DETECTION OF DNA AT THE MICROCHIP
Study of DNA Detection Microchip by Oxidation Peak of DNA

Gi-sung Joo, You-Cheol Jang, Islam Atm Kamrul
Department of Nano Science and Engineering, Myongji University, Gyeonggi, Korea

Yong-Sang Kim
Department of Electric Engineering, Myongji University, Gyeonggi, Korea

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Abstract: We have demonstrated the separation and detection of DNA on the microchip based on amperometric with capillary electrophoresis. To enhance analytic perfermance such as throughput and analysis time, electrophoretic separation of DNA in capillaries or on microchips has been investigated using various microchip structures. Compared with commonly used laser induced fluorescence method, this method is more compatible with microchip and offer improved portability and miniaturization. Through Cyclic voltammetric experimental, we could optimized detection voltage for detection of DNA. At the optimal detection voltage, DNA fragments were successfully separated and detected with high sensitivity and stable baseline.

1 INTRODUCTION

DNA analysis is important step in area of biochemistry as well as molecular biology. In most cases DNA is detected by fluorescent or optical spectroscopy after agarose gel electrophoresis. However, this procedure is tedious, time consuming and require expensive equipments. Therefore miniaturization of DNA analysis is necessary. Capillary electrophoresis (CE) microchip, introduced by Manz et al. in the early 1990s, alternative for separation of biological compounds. To enhance throughput and to shorten analysis time, electrophoretic separation of DNA in capillaries or on microchips has been suggested using different microchip structures. Optical microarrays are arguably the most widely used type of biosensors in DNA analysis where detection of specific DNA sequences are based on labeling sample DNAs with fluorophores. While fluorescence-based detection technologies have shown tremendous utility, they suffer from the drawbacks of labor-intensive sample preparation, high cost, and complex and bulky fluorescence detection instrumentation. However, amperometric method is measured to change current according to oxidation of analytes. Guanine and adenine electro-oxidation is useful for the amperometric method. Using this technique, fragments of negatively charged DNA can be resolved inside a capillary by application of potential. The resolved DNA can be detected amperometrically using oxidation peak of adenine base.

In the present work, we have attempted to develop an amperometric. The microchips are usually fabricated from silicon and glass. However, polymeric materials are also used due to their properties such as low cost, high flexibility, and simply fabrication procedures. Several polymers such as poly(dimethylsiloxane) (PDMS) and poly (methyl methacrylate) (PMMA), polyester have been reported for fabrication of microchip. Our microchip was fabricated on glass substrate and microchannels were laid in PDMS mold. The capillary was filled with polyacrylamide gel and separation was achieved by application of DC potential. This technique was used in resolving DNA fragments

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2 EXPERIMENTAL

2.1 Chemicals

The testing analyte was DNA ladders (100–1500 bp, 9 fragments) (Biosesang). We have used Sylgard 184 from Dow Corning Corp. (Midland, MI,USA) and SU-8 50 photoresist and XP SU-8 developer from Micro-Chem Co. Acrylamide: bisacrylamide (29:1) solution was purchased from Bio Basic. Ammonium persulphate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were bought from Biosesang. Other reagents were purchased from Biosesang. Deionized water (DIW) was used throughout this research.

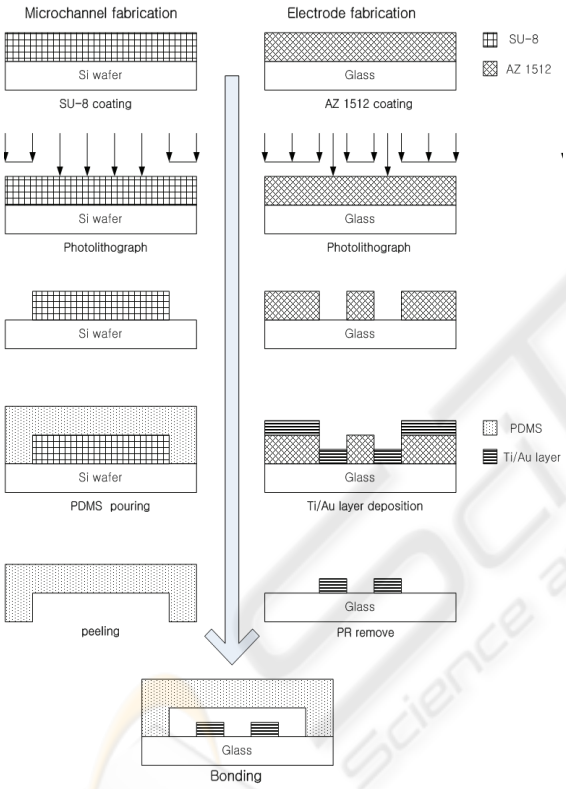

Figure 1: Fabrication process for Microchip.

