NANOELECTROKINETIC RADIAL PRECONCENTRATOR/EXTRACTOR BASED ON ION CONCENTRATION POLARIZATION

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ABSTRACT

This paper reported the experimental demonstration of a radial preconcentrator/extractor in a micro/nanofluidic platform based on ion concentration polarization (ICP) phenomena. While various biomolecular preconcentration mechanisms have been developed for BioMEMS applications, an integration with downstream analyzer or an efficient recovery of concentrated sample still remain challenging. Here we designed a simple radial device without complex channel network and one can complete sample preparation steps from the preconcentration of samples to the recovery of concentrated samples with this single platform. Various charged species such as fluorescent dye, dsDNA and polystyrene microparticle are exemplified for the process (preconcentration factor ~ 10 and throughput ~ 1 μL/min). This simple device configuration would serve a key component for point-of-care-test applications and biomedical analysis tools.

INTRODUCTION

Preconcentration method based on ICP

Since a preconcentration is an essential step for sensing various analytes in bio- and environmental-applications, a number of mechanisms such as isotachophoresis [1] and field amplified stacking [2], etc. have been competitively reported using micro/nanofluidic platform. Among the advances, ICP mechanism has critical advantage of high preconcentration factor because target samples from infinite volume of reservoir would be stacked at specific locations [3]. ICP is the fundamental electrochemical process that describes the imbalance of electrolyte concentration near the nanoporous membrane attached to the microchannels under dc-bias [4]. Since an ion depletion zone forming at the anodic side in the case of cation-selective membrane mostly rejects the penetration of charged species, the combination of any force field across the zone would accelerate the stacking of sample at the zone boundary. While two microchannels connected with a nanojunction has been served a basic platform of the ICP preconcentrator, a simple straight microchannel with nanoporous membrane at the bottom of microchannel has reported to realize the ICP preconcentrator as well [5]. This simple device minimizes the unnecessary electrical connections while keeping similar preconcentration factor so that it would provide more commercializable platform.

For further development of preconcentration factor, we employed a radial type microchannel network to significantly enhance throughput and easy recovery of the preconcentrated sample. The multiple straight microchannels were radially connected to one reservoir located at the center as shown in Figure 1 (a). The circular patterned nanoporous membrane on the glass was assembled with a microchannel layer to generate the same ICP phenomenon. This type of ICP device was named as a radial preconcentrator.

Operating procedures

Step-by-step operation procedures of the radial preconcentrator were shown in Figure 1. First step is device and solution preparing. Microchannel was integrated on nanoporous membrane and inject electrode into each rims. A commercial pipette tip containing target molecules were inserted at the center of the radial preconcentrator (Figure 1 (b)). Second step is sample loading and concentration. Buffer solution without sample was injected into the microchannel by pressure in advance, and sample solution was filled in the pipette tip. A dc bias was applied to the radial electrode (ground) and the pipette tip (+voltage). Then depletion zones formed at each microchannels, operating as electrical barrier so that the samples in the pipette tip were confined in the center, while solvent flowed outward from pipette tip by electroosmotic flow. Note that any pressure field was excluded during the preconcentration step. (Figure 1 (c)) Third step is sample recovery. The pipette tip containing concentrated samples was easily recovered by pulling out the tip (Figure 1 (d)).

