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Amperometric Detection of Bisphenol-A on Laser Fabricated Capillary Electrophoresis Device

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Abstract

In this study, poly dimethyl siloxane (PDMS) capillary or microchannel electrophoresis devices were fabricated and tested by laser writer. A programmable laser writer was controlled to make microchannel on PDMS mold instead of a conventional photolithography with SU-8. Indium tin oxide (ITO) electrodes for electrochemical detection of bisphenol-A were made by photolithography. UV-ozone treatment was used to bind laser patterned PDMS mold and patterned ITO glass. During electrophoresis, changes of current measured at working electrode were recorded with time by source measure unit. The peak current representing for detection of bisphenol-A was analyzed and compared with devices fabricated by laser and conventional lithography, respectively. Relationships among microchannel size, separation voltage, and peak currents were established.

Keywords: Capillary, Microchannel, Electrophoresis, PDMS, Laser writer

Introduction

Many researches about detection of hormones and endocrine disruptors in capillary or microchannel electrophoresis have been investigated for an issue of human health[1-5]. For example, bisphenol-A is an environmentally hazardous compound, which is a resource for carcinogenesis, mutation, and dermal problems[1,5]. The problems with the endocrine disruptor are its chronic nature and wide range of contamination area, which includes most of common-life spaces using plastic containers and additives[1,5]. Typically, its detection can be easily performed with HPLC. However, simple detection of bisphenol-A, for example, with portable instruments has been very limited[5].

Capillary electrophoresis using electrochemical or electrical detection has been developed as one kind of LOC (Lab-On-a-Chip) system, which is breakthrough technology for detecting hormones and environmentally hazardous chemicals[2-6]. In addition, electronic devices such as transistors for electrical detection targeting to biomolecules have been introduced[7-9]. As a consequence, organic molecules like catecholamine[3], dopamine[4], bisphenol-A[1,5], etc have been successfully detected by electrochemical measurement. Additionally, protein[7,8] and DNA[6,9] have been detected by electrical methods. Here, the biggest benefit of the electrochemical or electrical detection lies in a fact of simplicity with a label-free detection system.

Among electrochemical and electrical detections, amperometric detection is used to monitor substantial change in current (I) at a constant potential[2,3]. Typically, amperometric method has been proven to be a highly sensitive method for determination of a wide range of electro-active chemicals, from small molecule of bisphenol-A to large molecule of DNA. In an aqueous environment of capillary electrophoresis, molecules can be dissolved and charged into either positive or negatively charged ions depending on their chemical functional groups and buffer pH. When an external electric potential is formed to the aqueous phase, the positive and negatively charged species move towards the end of opposite charging electrodes. Electroosmotic and electrophoretic flows attribute to the separation of charged species depending on their sizes in a capillary. The surfaces of capillary are changed into negative charges as a result of ionization of silanol (Si-OH) groups. In this case, the motion of the charged species is facilitated through this region when an electric field is applied[3]. In a certain voltage, the electric field can drive a plug flow. At the same time, target molecule to be detected, such as bisphenol-A, can move towards electrode by electrophoretic motion[3,4].

For amperometric or electrochemical detection, three electrodes are generally required to constitute a detecting module: working, counter and reference electrodes. A potentiostat should be served to control applied voltage and stabilize the measurement of current (I). Capillary electrophoresis is usually performed by applying high voltage (~300 V) from injection end while keeping the detector end at electrical ground. Standard potentiostat circuitry (as setted up in most electrical

instruments) modulates the applied potential against reference electrode by adjusting the voltage at counter electrode. In this case, current can be measured at the working electrode. Therefore, total of four electrodes at the detector end of the capillary are fabricated[3-5]. Another set-up has been the use of a conventional reference electrode as counter electrode for the detector cell. In both circumstances, at least, total of three electrodes are still required. In this study, three electrodes were used to sense the current changes.

