미세 유체장치 내에서 마이크로 비드를 이용한 생물분리

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Bioseparation Techniques in Microfluidic Devices Using Micro-Bead

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Introduction

There has been a significant increase of interest on microfluidic device as miniaturized analytical system, recently. Micron-size fluidic paths and other components are integrated in microfluidic device, performing essential procedures for the analysis of chemical and/or biological materials (Harrison et al., 1993, Jacobson et al., 1994). The device that has dimensions of a few centimeters is capable of providing rapid identification of molecules and enhanced sensitivity with reduced consumption of reagents and samples (Stone and Kim, 2001). Various separation processes have been applied to microfluidic device such as zone electrophoresis, gel electrophoresis, isoelectric focusing, micellar electrokinetic chromatography (MEKC) and electrochromatography, resulted in the increase of its applications.

The applications using polymer-based microfluidic devices are increasing due to their ease of fabrication, inexpensive fabrication costs and increasing versatility (Becker and Locascio, 2002). These devices have been fabricated from various polymers including poly(methyl methacrylate), polycarbonate, polystyrene, and poly(dimethylsiloxane) (PDMS). The usages of PDMS are increasing among these polymers because of relatively lower expense and simpler procedures for fabrication than others. The control of minute volume of liquid in PDMS microfluidic device using electroosmotic flow (EOF) enhanced the increase of its application.

In this work, capillary electrochromatography (CEC) and preconcentration of neutral compounds have been realized on poly(dimethylsiloxane) (PDMS) microfluidic devices. The micro-structures of PDMS were fabricated in micro-channel for the packing of micro-beads. In addition, rapid prototyping technique for the fabrication of micro-channels using micro-fiber and PDMS slab was developed. The flexible characteristic of PDMS makes simple fabrication of micro-channel possible, by directed placement of glass micro-fibers on solid substrate. The applications of developed microfluidic device are tested.

Experimental

PDMS microfluidic device for CEC was fabricated by the photolithographic replica molding method. Embossed pattern of negative photoresist (SU-8 50, 25 μm) was manufactured on silicon wafer, then silanized by trichloro(3,3,3-trifluoropropyl)silane (Aldrich). Sylgard 184 (Dow Corning) was used for the casting of PDMS microfluidic device. PDMS replica and flat slab were bonded each other after oxidized by corona discharger. The channel pattern of CEC microchip is shown in Figure 1 (A), where the exploded view shows the chamber for bead packing with frit structures. The channel length and width of the bead chamber is 3 mm and 150 μm, respectively. The size of each frit structure is 25 μm x 20 μm and the gap between the frit structures is 3 μm. All channel widths are 50 μm, except the chamber for bead packing (K. W. Ro et al, 2003). The suspension of octadecylsilane (ODS) coated silica beads (5 μm diameter, 2% in acetonitrile) was injected from reservoir 4 by applying pressure at the inlet port of the microfluidic device. The electro-osmotic flow (EOF) of the reagents were generated by applying 100 to 1000 V to one of the reservoirs, while counter reservoir was grounded for the injection of sample, buffer and eluent solutions. The fluorescent dye, BODIPY, was concentrated from 10% acetonitrile/90% Tris-Borate buffer solution and eluted by increasing the concentration of acetonitrile in the buffer solution to 70%.

A rapid prototyping technique to make microfluidic channels was tried using 1 mm thick PDMS slab and glass fiber (diameter 12 μm) (figure 1 (B)). Around 12 μm wide micro-channels were fabricated by placing flexible PDMS slab on top of glass fiber located on solid slide glass. The width of micro-channel was varying depends on the diameter of glass fiber located on slide glass. The glass fiber was functionalized by dimethylamino (DMA) beads (diameter 0.8 μm) when it is dipped into an aqueous slurry of the beads. The straight micro-channel was fabricated using DMA coated glass fiber and developed method, and applied for the separation of fluorescent labeled avidin and BSA. The micro-channel was filled with mixture solution of fluorescent labeled avidin and BSA by dropping a solution at one exposed end of micro-channel (Tom T. Huang et al., 2003).

