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Voltammetric Analysis on a Disposable Microfluidic Electrochemical Cell

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A microfabricated electrochemical cell comprising PDMS-based microchannel and in-channel gold microelectrodes was fabricated as a sensitive and a miniature alternative to the conventional electroanalytical systems. A reproducible fabrication procedure enabled patterning of multiple microelectrodes integrated within a PDMS-based fluidic network. The active area of each electrode was 200 μm × 200 μm with a gap of 200 μm between the electrodes which resulted in a higher signal to noise ratio. Also, the PDMS layer served the purpose of shielding the electrical interferences to the measurements. Analytes such as potassium ferrocyanide; amino acid: cysteine and nucleoside: guanosine were characterized using the fabricated cell. The microchip was comparable to bulk electrochemical systems and its applicability was also demonstrated with flow injection based rapid amperometric detection of DNA samples. The device so developed shall find use as a disposable electrochemical cell for rapid and sensitive analysis of electroactive species in various industrial and research applications.

Key Words : Microfluidic chip, Microelectrochemistry, Voltammetry, Hydrodynamic voltammetry, Flow-injection amperometry

Introduction

Various methods are routinely used for qualitative and quantitative analysis of electroactive organic and inorganic compounds, out of which cyclic voltammetry (CV), pulse voltammetry and others have widespread use because it provides an easy procedure for direct and selective detection. Electrochemical studies are an integral part of many chemical and biological sensors.[1-3] CV in particular, determines the oxidation and reduction potential and electrochemical reaction rates of an analyte which can be used for detecting compounds like heavy metals, pesticides and biomolecules in different samples.[4-8] These voltammetric procedures require ultra-clean electrodes for conducting electrochemical reactions. Electrode cleaning is a cumbersome task and requires hazardous and expensive chemicals. For example a typical electrochemical cleaning of gold or platinum working electrode requires several steps such as putting the electrode overnight in chromic acid followed by electrode polishing and electrochemical cleaning by cycling the potential in strong acid. Disposable electrochemical cells on the other hand provide the opportunity to reduce the number of these cumbersome steps for electrode cleaning. Most of these disposable electrodes are employed in handheld biosensors or chemical sensors and are usually made up of carbon, silver or low cost materials with ideally the screen printing method.[9] These electrodes usually operate by placing them in analyte or solutions which limits the wider applicability of these electrodes. For example, complex and long running tasks such as CV, chronoamperometry etc. can be difficult to implement with these disposable electrodes without sacrificing the sensitivity. Moreover, continuous monitoring of drugs and pharmaceuticals in a production environment is rather difficult with these disposable or conventional electrochemical electrode setup.

In these regards, a microfluidic platform with microelectrodes can greatly facilitate sample handling, electrode cleaning, on-line/in-line detection and can enhance sensitivity by reducing interferences. The concept of microelectrodes is receiving large attention recently.[10,11] The advent of microfabrication technology has widened the scope and applicability of microelectrodes. The recent trend to miniaturize chemical and biological assays is stimulating the development of the field of microfluidic devices.[12-15] The small scale of the experiments result in an amazing reduction in solution consumption, meaning that lower volume of sample and reagents are required and less waste is generated. Improved fabrication techniques and the use of new materials are helping the field to move toward its ultimate goal of producing functional and low-cost micro total analysis systems. Microelectrodes have been employed for those applications demanding electrochemistry in restricted volumes, in solutions with high resistance as well as in short-time regimes.[16,17]

The behavior of microelectrodes differs from conventional sized electrodes (radius 1 mm or greater) in that nonlinear diffusion is the predominant mode of transport. This difference in mass transport from the bulk solution toward the electrode has several important implications that make microelectrodes very attractive in many areas of electroanalytical chemistry. These include reduced ohmic potential drop, a decreased time constant, a fast establishment of steady-state signals, and an increased signal-to-noise ratio.[16,17] In the

past, various groups have reported use of microelectrodes for classical electrochemical studies. Kang *et al.* reported fabrication of nano sized gold electrode using etching of gold wire but such electrodes lacked the aim of miniaturization.[18] Brett *et al.* and Chen *et al.* proposed platinum disc electrodeposited with mercury and polystyrene sheets electrodeposited with gold to fabricate electrode which makes the process expensive and tedious.[11,19]