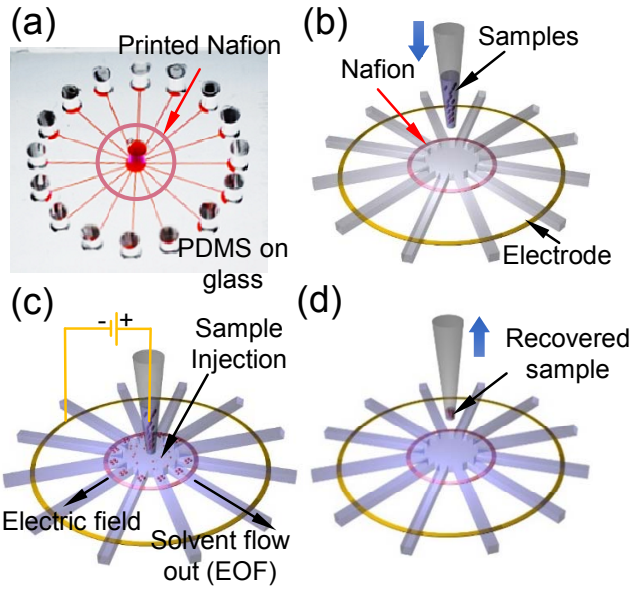

Figure 1. The optical image of the real device and the schematic procedure of the radial preconcentrator. (a) Fabricated 16 channel radial preconcentrator device. (b) Preparation step. (c) Preconcentration step. (d) Recovery step.

Materials and method

Fabrication

Figure 1 (a) showed the optical image of the fabricated radial concentrator device. Radially connected multiple microchannels were assembled in a single device for a massive throughput. Each straight channels had the dimension of 50 μm depth, 100 μm width and 7 mm length. The microchannel mold was loaded on the silicon wafer by photoresist fabrication method. To imprint the fabricated microchannel mold on the polydimethyl siloxane (PDMS, Sylgard 184 Silicon elastomer kit, Dow Corning, USA), a polymer solution, mixture of pre-polymer and curing agent at the raion of 10:1, was poured on the silicon wafer which had microchannel mold, and cured in an oven at 75□ for 4 hours. Nafion® resin solution (wt 20%, Sigma-Aldrich, USA) was used as a cation perm-selective nanoporous membrane. Nafion printing method was used to pattern atypical shape of nanoporous membrane on the glass. To adjust the viscosity between original EPSON ink and Nafion solution, Nafion was diluted by DI water at the ratio of 1:7 as shown in the Table 1. Then, 1:7 diluted Nafion with DI water was printed on the glass substrate by a conventional inkjet printer (K-100, EPSON, JAPAN). Finally, printed Nafion was heated on the hot plate at 95□ to remove solvent from Nafion solution, remaining only solid Nafion solute on the glass. Then, PDMS mold with microchannels was bonded with the Nafion patterned glass by O_2 plasma treatment (CUTE-MP, FemtoScience, Korea) and cured on the hot plate at 95□ for 2 hours.

TABLE1. Comparison of the viscosity between Nafion solution and original EPSON ink

Mixture ratio*	Nafion solution	EPSON ink	DI water
1:4	6.15	3.89	1
1:5	5.32		
1:7	3.26		
1:9	2.49		

* Mixture of Nafion and DI water

Experiment method

The mixture of 1mM KCl and fluorescence dye (Alexa 488, Invitrogen, USA) solution, 947 bp length of A260 dsDNA with 10 mM buffer, mixture of 1mM KCl and polystyrene 1 μm size particle were used to demonstrate the operation of radial preconcentrator. An electrical voltage was applied by source measure unit (Keithley 236, USA) through Ag/AgCl electrode. The behaviors of dye were traced by an inverted fluorescent microscope (IX-53, Olympus, Japan) and analyzed by ImageJ and CellSense.

Results and discussions

Fluorescence dye demonstration

Fluorescence dye was used to verify the basic performance of the device as shown in Figure 2. As electric field applied, the dyes in each microchannel were preconcentrated and plugs of dye were migrated toward the center, while only solvent in pipette tip was flushed toward the rim from each branch. The plugs completely reached to the center within 35 sec at 25 V and the concentration plug propagated with the average velocity of 41.5 μm/sec. The concentrated dyes in the pipette tip were able to be recovered simply by pulling out the tip. Assuming the loss of dyes in the pipette tip was negligible, the concentration ratio of dye was able to be calculated from the ratio of remained volume in pipette tip. The enhancement of fluorescent intensity enhancement was 7.5 fold from 15 μL of initial volume to 2 μL of final volume after 20 minutes operation (Figure 3 (a)). The operation time with 30 μL solution in 1 mM KCl buffer at 10 V bias depended on the number of branches are shown (Figure 3 (b)). The average concentration time of 4, 8, and 16 branched channel was 180, 120, and 90 minute, respectively. From the experiment results, concentration time (t) is proportional to the inverse of square root of number of branches (N).