2.2 Fabrication of the Microchip

Fig. 1 shows the simple procedure for the fabrication of the CE–AD device. A silicon wafer was cleaned. The wafer was then coated with SU-8 negative photoresist using a spin coater. The molding masters were made by photolithographic process. The height of the positive patterns on the molding masters, which were equal to the channel depth created on the PDMS layer, was 200 μm when measured with a surface profiler. The PDMS layer was fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto a molding master, followed by curing for at least 1 h at 72 ℃. The cured PDMS was peeled off from the mold, and reservoirs were made at the end of each channel using a 3 mm circular punch. The channels had a width of 250 μm. The separation channel was 2 cm long. The Au-electrodes for use in CE–AD were deposited on a glass substrate by thermal evaporation system. For this purpose, 1.8 μm thick photoresist (AZ-1512) was spin-coated on the bare glass and patterned for Au-electrodes. After evaporation, 320 nm thick Au layer was deposited on an adhesion layer of 50 nm thick Ti. In order to avoid the interference of high separation electric field on amperometric detection, two decoupling-ground electrodes were positioned in front of the three-electrode amperometric detection system that consisted of Au-electrodes of 250 μm width (Fig. 2). Finally, the PDMS mold was bonded with glass substrate after UV-ozone treatment for 40 min.

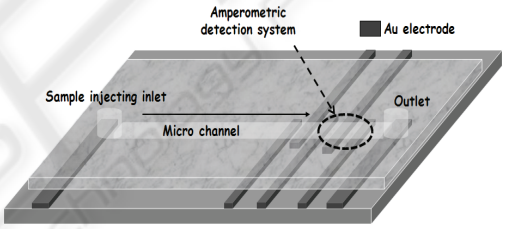

Figure 2: Schemetic of PDMS/Glass device.

2.3 Microchannel Treatment

Before use in CE–AD procedure, the microchannel was cleaned by flushing with 1 M NaOH for 45 min followed by D.I. water for 15 min at 5 μl/min flow rate using a precision pump (KD Scientific, USA) and then dried. Thereafter, 5% polyacrylamide prepolymer solution consisting of a mixture of 875 μl Phosphate Buffered Saline (PBS), 125 μl 40% (29:1) acrylamide/bisacrylamide solution, 1 μl TEMED and 4 μl 10% APS was introduced into the microchannel. The microchip was ready for DNA separation after 30 min of polymerization time.

2.4 CE-AD Procedure

For CE–AD, 5 μl testing sample was introduced in the injection reservoir using a micropipette. After the sample loading, an electric field was applied between the sample reservoir and the sample waste reservoir. Amperometric detection was performed with three-electrode configuration (Fig. 2) placed in the path of buffer flow. The potential between

working and reference electrode was +1 V DC. Redox reaction of adenosine from testing analyte on the working electrode generated current peaks, which was detected, recorded and stored directly on a notebook computer using a CHI 800B potentiostat. This instrument recorded 100 data points per second. The testing analyte consisted of 9 fragments (dsDNA).

3 RESULTS AND DISCUSSION

The CE–AD microchip developed in the present research was used in separation and analysis of DNA fragments. The chip was fabricated on transparent glass substrate, which assisted in Uvozone bonding with PDMS mold containing microchannel as well as loading of samples into the reservoir. The benefits of using PDMS as the material for fabricating microchannel were its transparent color, ease in fabrication using negative molding method, flexibility, mechanical strength and stability. The amperometric detection system consisted of in-channel working, counter and reference electrodes. Two decoupler electrodes were used to ground the separation current in order to minimize electric noise. The choice of gold Microelectrodes was based on its inertness and ease in patterning over glass substrate.

