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# A capillary electrophoresis microchip for amperometric detection of DNA

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ABSTRACT

Capillary electrophoresis amperometric detection (CE–AD) microchip was fabricated for separation and detection of DNA fragments. The chip was fabricated on glass substrate and microchannels were laid in PDMS mold. The capillary was filled with 5% polyacrylamide gel and separation of DNA fragments of different molecular weight was achieved by application of 100 V DC potential across sample and waste reservoirs made at the capillary ends. The amperometric detection (AD) system involved in-channel gold microelectrodes to analyze oxidation peak for adenine residues in DNA chain. This technique was used in resolving single as well as double stranded DNA fragments.



## 1. Introduction

DNA analysis is an important step in clinical and forensic analysis and other areas of biochemistry as well as molecular biology. In most cases DNA is detected by fluorescent or optical spectroscopy after agarose or polyacrylamide gel electrophoresis [1]. These procedures are time consuming and require sophisticated and expensive equipments. Therefore, miniaturization of DNA analysis is necessary. Capillary electrophoresis (CE) microchip, introduced by Manz et al. in the early 1990s [2,3], is fast becoming an attractive and powerful technique for DNA analysis due to its performance speed, small reagent consumption and miniaturization. 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 [4]. The microchips are usually fabricated from silicon and glass. However, polymeric materials are also used because of their properties such as low cost, high flexibility, and simple fabrication procedures facilitating mass production of device. Several polymers such as poly(dimethylsiloxane) (PDMS) [5] and poly(methyl methacrylate) (PMMA) [6], poly carbonate (PC) [7], polyester [8], and poly(ethylenterephthalate) (PET) [9] have been reported for this purpose.

In the present study, we demonstrated a microchip capillary gel electrophoresis with amperometric method for analysis of DNA fragments. The chip 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. The amperometric detection (AD) system involved in-channel gold microelectrodes and this technique was used in resolving single as well as double stranded DNA fragments.

## 2. Experimental

### 2.1. Chemicals

The testing analytes included 100 μM 25-mer single strand poly-A DNA (Bionics), and 100 μM 25 bp double strand (poly-A/T) DNA (Bionics) and DNA ladders (100–1500 bp) (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

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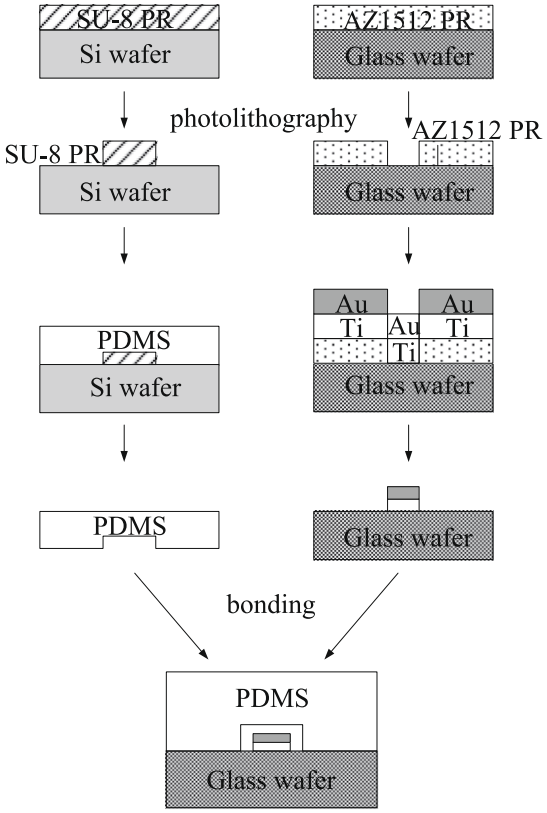


**Fig. 1.** Microchip fabrication process.

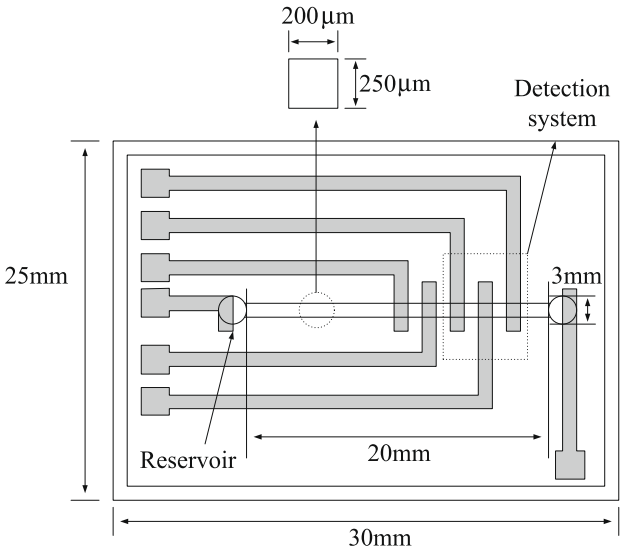


**Fig. 2.** Schematic diagram of CE–AD microchip.

Biosesang. Other reagents were purchased from Biosesang. Deionized water (DIW) was used throughout this research.