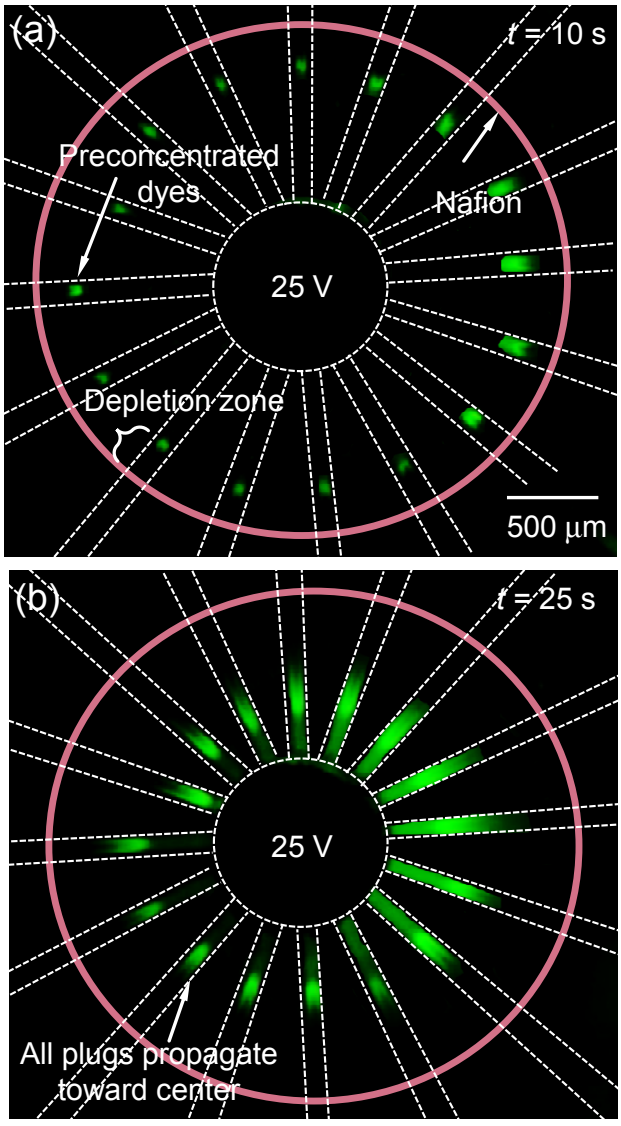

Figure 2. Fluorescence dye (Alexa 488) demonstration. (a) Formation of Preconcentrated dye plugs (b) Propagation of Preconcentrated dye plugs with continuous bias.

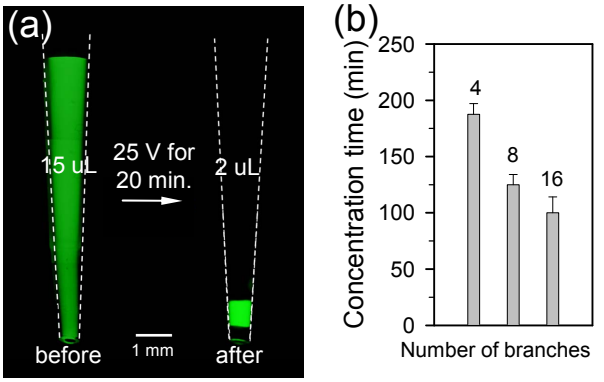

Figure 3. (a) Recovered dye in pipette tip after concentration. (b) Concentration time depending on the number of branches.

A260 dsDNA demonstration

The preconcentration of dsDNA (947bp) was also demonstrated as shown in Figure 4. The concentration of buffer solution was 10 mM and 10 V bias was continuously applied. Preconcentrated plugs of dsDNA was successfully converged to the center while solvent was flushed out from the center pipette tip. The amplification ratio would be ~10 fold within 1.5 hour.