For these reasons, in the present work, we developed an electrochemical cell on the glass substrate with sub-millimeter sized gold electrodes using microfabrication technology. The microelectrode for electrochemical study *i.e.* working, reference and counter electrode were thermally evaporated under high vacuum through a shadow mask. The microchip contained negatively molded polydimethylsiloxane (PDMS) layer containing a microchannel for electrode pretreatments, reservoirs for sample application and injection. The device was conceived with a view to reduce sample and reagent consumption and to increase portability and sensitivity. The proposed device was tested for cyclic voltammetry of potassium ferrocyanide ($K_4Fe(CN)_6$), cysteine (amino acid), and guanosine (part of nucleic acids and GTP) while comparing their electrochemical characteristics with respect to conventional bulk electrodes. The device proved useful in electroanalysis of complex molecules such as amino acids and nucleic acids. Cysteine is a thiol group containing amino acid and is of typical interest to many researchers due to difficulties in its analysis using bare-gold electrode and had to rely on modified electrodes.[20,21] Rapid detection of pharmaceuticals and complex molecules such as DNA is always a challenging task. In this view, we also demonstrated a rapid protocol for analysis of fish sperm DNA, using flow-injection amperometric detection (FI-AD) on the proposed device.

Experimental

Cysteine, guanosine, potassium ferrocyanide, and sodium salt of fish sperm DNA were purchased from Sigma-Aldrich, Korea. PDMS Sylgard 184 was from Dow Corning Corp. (Midland, MI, USA). SU-8 2075 negative photoresist and XP SU-8 developer were from Micro-Chem Co., USA. The other chemicals of ACS grade were purchased from Sigma-Aldrich, Korea and used without further purification. All solutions were prepared fresh, stock solutions were made using double-distilled deionized water (DI) and further diluted to required concentration using the supporting electrolyte. Experiments were carried out without removal of dissolved oxygen.

The microfluidic device was fabricated by the simple photolithographic technique described earlier.[8] The photomask for such fabrication was designed with AutoCAD software. For fabrication of microchannels, 120 μm thick, spin-coated photoresist (SU-8 2075) was exposed to UV light through a photomask containing the desired channel design. The PDMS layer was fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto the molding master, followed by curing for at least 1 h at 72 °C. The cured PDMS was separated from the mold, and inlets and outlets were made at the end of each channel using a 3-mm circular punch.

At the same time, gold electrodes were deposited on a glass substrate using a high vacuum thermal evaporator. The glass wafers were first cleaned in a piranha solution containing 70% H_2SO_4/30% H_2O_2 (v/v) for 15 min at 60 °C. For laying the electrodes, a shadow mask containing the elec-

