Son et al. Micro and Nano Syst Lett (2017) 5:8
DOI 10.1186/s40486-017-0042-1

Micro and Nano Systems Letters

LETTER

Open Access

Paper-based ion concentration polarization device for selective preconcentration of muc1 and lamp-2 genes

Seok Young Son[1], Hyomin Lee[1] and Sung Jae Kim[1,2,3*]

Abstract

Recently, novel biomolecules separation and detection methods based on ion concentration polarization (ICP) phenomena have been extensively researched due to its high amplification ratio and high-speed accumulation. Despite of these bright advances, the fabrication of conventional ICP devices still have complicated and times-consuming tasks. As an alternative platform, a paper have been recently used for the identical ICP operations. In this work, we demonstrated the selective preconcentration of a muc1 gene fragment as human breast cancer marker and a lamp-2 gene fragment as the cause of Danon disease in paper-based ICP devices. As a result, these two DNA fragments were successfully concentrated up to ~60 fold at different location in a single paper-channel. The device would be a promising platform for point-of-care device due to an economic fabrication, the easy extraction of concentrated sample and an easy disposability.

Background

Over the past several decades, the separation and concentration of biomolecules including DNA and proteins have been developed for micro-scale assay such as genetics, disease diagnostics and point-of-care applications [1]. Recently, novel biomolecules separation and detection methods based on ion concentration polarization (ICP) phenomena have been extensively developed [2–6]. The ICP phenomena that is generated near the interface of a microfluidic channel and perm-selective nanojunction causes the depletion zone at the anodic side of the membrane. Due to electro-neutrality requirement at low concentration inside the depletion zone, any charged species were rejected into the ion depletion zone so that the molecules were accumulated at the boundary of the zone with a proper application of tangential electric (or pressure) field. Unfortunately, the fabrication of conventional ICP devices still have demanded complex photolithography and high cost reactive ion etching. Moreover an interfacing to downstream analyzer would be restricted due to the minute volume of preconcentrated samples and fast dispersion. While the accuracy, performance and reproducibility of paper-based ICP device were lower than conventional ICP device, it was always suffered from the extraction of preconcentrated sample and the instability. Since a paper is easily cut by any methods, the endeavor for the sample extraction for further processes become extremely simple [7–10]. With the advantage of cost-effective fabrication and easy extraction, paper-based ICP platform would be one of the major field of this ICP research society. In terms of electrokinetics, the cellulose-matrix of paper was able to suppress undesirable vortical flow [3] so that overall ICP process become significantly stable.

In this work, we demonstrated an economic and fast-fabricable paper-based microfluidic devices, which would be an alternative platform to easily generate ICP phenomena and selective preconcentrate biomolecules. The first experimental results demonstrated that the fluorescent tracers (Alexa) was successfully concentrated and migrated in a wetted paper channel using the reversal of the electric field, leading to the preconcentration factor of ~10. Moreover, the mixture of the target genes (cell surface associated mucin 1 (muc1, 945 bp) as the human

*Correspondence: gates@snu.ac.kr
[1] Department of Electrical and Computer Engineering, Seoul National University, Seoul 08826, Republic of Korea
Full list of author information is available at the end of the article

cancer marker [11] and lysosome-associated membrane protein 2 (lamp-2, 185 bp) encoding gene as Danon disease marker [12]) was injected and they were selectively preconcentrated at different locations having the amplification ratio of 20 and 60, respectively.