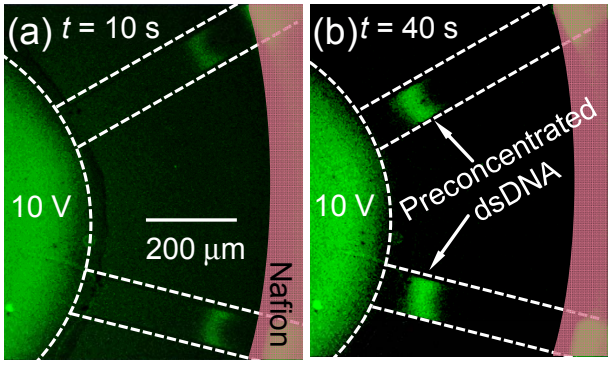

Figure 4. A260 dsDNA 947bp length demonstration. (a) Formation of Preconcentrated DNA plugs (b) Propagation of Preconcentrated DNA plugs with continuous bias.

Polystyrene microparticle demonstration

The preconcentration of polystyrene microparticle (1 µm size) was demonstrated by visualizing the fluid motion of microparticles. After applying 10 V bias to the center pipette tip, microparticle was preconcentrated to the anodic side of the Nafion while microparticle was also focused inside the pipette tip (Figure 5 (a)). Since a vortex motion of particles inside the depletion zone were also able to be observed, the shape of preconcentrated particles involved both compact plugs and vortical cloud, not like dsDNA cases. To verify the result of preconcentration step, comparison of the density distribution of microparticle between before and after preconcentration inside the pipette tip were shown (Figure 5(b)). Region of interest (ROI) was set to be 300 µm X 300 µm square and the density of particles inside the ROI was manually counted. According to this method, 5 particles/square were counted before preconcentration and about 57 particles/square were counted after preconcentration, which result in 11 fold of preconcentration factor, experimentally. In this experiment, 35 µL microparticle solution was concentrated to 3 µL solution, result in ~11.7 fold preconcentration factor, theoretically. From this result, we was able to verify that most of the sample was confined in the pipette tip by the effect of ICP during preconcentration phase.

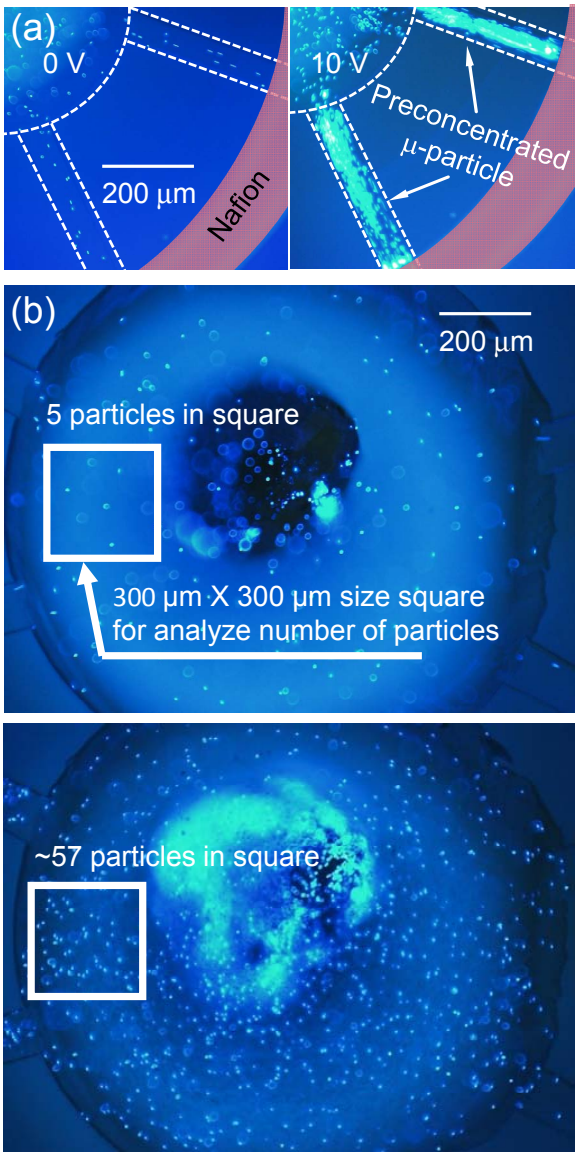

Figure 5. Polystyrene microparticle (1 µm) demonstration. (a) Preconcentrated microparticle. (b) Density distribution of microparticle inside the pipette tip.