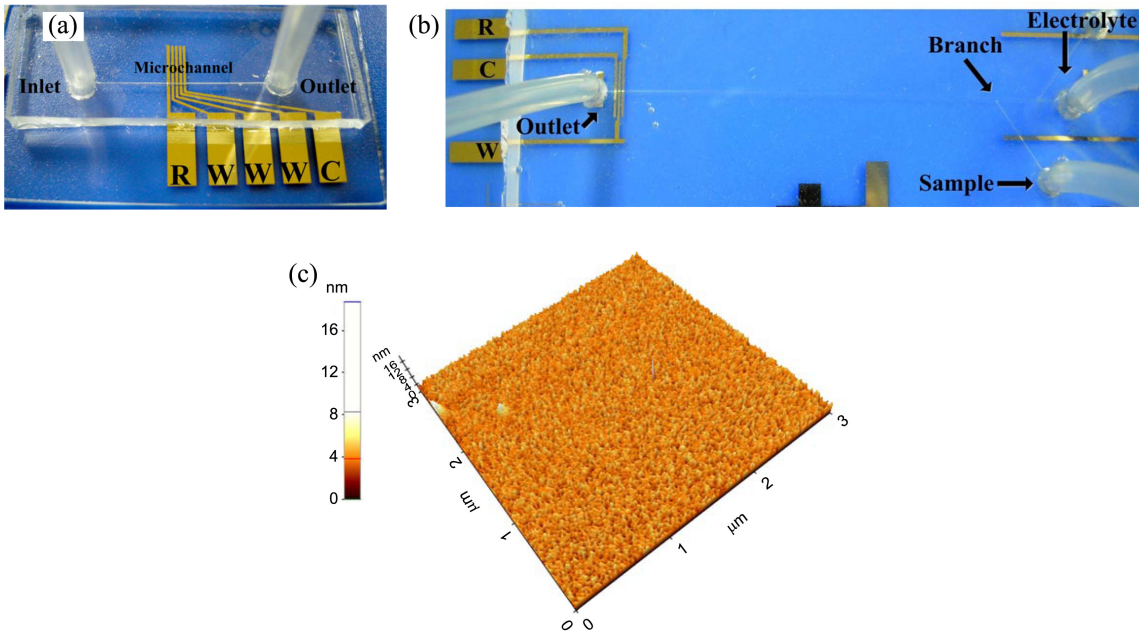

Figure 1. (a) Image of on-chip CV system showing gold working (W), reference (R) and counter (C) electrodes, inlet and outlet reservoirs along with microchannel; (b) Image of Y-shaped microchannel for FI-AD detection (c) surface profiling of the gold electrode through atomic force microscopy.

trode pattern was attached to the cleaned glass wafer. Then, in a thermal vacuum evaporator, titanium layer was deposited on the glass wafer as an adhesion layer followed by a layer of gold deposited over it. Finally the mask was removed from the wafer, leaving electrodes over glass wafer. At the end, the PDMS layer containing microchannel structures was covalently bonded on glass substrate having Au-electrode features using UV-Ozone cleaner. In order to study the surface of these gold electrodes, the AFM images were acquired on a PSIA XE 100 (Park Systems Co.) in non-contact mode with PPP-NCHR tips (silicon cantilever, Nanosensors Co.).

Electrochemical detections were performed using an electrochemical analyzer (CHI 800B, CH Instruments, USA). The three-electrode electrochemical system was used for electrochemical studies; where Ag/AgCl reference electrode (RE-5B, BASi), a platinum wire counter electrode (CHI 115) and a gold working electrode (CHI101) was used for the bulk system while microfabricated gold microelectrodes were used for on-chip voltammetry. Prior to voltammetry, the conventional (bulk) gold and platinum electrodes were cleaned using chromic acid, polished using electrode polishing kit (CHI 120) and electrochemically cleaned and activated by cyclic sweep, performed in the range of 2 V to −2 V at a scan rate of 100 mV/sec in 0.1 M sulfuric acid until a stable curve was obtained. Microelectrodes were cleaned using acetone and 2-propanol and dried using nitrogen gas. In this case no other pre-treatment steps, electrochemical cleaning or polishing were performed.

Studies involving CV and hydrodynamic voltammetry (HDV) were performed in a straight microchannel with a length of 1 cm (Fig. 1(a)) while FI-AD was performed in a Y-shaped microchannel. The Y-shaped channel (Fig. 1(b)) had a straight channel of length 5 cm with branching at a distance of 4 cm from the electrodes. For cyclic voltammetric study, samples were directly injected into the channel using a disposable syringe whereas for HDV and FI-AD; syringe pump (KD Scientific, U.S.A) was used for buffer flow in the central channel and sample injection in the branched channel.

Voltammetric studies of 100 μM $K_4Fe(CN)_6$ were performed in 200 mM potassium chloride (KCl) as an electrolyte, while 1 mM cysteine, 1 mM guanosine and 1 mg/mL fish sperm DNA were analyzed in 200 mM sodium hydroxide (NaOH) at a voltammetric scan rate of 100 mV/sec. For reference, control voltammetry of KCl and NaOH were also performed in a similar fashion as the analytes. All the measurements were performed at least 3 times for each condition (n = 3) except otherwise stated.

Results and Discussion

The microfabricated electrochemical cell depicting all the microelectrodes for electrochemical study is shown in Figure 1(a) and 1(b). Out of the three gold electrodes, one each can be used as reference, counter and working electrodes. The widths of the electrodes were 200 μm and the