### 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 and oxidized with piranha solution (6:1 $H_2SO_4/H_2O_2$). 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 °C. 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 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 [10].

### 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 of 100 V 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 +700 mV DC. Redox reaction of adenosine from testing analytes on the working electrode generated current peaks, which was detected, recorded and stored directly on a notebook computer using a Kiethly 236 Source-Measure meter with GPIB connection and Labview software interface. The acquisition rate of this connection was 45 data points per second.

The testing analytes consisted of 25-mer single strand poly-A DNA (ssDNA) as well as 25 bp poly-A-poly-T hybridized double strand DNA (dsDNA). On the latter half of the research 1.5 kb commercial DNA ladder comprising of a mixture of DNA fragments of nine different sizes was resolved on CE–AD chip.

## 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 UV-ozone bonding with PDMS mold containing microchannel [11] 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 [11]. The amperometric detection system consisted of in-channel working and reference electrodes, whereas, counter electrode was placed inside waste reservoir, so as to avoid formation of air bubble inside the microchannel. Two decoupler electrodes were used to ground the sepa-

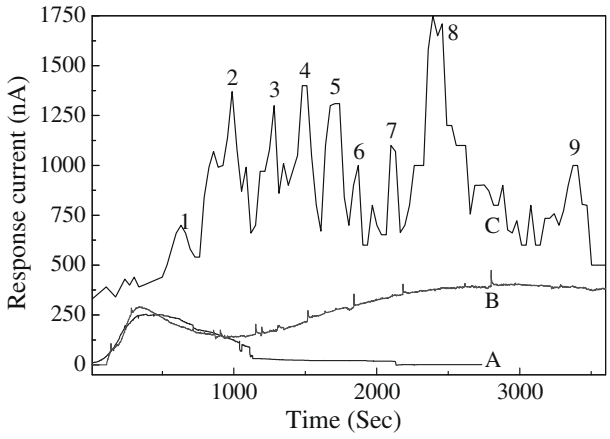


**Fig. 3.** Electropherograms of: (A) 25-mer ssDNA (poly-A); (B) 25 bp dsDNA (polyA–T); (C) 1.5 kb DNA ladder detected using amperometric method. Separation channel length = 2 cm, separation voltage: 100 V, detection voltage: 0.7 V, buffer solution: 1X PBS, pH 7.4. Peaks: (1:100, 2:200, 3:300, 4:400, 5:500, 6:600, 7:800 and 9:1500 bp DNA).

ration current in order to minimize electric noise. The choice of gold microelectrodes was based on its inertness and ease in patterning over glass substrate. The adenosine base in the DNA chain is known to produce oxidation peak at +0.7 V DC [4]. 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 sizes were separated in the process due to difference in molecular weight.

In order to verify the concept, we first tested 25-mer poly-adenosine in pure form to generate amperometric peak (Fig. 3A). Subsequently, the hybridized double stranded poly-A-polyT DNA (Fig. 3B) was used, which produced similar peak as to ssDNA, thereby proving the concept that the amperometric peaks correspond to adenosine residues. The sensitivity of such detection was 0.543 nA/pmole of ss/dsDNA with 93% reproducibility. With a baseline electrical noise of 80 nA in running buffer devoid of DNA, the detection limit of device (at $S/N = 3$) was 130 pmol ss/ds DNA, compared to 2–10 nmoles using conventional ethidium bromide staining in standard 0.5 cm agarose gel DNA band [12].

Our final goal in the present study was to be able to resolve DNA mixture of different lengths. Routine molecular biology experiments such as PCR analysis involve detection of DNA on agarose gel electrophoresis [1]. The process is tedious and requires manual input to a large extent. Therefore, in the present work we used commercial DNA ladder comprising of a mixture of different DNA sizes. The DNA fragments could be resolved during CE–AD process and all nine fragments could be identified (Fig. 3C) from the Electropherogram. This proved the feasibility to build a cost-effective and power efficient microchip to analyze DNA sizes in rapid time. The specificity of proposed CE–AD method shall depend on the presence of additional electroactive species producing amperometric peak at 0.7 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. Conclusion

In the present study, we devised a PDMS-based microchip for capillary electrophoresis amperometric detection of DNA fragments. The capillary was filled with polyacrylamide gel for effective separation of DNA fragments under the influence of separation potential. The amperometric detection (AD) system involved in-channel gold microelectrodes and this technique was used in resolving single as well as double stranded DNA fragments.

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