Extraction results

The concentration of extracted samples were measured. To collect all the samples preconcentrated in the device, we followed the steps at the schematic in Figure 6 (a). After the preconcentration step, the solvent are almost flushed out while samples were confined inside the pipette tip. Then, the first step for extraction was remove all the DI water at the rims. Since, the volume of the preconcentrated solution was about 3~5 µL, and the volume of the DI water at each rim was about 5 µL, removing the DI water at the rims were important to prevent the dilution of the preconcentration samples by the DI water at the rims while extraction. The second step for extraction was simply pull out the tip by pipette. The final step was pulling out the

remained sample solution in the device by the suction of negative pressure. Fluorescence dye (alexa 488) was demonstrated for the extraction step (Figure 6 (b)). In the histogram, black bar, and gray bar represented the fluorescence intensity before, and after preconcentration step, respectively. Considering the references signal, fluorescence intensity extracted from the rims was more less than 200 nM. Because the fluorescence dye diffused toward the rims during the experiment, fluorescence dye was measured at the rims. 30 µL solution was concentrated to 3 µL with the recovery ratio of ~79 %, and ~4.5 fold of preconcentration factor while 50 µL solution was concentrated to 3 µL with the recovery ratio of ~75 %, and ~7.5 fold of preconcentration factor.

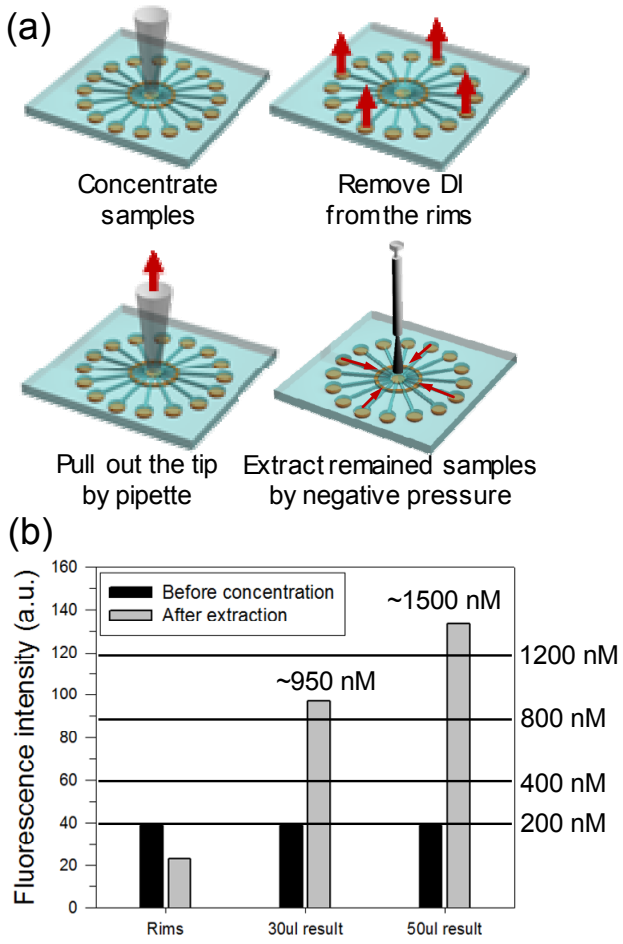

Figure 6. (a) The schematic procedures of extracting step. (b) Fluorescence dye (alexa 488) demonstration of extraction step.

CONCLUSIONS

In this paper, we proposed the simple and effective radial preconcentrator by printed Nafion and ICP phenomenon. The fluorescence dye, dsDNA, and µ-particle were successfully demonstrated with the preconcentration factor up to ~10 fold, throughput of 1 µL/min. Since these results guaranteed the preconcentration of the charged molecules in high-throughput manner and the easy-extraction without loss of invaluable target molecules, the device is expected for BioMEMS and environmental applications such as disease diagnostics, point-of-care test and water monitoring, *etc*.

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