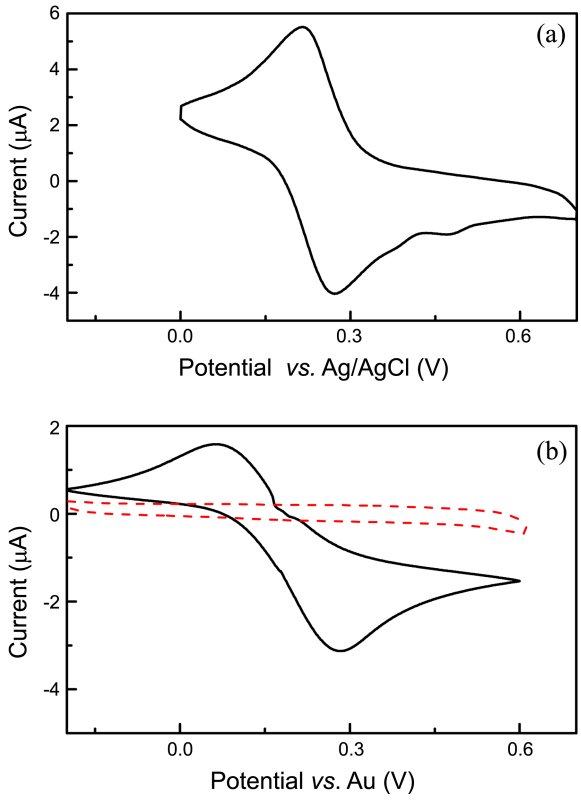

Figure 2. CV of 100 μM K4FeCN6 in 200 mM KCl on (a) bulk and (b) on-chip system. The dotted line represents reference CV of KCl solution. Scan rate: 100 mV/s.

distance between two electrodes was 200 μm. As the dimension of microchannel was 200 μm (width) × 120 μm (height), the effective active electrode area used for electrochemical study was 200 μm × 200 μm with a working volume of ~10 μL inside the microchannel for CV. Gold was used as the electrode material due to ease of fabricating it on a substrate and also because of its wide use in other microchip devices. Another potential advantage of gold is that, the sheet resistance of gold is low (0.38 Ω/sq) as compared to other materials (*e.g.* ITO (10.0 Ω/sq). PDMS was chosen as the channel material because of its inertness with the reagents and transparency and was also expected to shield electrical interferences during electrochemical measurements. Apart from it, as evident from surface profiling of gold microelectrode using atomic force microscopy (Fig. 1(c)), the surface of gold electrode was quite smooth and therefore did not require further electrode polishing as in the case of bulk conventional electrodes. A clean and smooth active electrode area also ensured sensitive analysis of samples.

The fabricated device with 1 cm straight channel configuration was first characterized to establish its performance in voltammetric analysis. It was first tested for the cyclic voltammetric measurement of potassium ferrocyanide. Figure 2 represents cyclic voltammograms obtained by scanning the potential at 100 mV/s in the presence of 100 μM $K_4Fe(CN)_6$ in 200 mM KCl at stationary condition. For bulk system, 1 mL of sample was taken in a 5 mL glass beaker and for the on-chip voltammetry, microchannel was filled

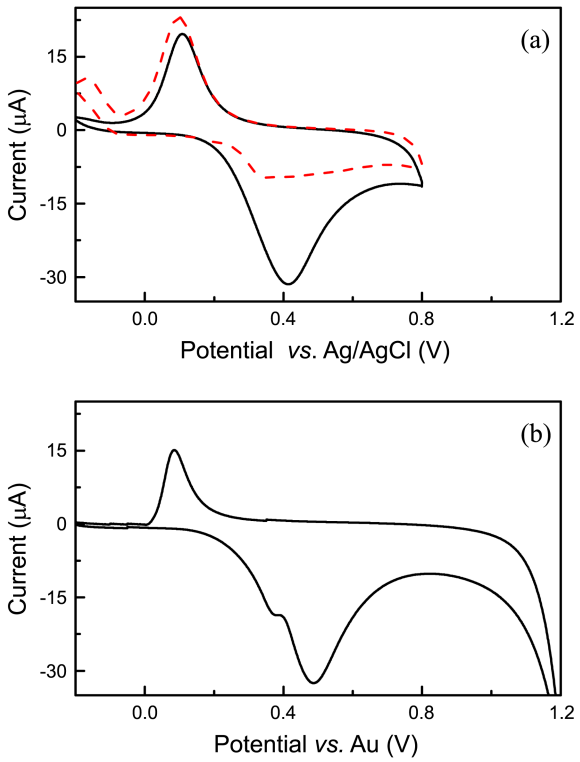

Figure 3. CV of 1mM cysteine in 200 mM NaOH on (a) bulk and (b) on chip system. The dotted line represents reference CV of NaOH solution. Scan rate: 100 mV/s.