PDMS (Poly dimethyl siloxane) is one of the most important materials for lab-on-a-chip applications. Recently, PDMS becomes a critical polymer and its modification is one of the most growing research fields[10,11]. An available approach for modification is application of UV irradiation. UV irradiation can convert the polymer surface from hydrophobic to hydrophilic, which enhances binding of PDMS to glass. With this property, numerous applications in biochip or microfluidic applications have been available. Among the PDMS modifications, laser fabrication of PDMS in lamination construction is meaningful[10-12]. Typical process for the fabrication of PDMS-based biochips including capillary electrophoresis requires a complicated photolithography using SU-8 to make a mold-master for PDMS mold[5,10]. It requires a high cost optical instrument for a pattern-mask exposure. However, since laser fabrication is very simple without the photolithography process, it can be a meaningful step for automated assembly mass production of biochip. In this study, the laser-ablated microchannels or capillaries are characterized revealing a modified gaussian contour. In addition, surface roughness issue from laser writing or ablation for electrophoresis is discussed. A strong relationship between PDMS cut profile and laser power and writing speed is drawn.

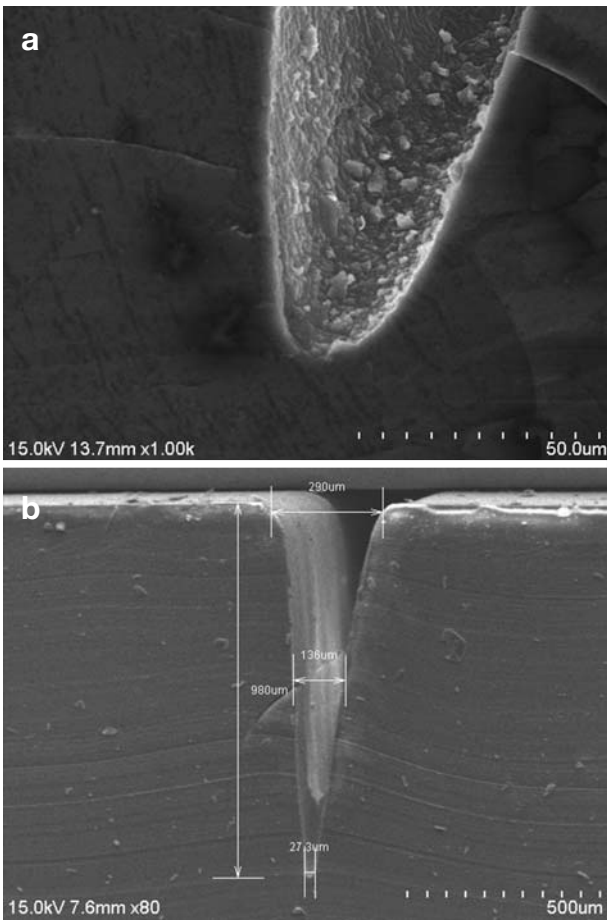

Figure 1. SEM images of (a) surface roughness of microchannel and (b) microchannel shape in electrophoresis device using laser writer.

Results and Discussion

Figure 1(a) and (b) show SEM cross-sectional images of microchannel in the electrophoresis device using programmable laser writer. Some extent of surface roughness is shown in channel in Figure 1(a). Figure 1(b) also shows that depth of the microchannel of device is quite deeper than previously reported microchannel of capillary electrophoresis chips and width. It was fabricated 40-80 μm wide microchannel of device, which is much smaller than microchannel in this study[5]. The surface of the microchannels was expected to be harsh because laser writer burnt or ablated PDMS to make microchannels. However, no substantial obstacles for both electrophoresis and amperometric measurement from the roughness were detected in this study. The reason can be originated from two aspects: first, the capillary or microchannel devices are pre-conditioned for a long time before measurement of bisphenol-A with buffer, which can remove any remained particles on surface. Second, the surface might be slightly swollen with buffer, which minimizes effects from surface roughness. No SEM image of swollen surface could be obtained in this experiment. Particularly, in a separate experiment adopting ATR (Attenuated Total Reflection) FT-IR with a normal surface of PDMS mold and an ablated surface of PDMS mold, there are no noticeable differences in spectra of chemical state (data not shown here). It was reported that laser irradiation often generate OH group or Si-O group depending on incubational condition[11]. However, PMMA was reported to be same in its chemical state before or after an excimer laser irradiation[12].