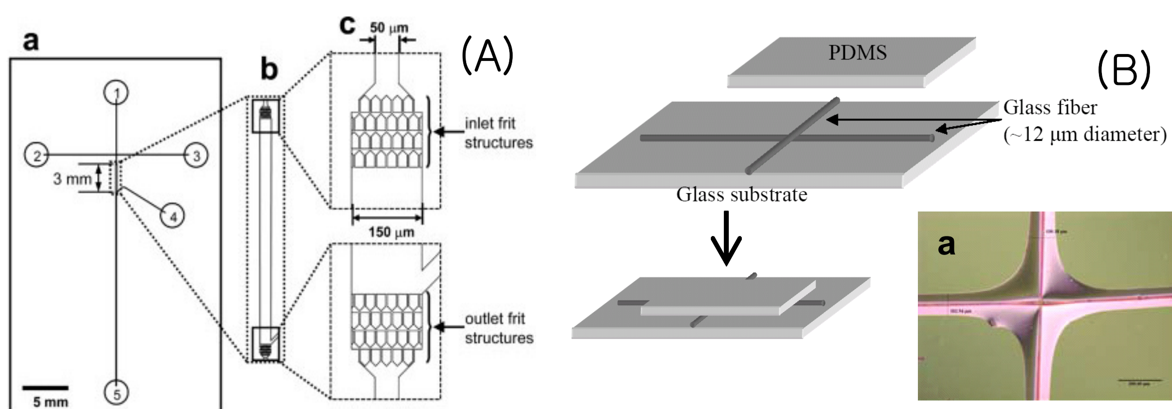

Figure 1. Schematic drawings of (A) PDMS microfluidic device for CEC, The reservoirs for sample(1), buffer(2), sample waste(3), bead introduction(4), waste(5) are shown in a. Detailed structure of frit is shown in c. (B) Rapid prototyping method for micro-channels using PDMS slab and glass fiber. Picture of intersection of micro-channels is shown in a.

Results and Discussion

Preconcentration of neutral compounds in a PDMS microchip packed with ODS-coated beads were tried. Fluorescent dye, BODIPY, in aqueous buffer solution was preconcentrated as a model compound. The BODIPY was strongly absorbed into the uncoated PDMS channel, however it was prevented when the surface of PDMS micro-channels were coated with polybrene (PB) and dextran sulfate (DS). Figure 2 (A) and (B) shows the preconcentration and elution of BODIPY on packed beads in the PB/DS-coated PDMS channel. After rinsing the packed micro-channel with 10% acetonitrile/90% Tris-Borate buffer, a solution of 10 μM BODIPY was loaded into the microfluidic device by EOF. A narrow band of fluorescence of adsorbed BODIPY was found at the inlet of the bead-packed micro-channel (Fig. 2 (A)). The adsorbed BODIPY was eluted with 70% acetonitrile/30% Tris-Borate buffer (Fig. 2 (B)), after rinsing of excess reagent in micro-channel. A significant increase of fluorescent signal was not detected during sample loading and rinsing steps, meaning there was no remarkable bypassing of BODIPY during preconcentration. The BODIPY was concentrated up to 100 times by developed preconcentration technique. The simultaneous preconcentration and separation of fluorogenic neutral compounds, coumarin 440 and coumarin 450, was successfully performed with same microfluidic device by reversed-phase CEC. The optimum CEC separation was accomplished in a buffer composition of 50% acetonitrile/50% aqueous Tris-Borate buffer (data not shown).

A static separation of a mixture of BSA and avidin labeled with a red (TRITC) and a green fluorescent dye (FITC), respectively, is shown in Figure 2 (C). The micro-channel was made of glass fiber coated with DMA-coated micro-bead and PDMS slab. A micro-pipette was used to place 2 μL of mixture solution onto an open end of a 6 nL volume micro-channel. The micro-channel was filled with the mixture within 10 seconds by capillary action. Fluorescence labeled BSA (red) was separated from the mixture with avidin (green) in 3 minutes due to diffusive de-mixing where the BSA (red) adsorbed on the DMA beads of glass fiber while the avidin remaining distributed across the width of the channel (picture in figure 2 (C)). Both of axial and radial concentration gradient of labeled BSA (red) is evident near the entrance of the micro-channel (picture in figure 2 (C)). Electrostatic interaction causes the positively charged DMA micro-beads to capture the negatively charged BSA (pI 4.8) when the BSA is resolved into PBS buffer (pH 7.2). In contrast, Avidin (pI 10.2) is positively charged in the same PBS buffer (pH 7.2), thus avidin does not adsorbed onto the DMA beads. The capture of avidin labeled microbeads (diameter 0.8 μm) by biotinylated glass fiber was also performed (data not shown). Avidin adsorbs on biotinylated BSA coated glass fiber, since biotin has a strong affinity for avidin with a dissociation constant of 10-15 M. The stability of coated micro-beads on glass fiber could be increased when biotin-avidin interaction is applied for the coating of micro-beads on glass fiber. In addition, other fibers could be used such as hydrophilic cellulose and glass, or hydrophobic polypropylene, aramid, and acrylic fibers in this microfluidic device, resulting the expansion of the application of developed technique.