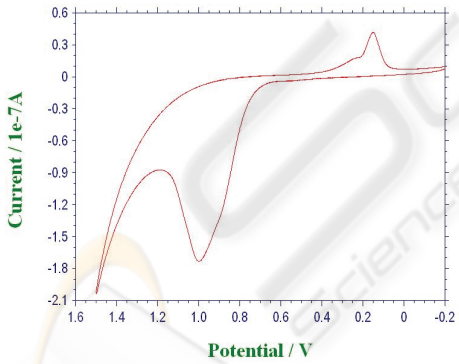

Figure 3: Cyclic voltammogram of DNA ladders in microchip. The scan rate is 10mV/s^{-1}.

The adenine and guanine base in the DNA chain is to produce oxidation peak at +1 V DC (Fig.3). Therefore, this potential was used for detection of DNA fragments being separated inside the microchannel filled with 5% polyacrylamide gel. Although technically it was possible to resolve DNA fragments in a narrow capillary without the use of polyacrylamide, but that would require quite long capillary length which may hinder the detection of DNA due to its adsorption on long PDMS capillary walls. Therefore, to minimize the detection time as well as increase detection sensitivity, we used 2 cm channel length filled with 5% polyacrylamide. The DNA fragments were separated in the process due to difference in molecular weight. Separation DC potential is important factor as well as buffer solution to enhance perfermance of device. We have demonstrated effect of separation potential. all nine fragments could be identified (Fig. 4 a,b) from the Electropherogram for 85min at 100 V/cm and 40min at 150 V/cm, repectively.

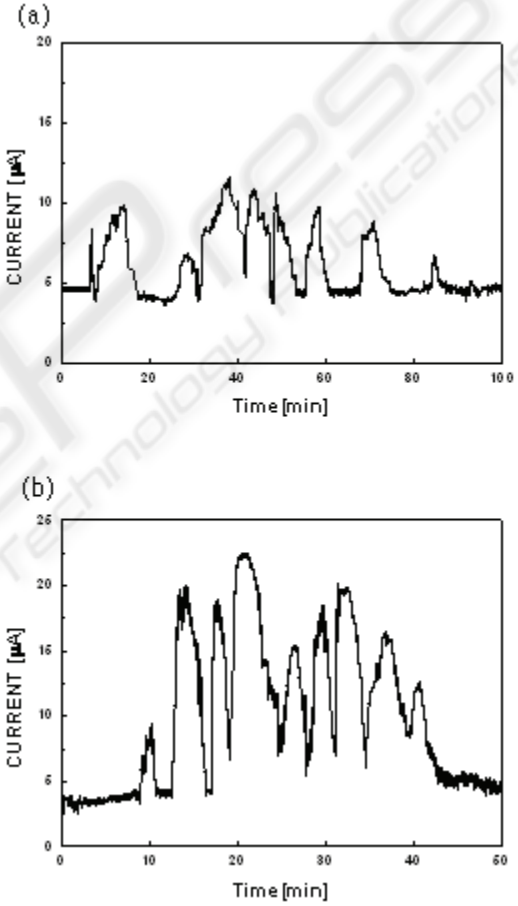

Figure 4: Electropherogram of DNA fragments using PBS with 5% polyacrylamide for applied separation field 100V/cm (a) and 150V/cm (b).

The DNA fragments could be resolved during CE–AD process and all nine fragments could be identified from the Electropherogram (Fig.4). This proved the feasibility to build a cost-effective and power efficient microchip to analyze DNA fragments. The specificity of proposed CE–AD method shall depend on the presence of additional electroactive species pro-ducing amperometric peak

at + 1 V. The numbers of such species are limited in most of the molecular biology techniques involving DNA electrophoresis, including PCR, therefore causing limited impact on the effectiveness of proposed method.

4 CONCLUSIONS

In the present study, we devised a PDMS-based microchip for capillary electrophoresis amperometric detection of DNA fragments. At the cyclic voltammetry experimental, oxidation peak of adenine and guanine was indicated at + 1 V. The capillary was filled with 5% polyacrylamide gel for effective separation of DNA fragments under the influence of separation potential. The amperometric detection (AD) system involved in-channel gold micro-electrodes and this technique was used in resolving DNA fragments.

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