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Electrical percolation-based biosensor for real-time direct detection of staphylococcal enterotoxin B (SEB)

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ABSTRACT

Electrical percolation-based biosensing is a new technology. This is the first report of an electrical percolation-based biosensor for real-time detection. The label-free biosensor is based on electrical percolation through a single-walled carbon nanotubes (SWNTs)–antibody complex that forms a network functioning as a "Biological Semiconductor" (BSC). The conductivity of a BSC is directly related to the number of contacts facilitated by the antibody–antigen "connectors" within the SWNT network. BSCs are fabricated by immobilizing a pre-functionalized SWNTs–antibody complex directly on a poly(methyl methacrylate) (PMMA) and polycarbonate (PC) surface. Each BSC is connected via silver electrodes to a computerized ohmmeter, thereby enabling a continuous electronic measurement of molecular interactions (e.g. antibody–antigen binding) via the change in resistance. Using anti-staphylococcal enterotoxin B (SEB) IgG to functionalize the BSC, we demonstrate that the biosensor was able to detect SEB at concentrations as low as 5 ng/mL at a signal to baseline (S/B) ratio of 2. Such measurements were performed on the chip in wet conditions.

The actuation of the chip by SEB is immediate, permitting real-time signal measurements. In addition to this "direct" label-free detection mode, a secondary antibody can be used to "label" the target molecule bound to the BSC in a manner analogous to an immunological sandwich "indirect" detection-type assay. Although a secondary antibody is not needed for direct detection, the indirect mode of detection may be useful as an additional measurement to verify or amplify signals from direct detection in clinical, food safety and other critical assays. The BSC was used to measure SEB both in buffer and in milk, a complex matrix, demonstrating the potential of electrical percolation-based biosensors for real-time label-free multi-analyte detection in clinical and complex samples. Assembly of BSCs is simple enough that multiple sensors can be fabricated on the same chip, thereby creating "Biological Central Processing Units (BCPUs)" capable of parallel processing and sorting out information on multiple analytes simultaneously which may be used for complex analysis and for point of care diagnostics.

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1. Introduction

Several types of direct detection biosensors such as surface plasmon resonance (SPR), piezoelectric and cantilever sensors were developed for label-free detection of analytes. Single-walled carbon nanotubes (SWNTs) (Iijima, 1991) have also been used for label-free detection in field effect transistor (FET)-based sensors (Kong and Dai, 2001; Kong et al., 2000). FETs sensors fabricated from single SWNTs grown by chemical vapor deposition (CVD) can measure biological interactions on the surface of SWNTs by measuring changes of electrical conductance in individual nanotubes. They have been used in chemical and biological sensors (Kong and Dai, 2001; Kong et al., 2000; Tans et al., 1997). In addition to single SWNTs-based sensors, submonolayer of SWNTs also fabricated by CVD (Chen et al., 2003) were shown to exhibit semiconductor-like behavior in which surface interactions of biomolecules were used for biosensing (Chen et al., 2003, 2001).

More recently, SWNTs percolation-based sensing has been carried out using non-porous material (Yang et al., 2010) in which SWNT–antibody complex was used to form a bio-nanocomposite network on plastic forming a "Biological Semiconductor" (BSC) for biodetection. In this SWNT–antibody complex, a recognition element which binds to a biological target was used to control the

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electrical conductivity of the bio-nanocomposite network via an electrical percolation principle.

In this model of electrical percolation-based system, the conductivity of the network is through the passage of current between the conductive ends of each SWNTs (the SWNTs ends within the network have to be connected for current to pass) and the overall conductivity dependent upon the continuity of the network (the number of connections). Binding of specific antigens to the SWNT–antibody complex disrupts the continuity by displacing the connected ends of the SWNT, resulting in increased tunneling distance and subsequent resistance. So the conductivity of the SWNT–antibody network increases with the increase in concentration of SWNT. At a specific SWNT concentration (the percolation transition point), the change in resistance begins to level off. Below this point, there is a still relatively low statistical distribution of "contacts" between the SWNT–antibody complexes in the network. Therefore, small changes in the SWNT–antibody complexes can lead to dramatic changes in conductivity.

Unlike the FET-based sensors in which changes on the surface of s single carbon nanotubes result in changed conductivity, in BSC the change in the connectivity of the carbon nanotubes network results in a change in conductivity of the network.

Based on this model, we have shown (Yang et al., 2010) that for immunodetection, the bio-nanocomposite prepared with 1 mg/mL of SWNT will be the most sensitive to molecular interactions, other concentrations, such as 0.5 mg/mL, 1.5 mg/mL, and 2 mg/mL, the response is much smaller. The best results have been obtained with 1 mg/mL of SWNT because it is near the percolation threshold. These measurements were conducted with dry SWNT–antibody complexes. However most biological interactions take place in fluids. In this work, we describe the adaptation of BSCs for measurements in fluids and the development of a real-time biosensor based on BSC technology for the detection of staphylococcal enterotoxins (SEs).

