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Free-flow isoelectric focusing microfluidic device with glass coating by sol–gel methods

Kwang Suk Yang [a,b], Philippe Clementz [a], Tae Jung Park [a,b,c], Seok Jae Lee [d], Jong Pil Park [a,c], Do Hyun Kim [a,b,*], Sang Yup Lee [a,b,c]

[a] Department of Chemical and Biomolecular Engineering, KAIST, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Center for Ultramicrochemical Process Systems, KAIST, Daejeon 305-701, Republic of Korea
[c] BioProcess Engineering Research Center, Center for Systems and Synthetic Biotechnology, Institute for the BioCentury, KAIST, Daejeon 305-701, Republic of Korea
[d] NEMS-Bio Team, Nano Fusion Technology Division, National Nanofab Center, Daejeon 305-701, Republic of Korea

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ABSTRACT

A device for microfluidic free-flow isoelectric focusing (μ-FFIEF) was fabricated in a glass-coated PDMS structure. The microfluidic channel was made by a PDMS replica molding and standard soft photolithography technique. For the prefractionation and preconcentration of a protein mixture, we patterned palladium electrodes on the glass substrate and coated with TEOS–sol to enhance the bonding with PDMS. The PDMS microchannel was coated with glass by flushing TEOS–sol and annealing at 100 °C. The optimum focusing condition was obtained by pH indicator with ampholyte solution made with MES. Model proteins, RFP and EGFP, were successfully concentrated around 214 μm and 357 μm from the anode, respectively. The optimum focusing condition in the μ-FFIEF device was an electric potential of 1.5 V/cm and pH 6.0. The focusing time in the channel without glass coating was approximately 30 s, but the breakdown of the electrode by electrolysis started immediately when the electric field was applied. However, the glass-coated device was kept intact under continuous application of voltage giving a stable pH gradient, but with twice the focusing time.

1. Introduction

A microfluidic system for lab-on-a-chip is the integration of sampling, processing, and detection techniques for micro total analysis systems (μTAS) [1–4]. Recently, the miniaturized system has gained significance, since it offers many practical advantages over classical technology: it reduces the analyzing time and the amount of samples needed for analysis of complex protein mixtures [5,6]. A requirement for a very small volume of sample can make a great breakthrough in medical analysis.

Among the various electrokinetically driven methods for separation and analysis of proteins, isoelectric focusing (IEF) based on pI is a very distinctive technique with high resolving power, rapid separation and easy operation [7,8]. For the prefractionation and concentration of protein samples, IEF is a very attractive tool for further separation (e.g., SDS–PAGE) or detection (e.g., electrochemistry, ELISA) [9,10]. However, reported FFIEF systems have required complicated fabrication and operations to prevent electrolysis and Joule heating. Continuous operation in micro-IEF requires a free-flow system with an electric field applied perpendicular to the sample stream. A microfluidic free-flow IEF (μ-FFIEF) system as a separation tool is a promising alternative to conventional electrophoresis [2,11].

Polydimethylsiloxane (PDMS) is a widely used material for microfluidic devices, due to its low cost, optical transparency and capability of sophisticated design by a soft lithographic molding technique [12–14]. Despite these advantages, researchers frequently suffer from permeability and swelling in organic solvents. Furthermore, the hydrophobic nature of PDMS can cause serious deformation in a multi-phase system. PDMS microfluidic devices for IEF separation work properly with no or uniform electro-osmotic flow (EOF). For the minimization or elimination of EOF in a PDMS microchannel, permanent coating is necessary for electro-

* Corresponding author. Address: Department of Chemical and Biomolecular Engineering, KAIST, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea. Tel.: +82 42 350 3929; fax: +82 42 350 3910.
E-mail address: DoHyun.Kim@kaist.ac.kr (D.H. Kim).

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chemical stability and further functionalizability by controlling the interfacial properties.

In this study, we used dynamic coating of SiO_2 on a PDMS microchannel and electrode surface with minimum EOF and electrode degradation [15,16]. Sol–gel chemistry in a thin-film coating is widely used in various applications because of its low reaction temperature and easy control of porosity to allow ion transport [17]. In this work, the compatibility of the sol–gel method for a μ-FFIEF device has been examined. To test IEF using a glass-coated PDMS microchannel, we used green and red fluorescent proteins (EGFP and RFP) as model proteins to demonstrate the prefractionation and preconcentration of proteomic samples. Here, we show that the dynamic coating of glass minimizes EOF, improves durability of electrodes under very low electric field and suppresses protein adhesion.

2. Materials and methods

2.1. Materials

EGFP (pI 5.73) and RFP (pI 6.55) as model proteins for IEF separation were obtained by the protein expression method using recombinant DNA technologies [18]. 2-(*N*-morpholino)ethanesulfonic acid (MES), tris(hydroxymethyl)aminomethane (Tris), tetraethoxysiloxane (TEOS), and palladium wire were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sylgard 184 was purchased from Dow Chemical (Midland, MI, USA). Two photoresists were used for this experiment: SU-8 50® negative photoresist and AZ 9260® positive photoresist, which were obtained from MicroChem (Newton, MA, USA) and Clariant Corporation (AZ® Electronic Materials; Somerville, NJ, USA), respectively. All aqueous solutions were prepared with deionized water from Human UP900 (Korea). All other chemicals and reagents were purchased commercially and used as received, without further step.