In this work, PDMS-based fabrication methods for microfluidic device has been developed, and it is expected to be done more easily, rapidly, and cost effectively than glass-based methods. A various applications of these techniques are supposed to be applied for the separation of various chemical and/or biological materials, and expanding applications of PDMS microfluidic devices.

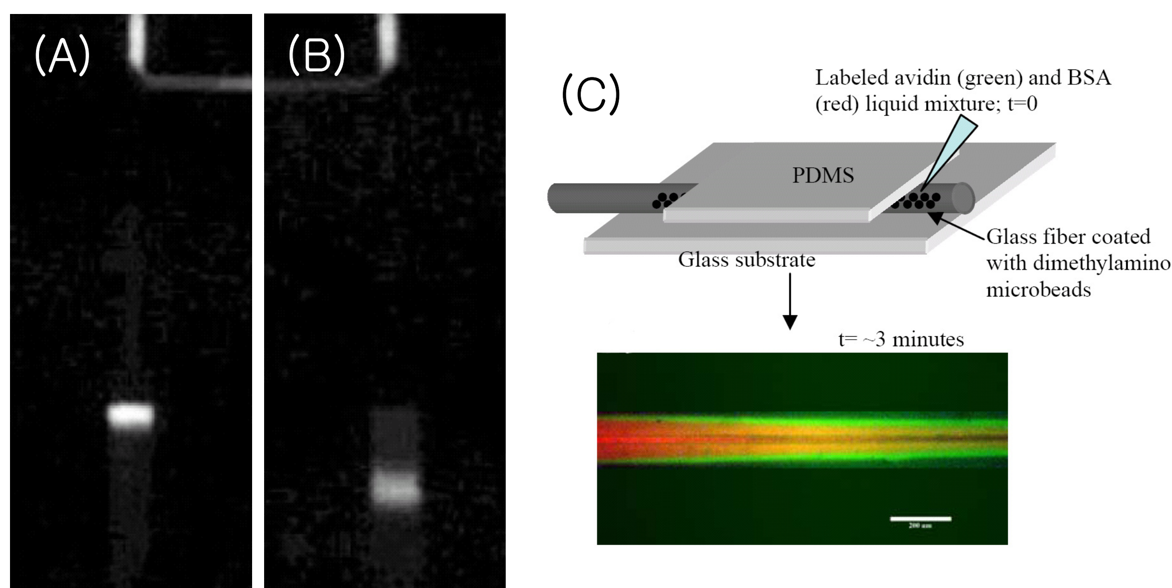

Figure 2. CCD images of (A) preconcentration and (B) elution of BODIPY in PDMS CEC microfluidic device. (C) Schematic drawing and picture of separation of fluorescence labeled avidin and BSA in microfluidic device fabricated by PDMS and DMA-bead coated glass fiber.

Acknowledgement

This work was supported by the National Research Laboratory Program of the Ministry of Science and Technology, Korea and supported through a cooperative agreement with the Agricultural Research Service of USDA (Project No. 1935-42000-035), USA. Dr. Woo-Jin Chang was partially supported by the ERC for the Advanced Bioseparation Technology, KOSEF, Korea and by an NIH grant R21EB000982-1. The authors thank Ho Kim at POSTECH and Dr. Rafael Gomez, Dr. Nathan Mosier, Dr. J. P. Robinson, Dr. Miroslav Sedlak, Winnie Chen, Dr. Richard Linton, Randy Woodson, Debra Sherman, Chia-Ping Huang, Dr. David Taylor and Richard Lowe at Purdue University for their help.

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