MICROCHIP CAPILLARY ELECTROPHORESIS DEVICE FOR AMPEROMETRIC DETECTION OF DNA WITH REDOX INTERCALATION

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Abstract: Microfabricated biochips are very efficient platforms for analysis of biologically important molecules such as DNA, RNA, enzymes, antibodies etc. These devices requires sample in micro/nano volume and produces faster and better results. For these reasons, we fabricated a simple, disposable microfluidic device for amperometric detection of DNA intercalated with methylene blue redox dye. The devices were fabricated using conventional photolithographic method. The microchannels were laid in PDMS using negative molding. The microchannel was 2 cm in length while the height and thickness were 250 µm and 200 µm respectively. The electrodes used for electrophoretic separation and amperometric detection were made of gold and were deposited by thermal evaporation on glass substrate. For the detection of DNA, fish sperm DNA was intercalated with methylene blue as an analyte. The cyclic voltammograms of free methylene blue and those of different concentrations of DNA intercalated with same amount of methylene blue was obtained in this study. The intercalated DNA was then injected in the sample reservoir of fabricated device and subjected to a separation electric field. The i-t curve was monitored for this process. The electropherograms thus obtained suggested a possibility of rapid detection of DNA with high sensitivity and reproducibility.

1 INTRODUCTION

Ever since the publication of DNA's double helix structure, electrophoresis has been a standard, indispensible analytical tool in modern biochemistry and molecular biology; electrophoretic procedures are used in almost every aspect of basic or applied biomedical and clinical research.

Traditional techniques as performed today in the majority of laboratories, is still typically a manual process which makes electrophoretic procedures often time consuming and labour intensive. Capillary electrophoresis (CE) on the other hand, is a relatively new separation technique that is ideally suited for handling small amounts of sample material. The advent of microfabricated fluidic devices in the past decade promises to address some of these issues by miniaturizing and automate these devices including the CE process (In-Je Yi, 2006).

More recently, electrochemical detection (ED) has been reported for microchip (MC) CE (Ju-Ho Kim, 2004). This mode of detection is ideally suited for miniaturization to the microchip format. If the power supply and electrochemical analysers are also miniaturized, it is possible to envision a complete µTAS (Dolnik, 2000; Woolley, 1998).

A typical MC-CE device has channel widths varying from 50 to 200 µm with typical straight separation channels between 1 and 5 cm in length. A serpentine or semi-circular design can be implemented to increase the separation channel length up to 15 cm (Jacobson, 1994; Culbertson, 2000).

DNA-binding or intercalating dyes have been

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used for fluorometric and amperometric DNA assays and in flow cytometry applications. Ethidium bromide (EtBr) was the first of such intercalators to be used for DNA assays. Interestingly, the resolution of dsDNA separations in CE can be improved by using intercalating dyes. This is usually done by adding dye to the running buffer (and/or sample) in concentrations of 0.5 to 5 mg/mL. The dye molecule inserts itself ("intercalates") between the base pairs of DNA, changing the molecular persistence length, conformation, and charge of the DNA.

Amperometric CE detection was first reported as a detection technique for CE by Wallingford and Ewing in 1987 for the quantitation of catechol and catecholamines. Amperometric detection is based on electron transfer to or from the analyte of interest at an electrode surface that is under the influence of an applied DC voltage. The result of electron transfer is a redox reaction at the electrode that produces a current that is directly related to the analyte concentration. Thus, by analysing the DNA intercalated redox-active dye by amperometric method; it should be possible to analyse the concentration of DNA after its capillary electrophoretic separation. In the present work, we fabricated a CE-AD device for CE separation followed by amperometric analysis of DNA-intercalated methylene blue (MB) dye, thus providing the basis for detection of DNA fragments by indirect means.

2 MATERIALS AND METHOD

2.1 Reagents and chemicals

DNA Sodium salt fish sperm (Ultra-Pure) was obtained from Bio Basic Inc., Korea. Methylene blue (MB) (Reagent grade) was purchased from Biopure, Canada. PDMS Sylgard 184 was from Dow Corning Corp. (Midland, MI, USA). SU-8 2000 negative photoresist and XP SU-8 developer were from Micro-Chem Co. USA and AZ 1512 positive photoresist and AZ developer was from AZ electronics material, Korea. The other chemicals of ACS grade were purchased from Sigma-Aldrich, Korea. All solutions were prepared afresh, stock solution were made using double-distilled deionized water (DI) and further diluted to required concentration using the supporting electrolyte.