Methods

Preparation of DNA samples

Nested PCR amplification of muc1 gene (NG_029383.1, NCBI) was accomplished with a template of human genomic DNA. Amplification conditions of 3.5 kb fragment gene were 94 °C for 90 s, 65 °C for 45 s, and 72 °C for 90 s, by 35 cycles. The specific oligonucleotides were designed as following: forward primer; 5′-CCACACC CTTTGCATCAAGC-3′, reverse primer; 5′-TCTTGG CACCCGGTTGTTAC-3′. The second PCR conditions of 947 bp fragment gene were 94 °C for 1 min, 65 °C for 45 s, and 72 °C for 1 min, by 35 cycles. The forward primer of 947 bp muc1 fragments gene with tagging fluorescence dye FAM was specific for the 5′-FAM-TTTCCAGCC-CGGGATACCTA-3′, whereas the reverse primer was designed as 5′-CTCCTCCCTCTGCTCTCCTT-3′. The primary and secondary PCR conditions of a lamp-2 fragment gene (NM_001122606.1, NCBI) fragment were 94 °C for 1 min, 60 °C for 30 s, and 72 °C for 1 min, by 32 cycles. The specific oligonucleotides were designed for primary amplicon with 510 bp as following: forward primer; 5′-TACATCACCACCCCTCTC-3, reverse primer; 5′-GCCCCTTCTTACTCTCCT-3′. The forward primer of 185 bp lamp-2 fragments gene with tagging CY5 for secondary PCR was specific for the 5′-CY5-TTG CAGCTGTTGTTGTACCG-3′, whereas the reverse primer was designed as 5′-GTAGCTTTGAACTGGTG CCC-3′. All PCR products were eluted from 1.0% TBE agarose gels and resuspended on autoclaved DI water.

Fabrication of the paper-based microfluidic device

The paper-based ICP device was fabricated by cellulose paper (Whatman grade 1, Sigma-Aldrich Co.) with slide glass and E-tube caps (Fig. 1). The paper had 180 μm of thickness and a mean pore diameter of 11 μm. The main paper-bridge was cut in 10 mm of length and 0.82 mm of height using an electronic craft cutter. The paper was floated above the glass as shown in Fig. 1 for avoiding the unwanted electrical connection through leaked liquid on the glass slide. 0.5 μL Nafion was dropped in a bridge of paper for forming cation-selective membrane. The Nafion-patterned paper was dried at 95 °C for 5 min and immersed in DI water for 30 min. The fabricated devices were dried in petri dishes with cover at room temperature until use. While dropping a Nafion drop to pattern the nanoporous membrane may result the non-uniform and irreproducible pattern, it had a minimal effect on the

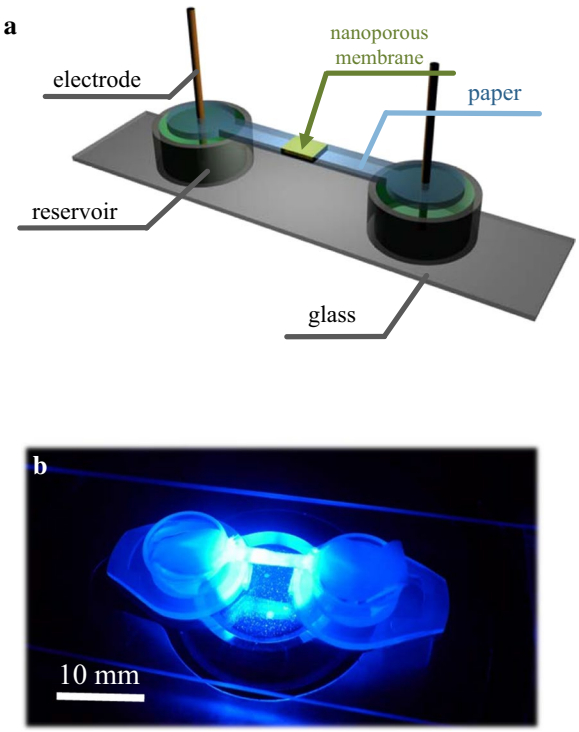

Fig. 1 Design of the paper-based ICP device: **a** device schematic with paper (*blue*), nanoporous membrane (*green*); **b** an optical image of an assembled device on the microscope

performance of ICP because the conductivity of Nafion is similar with 2 M of electrolyte [13, 14], leading ultrafast proton conduction regardless of the non-uniformity of the Nafion pattern.

