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Carbon nanotube/polymer composite electrodes for flexible, attachable electrochemical DNA sensors

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ABSTRACT

All-solution-processed, easily-made, flexible multi-walled carbon nanotube (MWCNT)/polydimethylsiloxane (PDMS)-based electrodes were fabricated and used for electrochemical DNA sensors. These electrodes could serve as a recognition layer for DNA, without any surface modification, through π–π interactions between the MWCNTs and DNA, greatly simplifying the fabrication process for DNA sensors. The electrodes were directly connected to an electrochemical analyzer in the differential pulse voltammetry (DPV) and cyclic voltammetry (CV) measurements, where methylene blue was used as a redox indicator. Since neither functional groups nor probe DNA were immobilized on the surfaces of the electrodes, the sensor can be easily regenerated by washing these electrodes with water. The limit of detection was found to be 1.3×10^2 pM ($S/N=3$), with good DNA sequence differentiation ability. Fast fabrication of a DNA sensor was also achieved by cutting and attaching the MWCNT–PDMS composite electrodes at an analyte solution-containable region. Our results pave the way for developing user-fabricated easily attached DNA sensors at low costs.

1. Introduction

DNA sensors have attracted much attention because of their application in point-of-care medical diagnosis (Hashimoto et al., 1994), genome research (Rasooly and Jacobson, 2006), and forensic science (Divne and Allen, 2005). The electrochemical method has been widely used for DNA detection (Wang, 2006) because it directly converts biological information into an electric current, enabling simple, low-cost, and accurate detection of DNA. In many studies, electrochemical DNA sensors have been made by using a Au electrode as the working electrode (Hashimoto et al., 1994). However, the cost for fabricating these Au-electrode-based sensors is very high owing to the high price of Au, which limits the commercialization of this type of sensor. Carbon-material-based electrodes have the potential to replace Au electrodes owing to their relatively low fabrication costs. For example, glassy carbon electrodes (GCEs) have been widely used in electrochemistry (Cheng et al., 2005; Wang et al., 2003). However, for detecting DNA, the surfaces of GCEs need to be either functionalized with specific groups to immobilize DNA (Millan et al., 1992) or coated with other materials such as pyrrole (Wang et al., 1999), carbon nanotubes (CNTs) (Wang et al., 2003), and gold nanoparticle/ploy (*p*-aminobenzoic acid)/CNT composites (Zhang et al., 2010). These processes are followed by an additional process for immobilizing the probe. Thus, the processes for fabricating GCE electrodes for DNA detection are sometimes complicated, labor intensive, and time consuming, possibly causing large variations in performance.

CNTs, which have unique electrical, chemical, and mechanical properties (Ajayan, 1999), are used as an active material in field-effect transistor-type DNA sensors (Jacobs et al., 2010; Star et al., 2006) and as charge transport or coating materials for Au electrodes or GCEs in electrochemical DNA sensing (Cheng et al., 2005; Dong et al., 2012). CNTs can be also fabricated as electrodes (Saleh Ahammad et al., 2009), meaning they may be a candidate for replacing Au electrodes. However, to the best of our knowledge, there have been no reports that carbon-nanotube-based electrodes without surface modification are directly connected to an electrochemical analyzer for DNA detection. This might be due to the fact that the surfaces of CNTs are so chemically inert that they are unsuitable for detecting biological reactions near the surface. Thus, similar to GCEs, the surfaces of carbon-nanotube-based electrodes usually need to be chemically modified (Saleh Ahammad et al., 2009) or coated by other materials (Zhang et al., 2010).

In this work, we have fabricated multi-walled CNT (MWCNT)/polydimethylsiloxane (PDMS) composite electrodes. Although

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similar CNT/PDMS composite electrodes have been used in other applications such as gas sensing (Woo et al., 2007), MWCNT/PDMS composite electrodes with no surface modification are for the first time directly connected to an electrochemical analyzer and used as a recognition layer for DNA, using methylene blue (MB) as an electrochemical indicator. This recognition function largely relies on π–π stacking interactions between DNA and the MWCNTs, similar to graphene–DNA systems (Tian et al., 2014). The current electrodes were stable against the analyte solution and reusable for DNA sensing by washing in water. They were simple to fabricate using all-solution processing, without the need for any surface modification and probe immobilization, which are usually required for DNA sensors based on carbon-nanotube electrodes (Jacobs et al., 2010) or GCEs (Millan et al., 1992; Wang et al., 2003). Our MWCNT/PDMS composite electrodes are flexible, stretchable, and attachable. The cost for fabrication of these electrodes is much less than that for Au electrodes. We show the fast fabrication of the DNA sensor by cutting a MWCNT/PDMS sheet into three electrodes of proper sizes with scissors and attaching them to a concave region containing the analyte solution. Our MWCNT/PDMS composite electrodes pave the way for developing user-fabricated easily attached DNA sensors.