2.2 DNA Precipitation

Fish sperm DNA obtained was precipitated using ethanol precipitation. After precipitation, the mixtures were then centrifuged to collect the precipitate. The pellet was washed twice with 1 ml of 70 % Ethanol. The pellet was air dried. Later, the pellet was dissolved in 800 µl of DI water and used for spectrophotometric study. For electrochemical study the samples were further diluted with 200 µl of 1 M KCl.

2.3 Fabrication of Microchip

A three-electrode detection system was used for CE-AD. The simple process flow for the fabrication of the CE-AD device is shown in Figure 1. We can divide the procedure in two parts: fabrication of microchannel in PDMS mold and laying gold electrodes on glass substrate. The electrodes were 200 µM each in width. Gold electrode was choice for the detection and separation electrodes due to its inertness to redox reaction. The microchannels, each

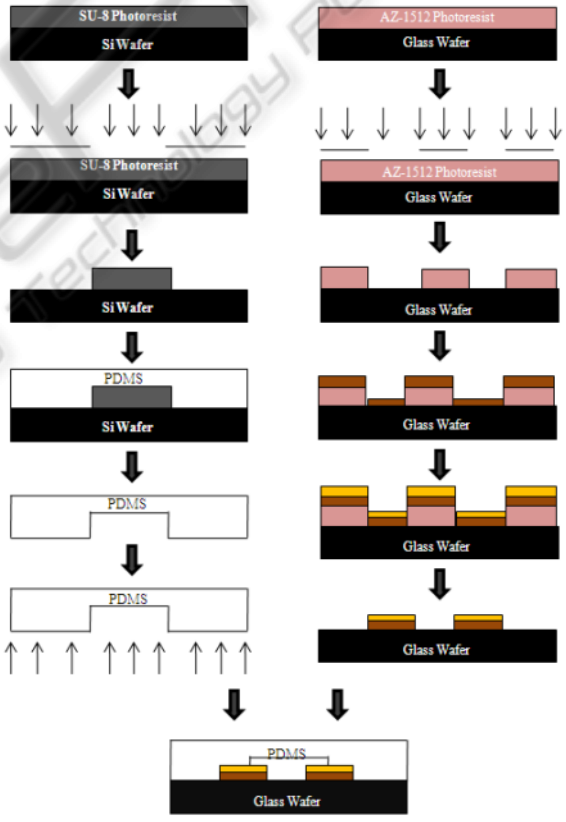

Figure 1: Fabrication of CE-AD device.

200 µm in width, 250 µm in height and 2 cm in length were fabricated using negative molding method (Gi-Sung Joo, 2009). Finally the PDMS was bonded on glass wafer using UV ozone bonding.

2.4 Electrochemical Detection

Electrochemical detection was performed using Electrochemical analyser, CHI 800B (CH Instruments, USA). The three-electrode electrochemical system was used for cyclic voltammetry, which consisted of an Ag/AgCl reference electrode (RE-5B, BASi), a Platinum wire counter electrode (CHI 115) and a gold working electrode (CHI101). Prior to voltammetry, the gold and platinum electrodes were cleaned using chromic acid, polished using electrode polishing kit (CHI 120) and cyclic sweep was performed in the range of 2 V to -2 V at a scan rate of 100 mV/sec in 0.1 M Sulphuric acid until a stable curve was obtained. Voltammetric sweep in sulphuric acid was repeated before every voltammetric study. Cyclic voltammograms of 200 mM Potassium chloride, 100 mM MB in 200 mM KCl, intercalated MB-DNA complex sample and the other two precipitated negative control were recorded at various potential range and scan rate.

3 RESULTS AND DISCUSSION

Spectrophotometric study of MB-DNA complex revealed that approximately 0.03 µM of MB binds to 1 mg/mL. Further electrochemical studies were carried out with the free MB and MB-DNA complex.

3.1 Electrochemistry of MB and DNA

A reversible redox cycle was obtained at 100 µM concentration (Figure 2) in scan range 0.2 V to -0.6

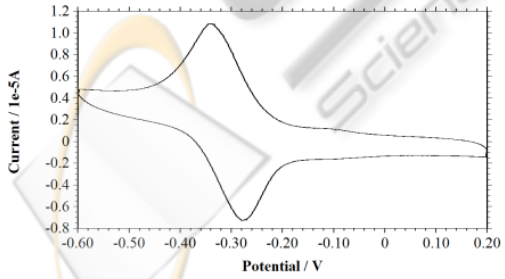

Figure 2: CV of methylene blue, Conc.: 100 µM, in KCl 200 mM, scan rate 0.1 V/s.