To operate the device, reservoirs were pre-wetted with 10 mM KCl and loaded the mixture of KCl and the fluorescent tracer (Alexa488) or prepared DNAs. Ag/AgCl electrodes were inserted into both reservoirs and connected to a power supply (Keithley 238, Keithley Instruments, USA). All fluorescent images were captured by inverted microscope (IX53, Olympus, Japan) and analyzed by ImageJ program (https://imagej.nih.gov/ij/). The external voltage was swept from 0 to 15 V at 0.2 V/30 s only for the electrical measurement.

Results and discussion

Overlimiting current in the paper-based ICP device

The I–V responses in the paper-based ICP devices with or without nanoporous membrane were plotted in Fig. 2a, b, respectively. The mechanism behind the overlimiting current behavior was able to be categorized into a strong electro-convection [3, 15], surface conduction [4, 16] and diffusioosmosis [5]. In this paper platform, the overlimiting current was initiated only by the surface conduction and diffusioosmosis because the cellulose

matrix greatly suppressed the electro-convection. Therefore, the observation of overlimiting conductance (OLC) is the evidence of generating ICP in nanofluidic platform. The current in Nafion-patterned device showed an ohmic region until 2 V and overlimiting current was initiated from 4 V, confirming the generation of ICP in this paper-based platform (Fig. 2a). In contrast, only ohmic response was measured without Nafion pattern so that one can judge the proper ICP operation (Fig. 2b).

Preconcentration in paper-based microfluidic device using the reversal of electric field

Nafion as a cation-selective membrane acts the barrier against co-ion in the solution due to electrical double layer (EDL) overlap, whereas count-ions easily pass through the membrane. Due to this physical background, ICP phenomena occurred near the Nafion membrane under an electric field. Nafion-submerged region in paper-bridge effectively worked as a cation-selective membrane in this paper-based ICP device (Fig. 1). Before starting the operation, the Nafion-patterned paper strip was pre-wetted with KCl 10 mM by capillary force. After 1 μL mixture of the fluorescent tracer (Alexa 488) and KCl were loaded to bridge of paper, 50 V was applied to both reservoirs and the depletion zone was generated at the anodic side of the membrane. Under dc bias, an electrokinetic flow was generated from anode to cathode a.k.a. electroosmotic flow (EOF). However, the ion depletion zone as an electrical barrier rejected the flowing tracer. Consequently, the charged species was started to be accumulated at the boundary of depletion region. The preconcentrated plug of the fluorescent tracer at the interface of depletion zone was shown in Fig. 3a. The preconcentrated tracer plug migrated at a rate of 280 μm/s to anodic side until the electrophoresis and electroosmosis were balanced out.

Figure 3b showed an effect of the electric field reversal. For first 10 s, the electric field was inversely applied to the device (snapshot from 0 to 8 s in Fig. 3b) so that the tracer initially flocked together near the left side of the membrane. The mechanism was the same ICP principle, but the left side of the membrane has the ion enrichment zone instead the ion depletion zone. After switching back the polarity of the electrode for preconcentration of samples at the ion depletion zone (snapshot from 10 to 20 s in Fig. 3b), the pre-accumulated tracer were preconcentrated further at the boundary of the depletion zone. The measured maximum concentration ratio of the tracer based on fluorescence intensity was ~10 fold. The reference fluorescent intensity of 2, 5, 10, 20 and 100 fold of samples were measured in advance and, then, the fluorescent from the preconcentrated signal was compared by ImageJ program.