For the laser ablation, etch rate or depth of microchannel can be determined by the effective absorption

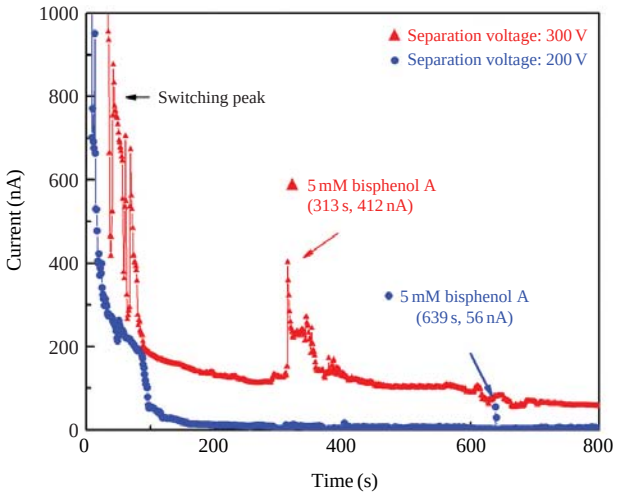

Figure 2. Electropherogram of bisphenol-A detection on device having width of 290 µm and a depth of 900 µm at separation voltage of (●) 200 V and (▲) 300 V.

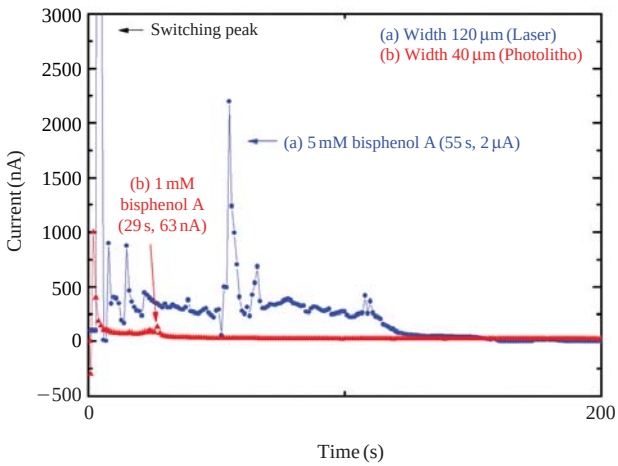

Figure 3. Electropherogram of bisphenol-A detection at separation voltage of 300 V on (a) laser fabricated device having width of 120 µm and (b) photolithography fabricated device having width of 40 µm.

coefficient of laser and fluence of laser[10-12]. In addition to these parameters, there are other parameters which determine PDMS patterning profiles. The most critical one will be traverse or moving speed of laser writer. In this study, 18 cm/s of its speed was chosen to be optimistic to have the smoothest surface.

Figure 2 shows electropherograms of bisphenol-A detection with patterned ITO electrode and PDMS which were made by programmable laser writer. Figure 2 shows the results of amperometric detection performance when applying +60 V/cm of separation electric field between buffer and detection reservoir at separation voltage of 300 V and +40 V/cm at 200 V. The depth of micro channels in the microfluidic device was about 980 µm and width of micro channels was about 290 µm. The peak current (●) at 200 V of 639 sec was shifted to left noticeably with the higher separation voltage of 300 V of 313 sec (▲), which originates from differences of the separation electric field between buffer and detection reservoir. At the same time, separation electric field also determines the height of peak currents (412 nA vs. 56 nA).

