areas under the same buffer concentration and electric field strength.

In addition to perm-selective membranes, we fabricated non-perm-selective nanoporous membranes in the microchannel by using agarose hydrogel and used them to concentrate functionalized nanobeads and bacterial cells in the anodic side of the channel. Although the HEMA-DAMEMA membrane possesses a clogging and degradation problem with negatively charged analytes, the agarose hydrogel membrane seems very useful to concentrate

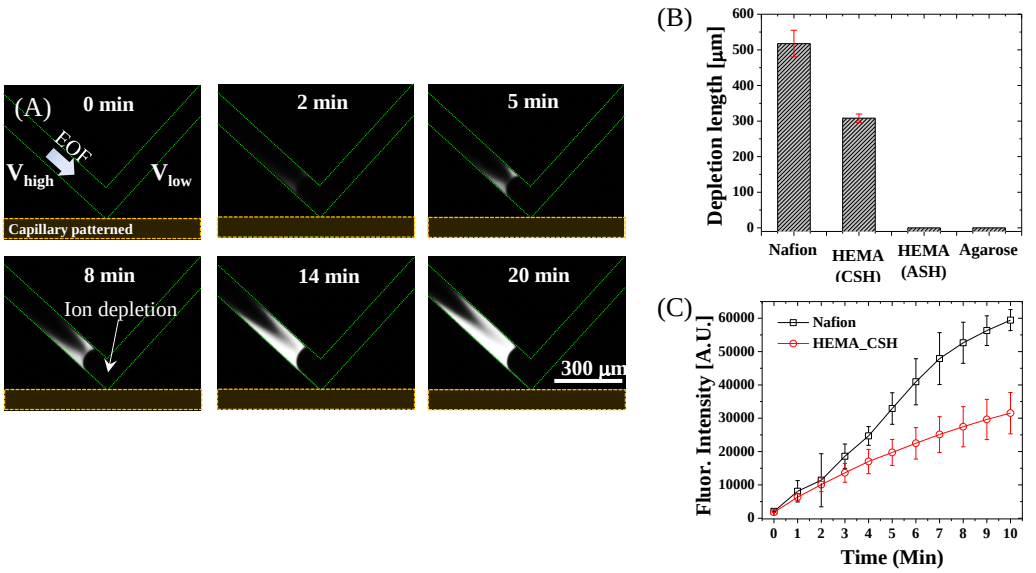

Figure 3. ICP based protein concentration and comparison of electrokinetic ion depletion areas. (A) ICP phenomenon produced in cathodic side of the permselective Nafion microplug enables concentration of proteins. (B) and (C) show comparisons of ion depletion areas (ICP strength) and protein concentration rates of various nanoporous materials

bio-samples at the cathodic side because electrophoresis based pre-concentration of negatively charged bio-samples is possible as illustrated in Fig 4(A) and (B). That is, since both EOF and PDF are not allowed to penetrate through the agarose hydrogel membrane, the net mobility remains only electrophoresis.

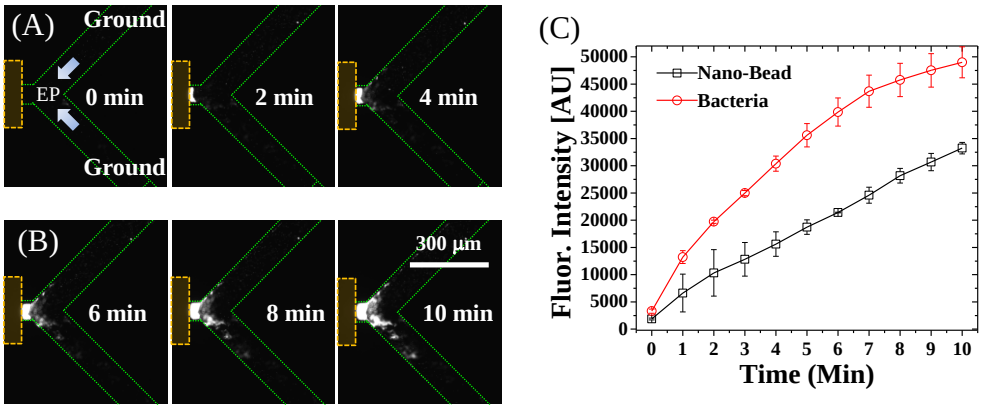

Figure 4. Electrophoresis based concentration of bio-samples. (A) and (B) show concentration of nanobeads and bacteria cells in anodic side using a non-permselective microplug (agarose). (C) Quantitative results of the time-lapse microscopic images in (A) and (B).

In summary, we developed a simple and novel method to fabricate microplugs/membranes in the microfluidic device using ion transport-tunable nanoporous materials and demonstrated that electrokinetic properties of the microplugs are very useful for pre-concentrating bio-samples at a desired location. Hence, it is believe that the method and characterized electrokinetic properties will be very useful in many micro total analysis systems.

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CONTACT

Taesung Kim +82-52-217-2313 or tskim@unist.ac.kr