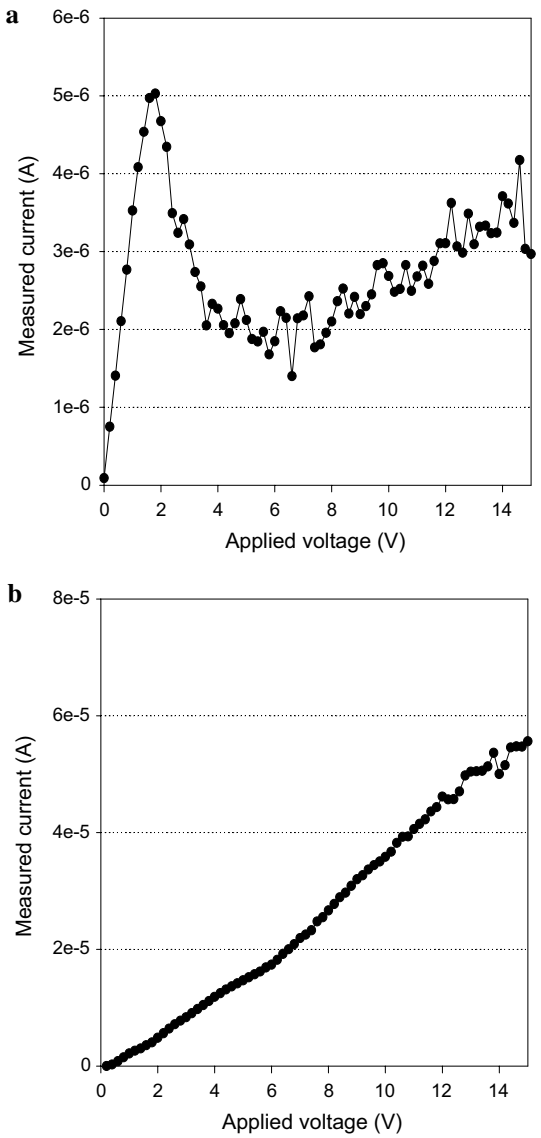

Fig. 2 The I–V responses of paper-based ICP device **a** with nanoporous membrane and **b** without nanoporous membrane

Selective preconcentration of two target genes in paper-based ICP device

The selective preconcentration of the mixtures (lamp-2 and muc1 gene fragments) were successfully demonstrated as shown in Fig. 4. Lamp-2 gene tagged by CY5 fluorescent dye and muc1 gene tagged by FAM dye emitted a red light and a green light, respectively so that one can observe clearer separation. The pairs of two snapshots at each time were taken by illuminating excitation light of different wavelengths, while actual experiment was conducted with single paper. Initially, the efficiency of selective preconcentration increased, while lamp-2 and

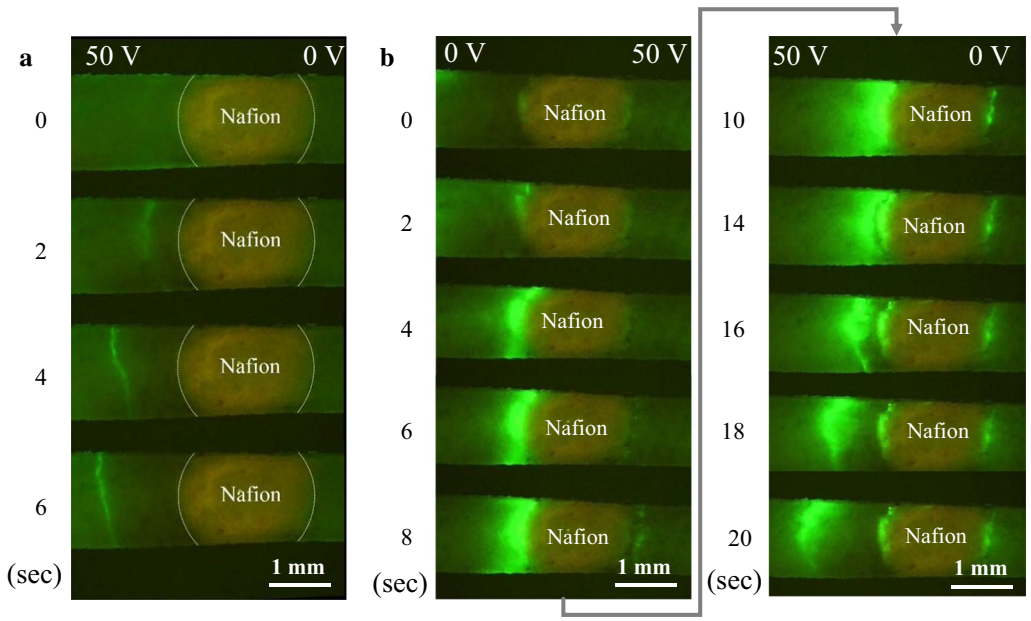

Fig. 3 **a** The preconcentrated fluorescent tracer out of the depletion boundary under normal electric field. **b** Two step preconcentration method in paper-based ICP device