Figure 3 shows electropherograms of bisphenol-A detection both on (a) laser fabricated microchannel and (b) photolithography-fabricated microchannel. It is clearly shown that the peak current for laser written microchannel was shifted to 55 sec at 300 V as compared with the microchannel by photolithography process which has a peak current of 29 sec. The peak current was shifted to right noticeably because of the large width and deep depth. In addition, roughness of the surface in channels could contribute to the shift of the peak current. In a word, in the larger and rougher channels, the more transport time the bisphenol-A molecules would need. However, it is believed that the shift of peak current from 29 sec to 55 sec is mainly from the difference of cross-sectional size of microchannel. Additionally, it was clearly shown that concentration of bisphenol-A for loading ((a) 5 mM and (b) 1 mM) and channel size of capillary ((a) 120 µm and (b) 40 µm) both influenced the size of peak currents ((a) 2 µA and (b) 63 nA) and even the size of switching peaks in Figure 3. In summary, channel size, electric filed, and concentration of analyte can determine the position and size of peak currents regardless of fabrication methods.

Conclusions

We have fabricated and tested the capillary electrophoresis and amperometric detection of bisphenol-A on a biochip fabricated using programmable laser writer. Microchannel or capillary biochips were fabricated with various widths, depths, and fixed length. Laser fabricated device showed a comparable separation performance to device fabricated by photolithography. The result demonstrates that laser writing process could dramatically simplify steps of fabrication to make electrophoresis device or microfluidic biochips.