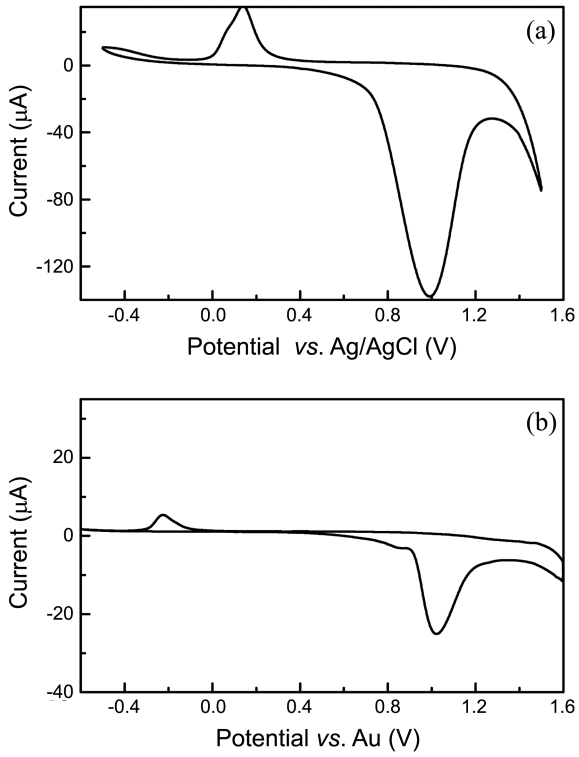

Figure 4. CV of 1 mM guanosine in 200 mM NaOH on (a) bulk and (b) on chip system. Scan rate: 100 mV/s.

with the sample using a syringe. Figure 2(a) shows a typical voltammogram of $K_4Fe(CN)_6$ in a three-electrode cell with a gold working electrode, Pt counter electrode, and Ag/AgCl reference electrode. Figure 2(b), shows CV curves obtained using microfluidic cell. As can be seen, the peak potential of the two curves is very similar to each other. A control experiment with electrolyte without any analyte produced no peak. These results clearly demonstrate that our microelectrodic system was comparable to the conventional electrode system and the microelectrodes could be useful to carry out further microvoltammetric experiments.

Microfluidic cell was later used to examine the electrochemical properties of cysteine. Cysteine is an amino acid containing a thiol group. Biological thiols are compounds of main interest due to their importance in biological processes such as antioxidant defense network, methionine cycle and protein synthesis. Cysteine deficiency is involved in slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness.[22] Therefore, rapid electrochemical detection of this chemical with minimal sample requirement is in demand. For this, CV of 1 mM cysteine in NaOH was carried out with on-chip microelectrodes (Fig. 3) and the voltammogram showed an oxidation peak at 0.48 V, much similar as with conventional electrodes.[8] Electrochemical detection of cysteine was attempted by various groups in the past, but such studies required complex electrode modifications and fabrication processes. Whereas, such detection can be performed rather easily with our device, with little sample requirement and almost no need for complex electrode pretreatments. This proves the efficacy of present microfluidic setup having microelectrode configuration.

In continuation, we analyzed guanosine (nucleoside) on the microdevice, as it is one of the important compounds of biological origin and is a building block of nucleic acids. Quantitative analysis of nucleic acid such as DNA molecule is challenging yet requisite task in most of the biological laboratories. Thus, cyclic voltammetric analysis of guanosine bases could be pre-requisite for the detection of DNA electrochemically.[7] Therefore, the CV analysis of guanosine was performed using the three in-channel electrodes. NaOH was used as the supporting electrolyte. The electrochemical behavior of nucleosides is shown in Figure 4. Experiments were performed in 200 mM NaOH solution with 1 mM concentration of guanosine. Guanosine produced defined oxidation peaks in the anodic scan at 1.0 V using both, the bulk (Fig. 4(a)) as well as microelectrodes (Fig. 4(b)). This result suggested the feasibility to analyze complex biological molecule such as DNA on our microchip device.

Therefore, we tested fish sperm DNA with varied concentration using similar cyclic voltammetric analysis on the microchip. The cyclic voltammogram of fish sperm DNA produced similar oxidation peaks in the anodic scan at 1.0 V (Fig. 5(a)) with peak currents proportional to its concentration (Fig. 5(b)). This time, the experiment was performed on the microchip itself and the results indicated a good correlation between concentration of DNA and amperometric peak current. This confirmed the possibility to use