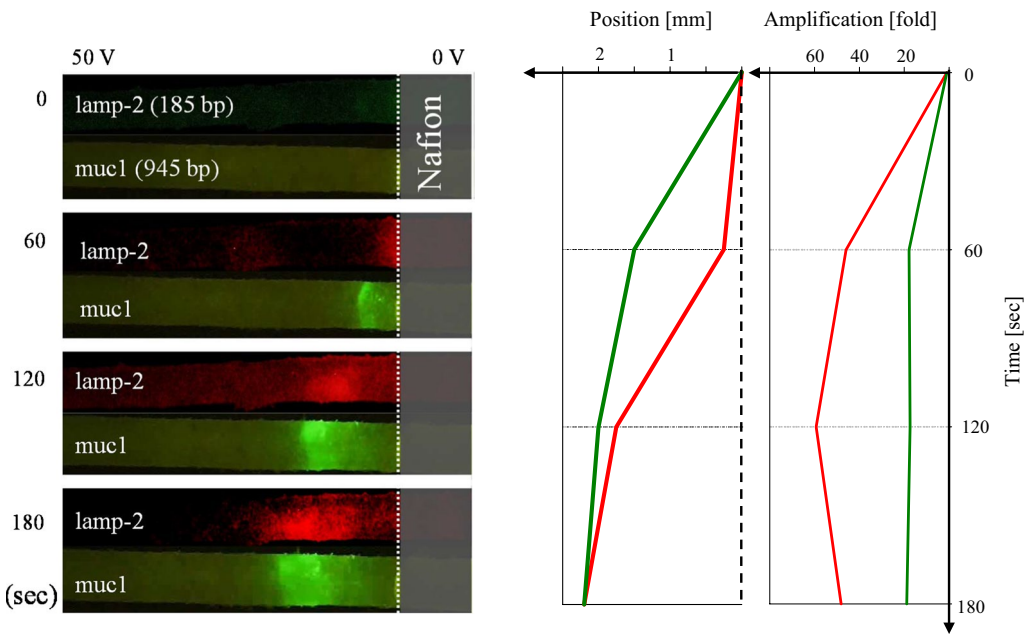

Fig. 4 Preconcentration and separation of muc1 and lamp-2 gene fragments in the paper-based ICP device under 50 V. The position of location where the fluorescent intensity was maximum of each target gene and the amplification ratio were plotted as a function of time during the ICP operation

muc1 were getting closer after 120 s as shown in position-time plot. This was because the repulsions of molecules were weak due to the absence of strong electrokinetic flow which was usually involved in conventional ICP operation. Thus, one needs to operate the process until 60 s for maximum separation. In the meantime, cutting the paper at the portion of either lamp-2 or muc1 would retrieve each target for further process. As shown in

amplification-time plot, the preconcentration factors of lamp-2 and muc1 at 60 s would be ~40 and ~20, respectively, while were reasonable amplification for further process.

Conclusions

Recently ICP phenomenon had been drawn significant attentions in biomedical and environmental research field as well as fundamental electrokinetic society, since its versatile capability of controlling charged species. In this work, we have demonstrated an economic and fast-fabricable ICP device on a commercial paper for selective preconcentration. The generation of ICP phenomena was confirmed by I–V responses of the paper-based device by comparing with to without nanoporous membrane. The fluorescent tracer (Alexa488) and DNA fragments (muc1 and lamp-2) associated with human diseases were successfully preconcentrated and separated. Such paper-based ICP devices would be a promising tool due to an economic fabrication, the easy extraction of concentrated sample and an easy disposability.

Authors' contributions

SYS performed the experiments; HL analyzed the dynamics of analyte; SJK supervised the project, designed experiments and wrote the paper. All authors read and approved the final manuscript.