Materials and Methods

Materials

PDMS substrate was made from Sylgard 184 (Sili-

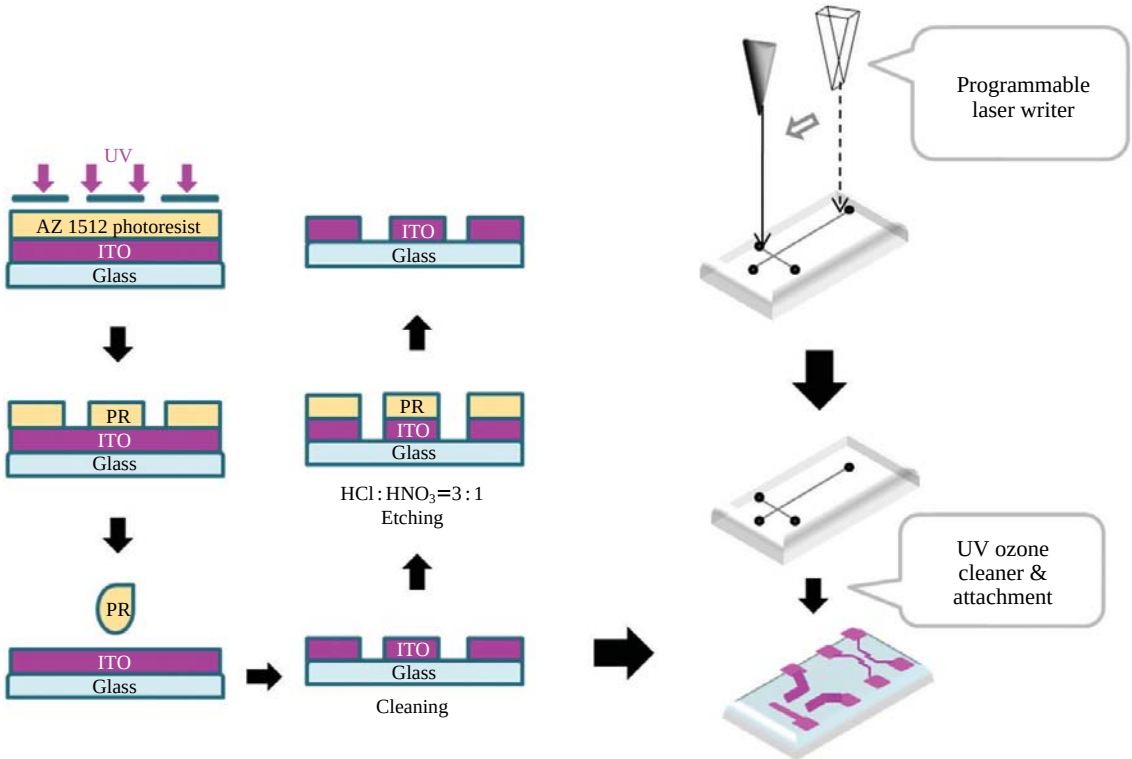

Figure 4. Schematic diagram of laser written PDMS capillary or microchannel fabrication and ITO electrodes for electrophoresis device.

cone Elastomer base, Dow Corning) and DC-184B (Silicone elastomer curing agent, Dow Corning) with ratio of 10 : 1. HMDS (Hexamethyldisilazane, Samchun pure chemicals Co. Ltd.), Positive photoresist (AZ 1512, AZ Electronic Materials KOREA Ltd.), AZ 300 MIF (2.38%, AZ Electronic Materials KOREA Ltd.), HCl/HNO_3 solution were used for the photolithography process which patterned the ITO electyodes. BPA (Bisphenol A, Wako Pure Chemical Industries Ltd.) was pruchased as a detecting sample. MES (2-(N-mor-pholino) ethanesulfonic acid) solution fixed to pH 6.5 using 10 N NaOH was used as buffer.

Device Fabrication

A schematic diagram of a simple fabrication method for PDMS CE device is shown in Figure 4. PDMS (Poly-Dimethyl Siloxane) was used for a material of capillary channel as mold and ITO (Indium Tin Oxide) glass as an electrode for electrochemical detection of chemicals. The programmable CO_2 laser (Infrared light) writer (PL-40K, Koreastamp, Korea) was used to make capillaries or microchannels on PDMS mold. For a device having photolithography process of PDMS microchannel, photosensitive SU-8 (Microchem Co., USA) was spin coated, baked, and exposed to UV light from mask aligner (MDE-8000, Midas System Co, Korea). The laser beam was focused with a lens (f=30 mm) onto the PDMS surface. The programmable laser writer can change its generating power at 40-50 Watts generating thermal energy to make microchannels of a chip in this experimental. A pristine PDMS and laser treated PDMS surfaces were analyzed with ATR (Attenuated total reflectance) FT-IR (Jasco Co. 400plus, Japan). Electrodes were shaped by photolithography process and were etched by HCl/HNO_3 solution. In order to bind laser patterned PDMS channel and ITO glass into a completed capillary electrophoresis device, UV-ozone treatment was used. Changes of current measured at electrode were recorded with time by the source measure unit. The whole measurement system was controlled by Labview program, which could automatically store a current (I) peaks for detection of the chemicals.

Bisphenol-A Detection

All microchannels or capillaries in devices were preconditioned before electrophoresis and measurment. For example, all capillaries were rinsed by Acetone and DI-water for one hour. Next, buffer solution was filled and placed in all capillaries for one hour. After

the preconditioning, the entire microchannels was full of buffer solution without any bubbles for capillary electrophoresis process. The electrophoretic run buffer solution was 10 mM MES at pH 6.5.

The 5 mL of 5 mM Bisphenol-A was injected to the sample reservoir by applying 60 V/cm for 50 s. Then, bisphenol-A was flushed to separation channel, the capillary electric field was set to 60 V/cm and 40 V/cm between buffer reservoir and detection reservoir. After applying voltage, sample flowed through the capillary between buffer and detection reservoir and among three electrodes, working, reference and counter electrode, amperometric detection was performed when bisphenol-A reached to working electrode and caused redox reaction at the surface of working electrode. Then, all current included a peak current were detected and recorded on a computer. More detailed methods are available in reference[5].

Acknowledgements

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