V with scan rate 100 mV/sec. The cyclic voltammograms shows a cathodic process of MB (Ep_C) at -0.280 V.The precipitated MB-DNA complex was studied in the same range as that of free MB. Figure 3 shows the cyclic voltammograms of different concentration of intercalated MB-DNA complex. The cyclic voltammograms of the complex shows the similar redox process as that of free MB with a cathodic process (Ep_C) at -0.28 V.

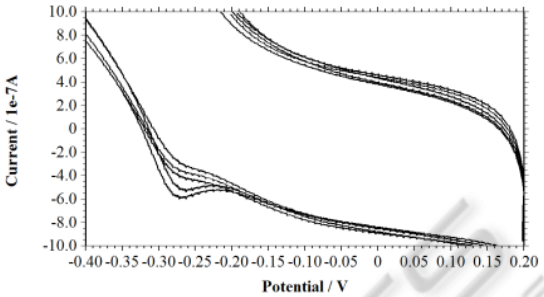

Figure 3: CV showing peaks for different concentrations of MB-DNA complex in 200 mM KCl, Scan rate: 0.1 V/s.

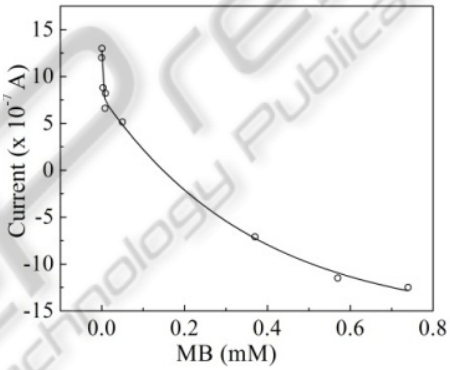

Figure 4: Shows a correlation between various concentration of intercalated MB and peak current produced by it using cyclic voltammetry. Suitable controls without DNA and without MB did not produce any corresponding peak, suggesting that the peak obtained in the DNA-MB complex is only due to the MB bound to DNA and not due to any other free MB.

3.2 Microchip CE-AD

Figure 5 shows the image of microchip CE-AD device. The microchannel in the device was filled with 200 mM KCl as a separation medium and support analyte. A blank i-t curve was observed without addition of any test analyte for the control purpose. The i-t curve of free MB was also monitored which showed no peak as free MB is positive in charge and will not migrate in the channel along the separation field for DNA.

The electrophoretic separation for MB-DNA complex was initiated by adding 2 µL of complex into the sample reservoir and a separation voltage of 100 V was applied. DNA-MB complex as migrating towards anode is detected amperometrically using in-channel gold working electrode. Figure 6 shows

the i-t curve of MB-DNA detection through CE-AD. Different peaks resemble MB attached to various bands of the DNA. The peak was detected after 2 min of sample injection. The detection sensitivity and LOD for this reaction were 640 nA/µg of DNA and 140 ng of DNA respectively.

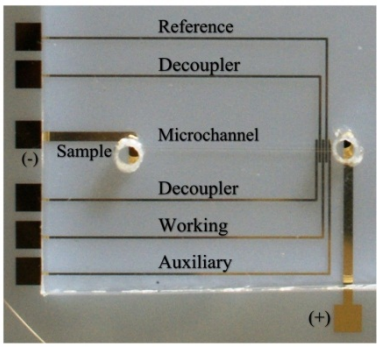

Figure 5: CE-AD microchip.

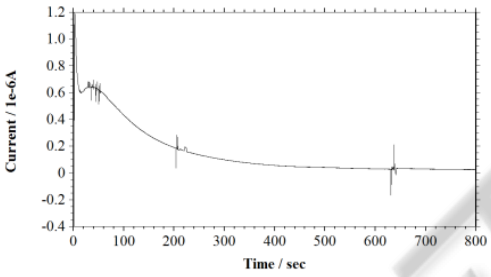

Figure 6: i-t curve of MB-DNA complex in KCl 200 mM.

Therefore, it would be possible to first determine peak current for MB from CE-AD experiment and then decipher its concentration using standard curve obtained under control conditions (Figure 4). Thereafter, DNA concentration can be calculated from the standard value obtained. This shows the potential of present study towards successful detection of DNA of any sizes and deciphers its concentration on our microchip. Further studies are underway to enhance the reproducibility and sensitivity of these devices and for detection of a mixture of short DNA fragments such as found in DNA molecular weight markers.

4 CONCLUSIONS

It was concluded in the present study that disposable CE-AD microchips with gold electrodes and PDMS channels can be used to prepare effective DNA detector in place of existing gel electrophoresis based system. The disposable electrochemical detector fabricated in this study displayed good performance in terms of sensitivity, stability, resolution and peak density. This type of microchip can also be used for detection of various other organic or inorganic compounds, or can be integrated with microfluidic modules on a µTAS.

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