Author details

[1] Department of Electrical and Computer Engineering, Seoul National University, Seoul 08826, Republic of Korea. [2] Big Data Institute, Seoul National University, Seoul 08826, Republic of Korea. [3] Inter-University Semiconductor Research Center, Seoul National University, Seoul 08826, Republic of Korea.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Research Foundation of Korea (CISS-2011-0031870, 2012-0009563, 2016R1A1A1A05005032, 2016R1A6A3A11930759) and Korean Health Technology RND Project (HI13C1468 and HI14C0559). All authors acknowledge the support from BK21+ program of Creative Research Engineer Development IT, SNU.

Received: 13 October 2016 Accepted: 4 January 2017
Published online: 11 January 2017

References

1. Kumemura M, Collard D, Yamahata C, Sakaki N, Hashiguchi G, Fujita H (2007) Single DNA molecule isolation and trapping in a microfluidic device. ChemPhysChem 8(12):1875–1880
2. Choi J, Huh K, Moon DJ, Lee H, Son SY, Kim K, Kim HC, Chae J-H, Sung GY, Kim H-Y, Hong JW, Kim SJ (2015) Selective preconcentration and online collection of charged molecules using ion concentration polarization. RSC Adv 5(81):66178–66184
3. Kim SJ, Wang Y-C, Lee JH, Jang H, Han J (2007) Concentration polarization and nonlinear electrokinetic flow near nanofluidic channel. Phys Rev Lett 99:044501
4. Nam S, Cho I, Heo J, Lim G, Bazant MZ, Moon DJ, Sung GY, Kim SJ (2015) Experimental verification of overlimiting current by surface conduction and electro-osmotic flow in microchannels. Phys Rev Lett 114(11):114501
5. Cho I, Kim W, Kim J, Kim H-Y, Lee H, Kim SJ (2016) Non-negligible Diffusio-osmosis inside an ion concentration polarization layer. Phys Rev Lett 116(25):254501
6. Kim SJ, Song Y-A, Han J (2010) Nanofluidic concentration devices for biomolecules utilizing ion concentration polarization: theory, fabrication, and application. Chem Soc Rev 39:912–922
7. Hong S, Kwak R, Kim W (2016) Paper-based flow fractionation system applicable to preconcentration and field-flow separation. Anal Chem 88(3):1682–1687
8. Han SI, Hwang KS, Kwak R, Lee JH (2016) Microfluidic paper-based bio-molecule preconcentrator based on ion concentration polarization. Lab Chip 16(12):2219–2227
9. Gong MM, Nosrati R, San Gabriel MC, Zini A, Sinton D (2015) Direct DNA analysis with paper-based ion concentration polarization. J Am Chem Soc 137(43):13913–13919
10. Yang R-J, Pu H-H, Wang H-L (2015) Ion concentration polarization on paper-based microfluidic devices and its application to preconcentrate dilute sample solutions. Biomicrofluidics 9(1):014122
11. Levitin F, Baruch A, Weiss M, Stiegman K, Hartmann M-L, Yoeli-Lerner M, Ziv R, Zrihan-Licht S, Shina S, Gat A (2005) A novel protein derived from the MUC1 gene by alternative splicing and frameshifting. J Biol Chem 280(11):10655–10663
12. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y (2000) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature 406(6798):906–910
13. Mauritz KA, Moore RB (2004) State of understanding of Nafion. Chem Rev 104:4535–4585
14. Ko SH, Kim SJ, Cheow L, Li LD, Kang KH, Han J (2011) Massively-parallel concentration device for multiplexed immunoassays. Lab Chip 11(7):1351–1358
15. Rubinstein SM, Manukyan G, Staicu A, Rubinstein I, Zaltzman B, Lammertink RGH, Mugele F, Wessling M (2008) Direct observation of a nonequilibrium electro-osmotic instability. Phys Rev Lett 101:236101
16. Dydek EV, Zaltzman B, Rubinstein I, Deng DS, Mani A, Bazant MZ (2011) Overlimiting current in a microchannel. Phys Rev Lett 107:118301