2.2. Preparation of TEOS–sol solution

Sol–gel solution for coating was prepared by mixing tetraethoxysiloxane (TEOS, 98%), ethanol, and H_2O in a flask with a mole ratio of 1:1:1 (TEOS:EtOH:water with pH 4.5 adjusted with HCl). The mixture was stirred for 2 h at 60 °C. Then, the flask was sealed and the temperature was raised to 80 °C and kept there for 2 h for reaction to give the sol.

2.3. Design and fabrication

In this study, the μ-FFIEF device was designed for both simple operation and cost efficiency. The palladium electrode normally shows poor bonding with PDMS. It is necessary to improve the bonding property with PDMS microchannel structure and electrochemical durability when an electric field is applied. The coating of silica–sol in the microfluidic channel minimizes EOF and allows ion transport through small pores. Furthermore, a layer of SiO_2 facilitates the adhesion to PDMS and chemical resistance.

2.3.1. PDMS molding process using lithography

Our microchip device was fabricated by using standard soft lithography and PDMS molding techniques as shown in Fig. 1A–E. The procedure to fabricate the PDMS microchannel pattern is described as follows. First, the negative photoresist, SU-8 50, was spin-coated on a silicon wafer in two steps, with 10 s at 500 rpm to spread SU-8 over the wafer and 30 s at 2500 rpm for a uniform thickness of the photoresist. A soft baking process followed the coating, to remove solvent from the photoresist and to harden the remaining photoresist. The wafer was processed by a two-step contact hotplate at 65 °C for 5 min and 90 °C for 15 min. After being cooled to room temperature, the wafer was placed under a PCG mask which was prepared from Microtech (Gyeonggi, Korea). UV (i-line 365 nm) was irradiated for 60 s with a power density of 300 mJ/cm^2. Fig. 2A shows the mask design. Post-baking was done by a two-step process at 65 °C for 1 min and 90 °C for 4 min. The photoresist was then developed using SU-8 developer, washed carefully with isopropyl alcohol, and dried with nitrogen gas. With the master pattern prepared above, a PDMS replica molding process was carried out. A PDMS mixture in the ratio of 10:1 by weight of base and curing agent was poured on the SU-8 master pattern and evacuated to eliminate the air bubbles from the mixing step. After evacuation, the PDMS mixture was cured at 70 °C for 4 h. Then, the cured PDMS mold was detached from the wafer. To introduce the protein sample, the PDMS was punched to install tubings.

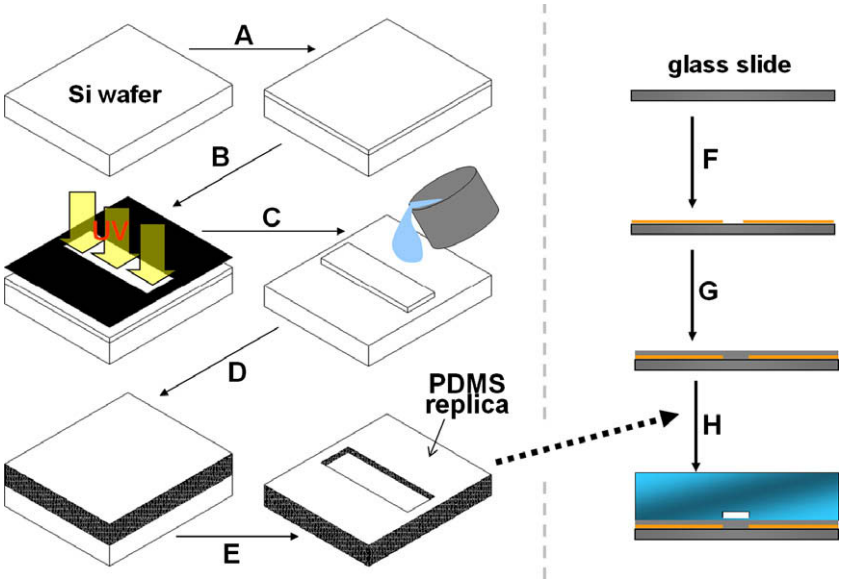

Fig. 1. Fabrication process of the PDMS microfluidic device using photolithography and replica molding: (A) negative PR (SU-8) spin-coating; (B) UV exposure with contact mask; (C) developing; (D) applying PDMS on master PR pattern and curing PDMS at 70 °C; (E) detaching PDMS from master pattern; (F) electrode patterning on a glass slide; (G) TEOS–sol spin-coating; (H) alignment and bonding between PDMS mold and glass substrate.