2. Materials and methods

2.1. Reagents and materials

MWCNTs whose diameters and lengths were 10–15 nm and 30–40 μm, respectively, were purchased from Hanwha Chemical (Korea). The target and probe DNA were purchased from Bioneer (Korea). We used one probe (P1) and three distinct targets (T1, T2, and T3) (Table 1). The T1, T2, and T3 targets had no-, single-, and all-base mismatches to the P1 probe, respectively. MB, which was used as a redox indicator, was purchased from Sigma-Aldrich (Germany). 2-Propanol (IPA) was purchased from J.T. Baker (USA). To prepare sample solutions for the DNA detection experiments, we first added 800 nM of the P1 probe to 1 mL of phosphate-buffered saline (PBS, pH 7.4). After 10 min, 800 nM of the target (T1, T2, or T3) was additionally mixed in the solution. After allowing 20 min for the probe–target interaction, the sample solution was prepared by adding 15.6 μM MB into the PBS solution. The sample solution was then stored for 30 min at room temperature before conducting the electrochemical experiments.

2.2. Preparation of MWCNT electrode

16 mg of MWCNTs was dispersed in 20 mL of IPA by ultrasonication for 1 h. The temperature of the water bath of the sonicator was maintained at approximately 40 °C. IPA is much less harmful to human beings than other solvents such as dimethylformamide (DMF), which was used in previous studies for the fabrication of CNT electrodes (Xu et al., 2004). Because no equipment for treating toxic solvents is required, the use of IPA is advantageous in terms of commercializing these electrodes. We developed our own method for fabricating MWCNT–PDMS composites as compared to methods used in previous studies on single-walled CNT (SWCNT)- or MWCNT–PDMS composites (Ding et al., 2012; Meitl et al., 2004; Wang et al., 2013). A schematic representation of the fabrication process of the MWCNT–PDMS–glass electrode is shown in Fig. S1. The process for forming a MWCNT layer on a Petri dish consisted of four cycles of casting and drying the solution. In the first cycle, 10 mL of the IPA solution containing MWCNTs was dropped onto the dish and dried at 95 °C for 1 h. The amounts of the subsequently applied solutions were 4, 3, and 1 mL for the second, third, and fourth cycle, respectively, and the time for drying was reduced to 30 min after the first cycle. This process yielded a fairly uniform and dry MWCNT layer on the dish. A PDMS elastomer and curing agent were mixed in a weight ratio of 10:1. The mixture was then cast onto a glass substrate and maintained at room temperature for 5 h for partial curing of the PDMS. The glass side of the PDMS–glass composite was faced up in the Petri dish, with the PDMS layer in contact with the MWCNT layer. For further curing of PDMS, the Petri dish was annealed at 95 °C for 11 h and a pressure of 11.2 kPa was exerted on the surface of the glass for the initial 30 min. The MWCNT–PDMS–glass composite was then easily peeled from the Petri dish, cut to a size of 6 mm in width and 12 mm in length (see Fig. 1A), and used for electrochemical detection of DNA. The thicknesses of the MWCNT and PDMS layers were ca. 5.9 μm and 95.5 μm, respectively. This composite was flexible, stretchable, and attachable owing to the material properties of the MWCNTs (Fig. 1B and C), similar to previous studies (Ding et al., 2012; Moon et al., 2012). As shown in Fig. 1C, this MWCNT–PDMS layer can also be fabricated on polyethylene terephthalate (PET). Although the MWCNT–PDMS layer can be formed on any hydrophobic substrate, glass is one of the most suitable substrates for PDMS in our experiments owing to its bioinert and rigid properties.

Table 1
Nucleotide sequences of probe and targets.

Type	Name	Sequence (5′→3′)	Mismatches between probe and target sequences
Probe	P1	GTG TTG TCT CCT AGG TTG GCT CTG	
Target	T1	CAG AGC CAA CCT AGG AGA CAA CAC	None
Target	T2	CAG AGC CAA CCT CGG AGA CAA CAC	One base-pair
Target	T3	ATA TCG ACC TTG GCC GAG ACG GTG	All base-pairs

2.3. Instruments

Surface images of CNT electrodes were obtained using scanning electron microscopy (SEM, Hitachi S4800, Japan). A CHI 622D electrochemical analyzer (CH Instruments, USA) was used to carry out cyclic voltammetry (CV) and differential-pulse voltammetry (DPV) measurements. Sheet resistance was measured using a DMR-1C sheet resistance analyzer (DM instruments, China).

2.4. Measurements

The sheet resistance was obtained from the MWCNT–PDMS composite. On the other hand, the rigid MWCNT–PDMS–glass electrode was used for DNA sensing in an electrochemical cell. The MWCNT–PDMS–glass electrode was used as the working electrode of the electrochemical analyzer, whereas a Ag/AgCl electrode and platinum wire were used as the reference and auxiliary electrodes, respectively. The MWCNT–PDMS–glass electrode and the other two electrodes were fixed in the cell such that half (6 mm × 6 mm) of the MWCNT–PDMS–glass electrode was immersed. The geometry of the electrodes in this typical setup is shown in Fig. S2. This was immediately followed by DPV or CV measurements in a voltage range from −0.4 to 0 V, with a sweep rate of 0.004 V/s. Thus, the electrochemical signals for detecting DNA could be obtained within 1 h, whereas some DNA sensors take much longer (10–20 h) to obtain response signals (Cheng et al., 2005; Wang et al., 2009). For experiments for the user-fabricated DNA sensors, the MWCNT–PDMS composite electrodes were used as the working, reference, and counter electrodes, and the experimental