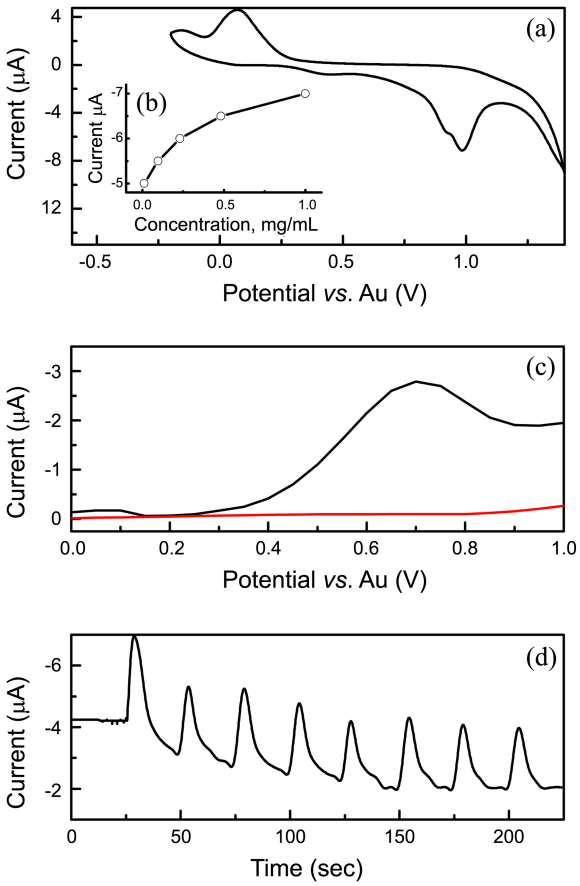

Figure 5. (a) CV of fish sperm DNA (b) Inset: concentration Vs. current of fish sperm DNA obtained using CV; (c) HDV of fish sperm DNA (black) and NaOH (red); (d) FI-AD of fish sperm DNA (for each, conc. Of DNA = 1 mg/mL, electrolyte = 200 mM NaOH, flow rate 5 μL/min). Scan rate: 100 mV/s.

this microfluidic electrochemical cell for various quantitative electrochemical studies in a convenient yet sensitive manner.

As a proof of concept and to strengthen the use of microdevice, flow-injection (FI) based direct and rapid detection of fish sperm DNA as a model analyte was carried out. An on-chip HDV analysis was first performed to ascertain the feasibility of carrying out FI analysis on the microchip. A sample of DNA prepared in NaOH was injected into the microchannel at a flow rate of 5 μL/min and HDV was performed by varying the electrode potential from 0-1 V, the results of which is shown in Figure 5(c). As shown in the figure, the HDV reaches a current limited plateau at +0.7 V. A comparison of Figure 5(a) and 5(c) proves the characteristic feature of HDV that the peak obtained using HDV is lower than that of the CV. These results show the micro patterned electrodes, when integrated with microchip based flow analysis, exhibit a similar analytical performance as conventional electrodes.

The microelectrodes were further used to detect repeated injections of a DNA solution into a Y-shaped channel with branched end for sample and electrolyte injection and other end as an outlet. For this, an off-chip syringe pump controlled using the LabVIEW program was used to inject 500 nL of sample plugs into the channel at an interval of every 25 sec. As shown in Figure 5(d), 8 injections of this DNA solution led to an average response of 2.32 nA (SD = 0.24, n = 8) with more than 90% response compared to first time detection current. Such setup could thus be used effectively in flow-injection based rapid analysis of pharmaceutical products and other analytes of biological origin, with a high degree of reproducibility, as can be seen from Figure 5(d). It also indicated that our setup does not have the disadvantages linked to conventional electrodes in which molecules such as DNA tend to adhere to the electrode thereby reducing the output detection current in a phenomena known as electrode passivation. This could be explained due to microenvironment around the electrodes and the optimum flow of electrolyte and sample within the microchannel. Such turbulence is seldom present in conventional electrochemical setup unless highly sophisticated systems such as rotating disk electrodes, *etc*. are used.

Conclusion

In the present work, fabrication and application of a simple disposable microfluidic electrochemical cell was demonstrated. It was shown that these devices having microelectrodes can be integrated with PDMS-based fluidic channels and used for voltammetric and amperometric detection of electroactive analytes. A microelectrode system thus facilitated an easy handling of experiment without worrying about the electrode polishing and pretreatment. Analysis of $K_4Fe(CN)_6$, cysteine, guanosine and fish sperm DNA was successfully performed using the proposed device and the voltammograms obtained showed good correlation with conventional bulk electrode system while having low background current and steady detection peaks even though a few microliters of analyte was used. The applicability of this microchip was demonstrated with flow-injection based rapid amperometric detection of DNA samples. The device so developed shall find use as a disposable electrochemical cell for rapid and sensitive flow injection based analysis of electroactive species in various industrial and research applications.

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