SEs are a group of 21 heat stable toxins implicated in foodborne diseases (Archer and Young, 1988; Bean et al., 1996; Bunning et al., 1997; Garthright et al., 1988; Olsen et al., 2000) and other diseases such as atopic eczema (Breuer et al., 2000; Bunikowski et al., 1999; Mempel et al., 2003), rheumatoid arthritis (Howell et al., 1991; Uematsu et al., 1991), and toxic shock syndrome (Herz et al., 1999). SEs are also recognized as potential bioweapons (Henghold, 2004; Ler et al., 2006; Rosenbloom et al., 2002; Wiener, 1996). Several immunological assays for SE detection have been described, including enzyme-linked immunosorbent assays (ELISA) (Bennett, 2005), which generally use optical detection. Several different biosensors have been used for SEs detection (Homola et al., 2002; Nedelkov et al., 2000; Rasooly, 2001; Rasooly and Herold, 2006; Rasooly and Rasooly, 1999; Sapsford et al., 2005; Shriver-Lake et al., 2003; Soelberg et al., 2005; Yu et al., 2005). In our previous work, we demonstrated that the sensitivity of sandwich immunoassays with a labeled secondary antibody detected with optical detectors could be enhanced by using single-walled carbon nanotubes (SWNTs) (Yang et al., 2008a,b, 2009) SWNTs enhanced the sensitivity of biodetection because of their large surface area to volume ratio.

Here we present a BSC-based biosensor for SEs (and for other microbial toxins), which offers advantages such as a rapid real-time detection (monitoring conductivity changes as they happen) and high-throughput detection. The biosensor consists of an array of sixteen BSCs on a PMMA-PC substrate with a computerized ohmmeter connected and controlled by the PC and the data from the ohmmeter is sent to the PC for continuous electronic measurement of molecular interactions (e.g. antibody–antigen binding) via the change in resistance can be used for point of care diagnostics and personalized medicine.

2. Materials and methods

2.1. Materials and reagents

Staphylococcal enterotoxin B (SEB) and rabbit anti-SEB affinity purified IgG were purchased from Toxin Technology (Sarasota, FL). Poly(diallyldimethylammonium chloride) (PDDA) was purchased from Sigma–Aldrich (St. Louis, MO). Single-walled carbon nanotubes were obtained from Carbon Solutions Inc. (Riverside, CA) and the food used for the analysis was purchased from a local grocery store.

2.2. Single wall carbon nanotube preparation

The single wall carbon nanotube solution was prepared as previously described (Yang et al., 2009, 2008b, 2010). SWNTs (30 mg) were oxidized by mixing with a concentrated sulfuric acid and nitric acid mixture (3:1, v/v) special care has to be taken care when handling sulfuric/nitric mixture. The SWNTs were shortened by sonication with a Fisher (FS-14) sonicator for 6 h, followed by extensive washing in water (100 mL) until neutralized (pH 7.0). The SWNTs were then dispersed in 100 mL 1 M NaOH solution for 5 min to achieve net negative charged carboxylic acid groups and again washed with water (100 mL). The positively charged polycation (PDDA) was adsorbed by dispersing the SWNT in 50 mL of 1 mg/mL PDDA containing 0.5 M NaCl for 30 min followed by centrifugation (10,000 rpm) in a Beckman centrifuge for 15 min, then washed with 100 mL of water. The role of PDDA is to immobilize antibody onto SWNT surface through electrostatic adsorption.

To create the bio-nanocomposite material, the SWNTs were functionalized by dispersing in a rabbit anti-SEB IgG phosphate buffer solution (20 mM, pH 8.0) at a concentration of 0.01 mg/mL for 1 h at room temperature, so that the antibody was adsorbed onto the SWNT surface. After centrifugation (15 min) and extensive washing with water (10 mL), the SWNTs–antibody complex is ready to use.

2.3. Fabrication of BSC chips

The chip's base (the non-conductive polymer) was fabricated as described in previous work (Sapsford et al., 2009; Sun et al., 2009; Yang et al., 2009, 2008b, 2010). The 16 BSCs array used in this study were designed in CorelDraw 11 (Corel Corp., Ontario, Canada). The poly(methyl methacrylate) (PMMA) used for fabrication was micro-machined using a computer controlled Epilog Legend CO_2 65W laser cutter (Epilog, Golden, CO). Before cutting, PMMA sheets were coated with 3 M 9770 adhesive transfer double sided tape (Piedmont Plastics, Beltsville, MD), and the polycarbonate (PC) film, which serves as the bottom of the chip, was bonded to it to form a PMMA-PC chip. The recesses for the silver electrodes were engraved into the top PMMA layer and the surface contacts for the SWNT–antibody complex were cut through.

The SWNTs–antibody complex (1 mg/mL) was applied to the chip surface by depositing pre-functionalized SWNTs with antibody to form a biological semiconductor layer into the PMMA-PC chip. For each BSC, 60 μL of SWNT complex solution was used.

To speed up the drying of the material, a simple evaporator with a variable speed 12 V computer fan facing a Minco clear heater (Minco, Minneapolis, MN) was fabricated (Fig. 2B). In this dryer, the chip is placed on the heater and a flow of air from the fan dry the material. After drying, electrodes were painted with silver contacts using "Silver Liquid" (Electron Microscopy Sciences (Hatfield, PA)) on both sides of the printed SWNT–antibody bio-nanocomposite.

The distance between the 2 silver electrodes is 15 mm, the thickness was not measured but empirically applied to cover completely