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Electrical percolation based biosensors

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ABSTRACT

A new approach to label free biosensing has been developed based on the principle of “electrical percolation”. In electrical percolation, long-range electrical connectivity is formed in randomly oriented and distributed systems of discrete elements. By applying this principle to biological interactions, it is possible to measure biological components both directly and electronically. The main element for electrical percolation biosensor is the biological semiconductor (BSC) which is a multi-layer 3-D carbon nanotube-antibody network. In the BSC, molecular interactions, such as binding of antigens to the antibodies, disrupt the network continuity causing increased resistance of the network. BSCs can be fabricated by immobilizing conducting elements, such as pre-functionalized single-walled carbon nanotubes (SWNTs)-antibody complex, directly onto a substrate, such as a Poly(methyl methacrylate) (PMMA) surface (also known as plexi-glass or Acrylic).

BSCs have been demonstrated for direct (label-free) electronic measurements of antibody-antigen binding using SWNTs. If the concentration of the SWNT network is slightly above the electrical percolation threshold, then binding of a specific antigen to the pre-functionalized SWNT dramatically increases the electrical resistance due to changes in the tunneling between the SWNTs. Using anti-staphylococcal enterotoxin B (SEB) IgG as a “gate” and SEB as an “actuator”, it was demonstrated that the BSC was able to detect SEB at concentrations of 1 ng/ml. Based on this concept, an automated configuration for BSCs is described here that enables real time continuous detection. The new BSC configuration may permit assembly of multiple sensors on the same chip to create “biological central processing units (CPUs)” with multiple biological elements, capable of processing and sorting out information on multiple analytes simultaneously.

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1. Introduction

Biosensors for direct measurements, in which the biological interaction is label-free, enable simple real time detection without the need for labeled secondary elements that are often catalytic elements, such as enzymes, receptors or antibodies. The most common direct detection biosensor systems are optical, employing evanescent wave or surface plasmon resonance (SPR). Other direct detection biosensors are based on resonant crystal biosensors (also known as quartz crystal microbalance (QCM), piezoelectric or acoustic wave), which measure changes in acoustic resonant frequency of a quartz crystal due to bound mass on the crystal surface. More recently nanomaterial-based biosensors have become very popular for biosensor detection using nanomaterials such as

carbon nanotubes, nanowires, nanoparticles, nanopores, nanoclusters and graphene [1]. The large surface area of single-walled carbon nanotubes (SWNTs) enabled increase sensitivity of various immunological assays [2–8]. In addition, the unique nanoscale electrical characteristics of nanomaterials have been used in several label free “electronic” detection modalities including field-effect transistors (FETs). The focus of this manuscript is on label-free electronic detection modalities based on SWNTs.

1.1. Field-effect transistors (FET) label-free biosensors

FETs used for electrical detection exploit the changes in conductivity that occur when the molecules of interest adsorb on the FET surface utilizing nanomaterials that are gated by changes in surface potential on their surface [9]. In a FET (Fig. 1), the ligands binding to the targets are immobilized on the gate surface, which modulates the channel conductance. For example, when positively charged analytes bind to the immobilized ligand on the gate, a

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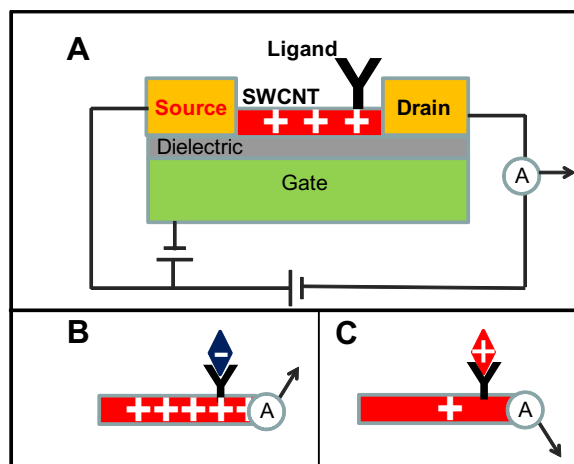


Fig. 1. FET biosensor. The SWCNT is placed between the source and drain electrodes with a gate electrode on the bottom to modulate the conductivity of the semiconductor channel. Target molecules can be recognized by the ligand on the SWCNT surface through binding affinity. If positively charged target molecules bind the ligand modified on the SWCNT, positive carriers (holes) are depleted in the SWCNT, resulting in a decrease in conductance. If, negatively charged target molecules captured by the receptor would make an accumulation of hole carriers, causing an increase in conductance.

depletion of charge carriers occurs in the conductance gate. FET sensors [10,11] based on single-walled carbon nanotubes (SWNTs) [12] were shown to be sensitive devices for directly detecting specific molecules without additional labeling. SWNT-based FETs have demonstrated a large conductance change in response to binding events on the surface. FETs rely on an electric field on the surface of individual carbon nanotube to control conductivity, and based on their ligands immobilized on the nanomaterial gate they shown to be highly sensitive to their environment. Conductance varies significantly with changes in electrostatic charges and surface adsorption of a variety of molecules [10,13,14]. Using tubes grown directly on the chip by chemical vapor deposition (CVD), it was shown that a large conductance change can be achieved when individual tubes are utilized as gates for FETs in chemical sensors [10,11,15]. In addition, a sub-monolayer of SWNTs fabricated by CVD [16] has been shown to exhibit semiconductor-like behavior (also based on an electric field on the surface of carbon nanotube to control conductivity), which can be gated to utilize surface interactions of biomolecules for biosensing [16,17].

In contrast to these approaches to FET-based electronic sensing based on the electronic characteristics of the surfaces for individual nanoscale elements, a biosensor has been developed based on a different physical principle known as “electrical percolation”. In electrical percolation, the electronic interactions within a randomly oriented and distributed network of nanoscale biological recognition elements is utilized as a transistor gate rather than the more ordered structures in the alternative surface-oriented approaches [18–20].

1.2. Electrical percolation based biosensors

Electrical percolation is based on the formation of long-range connectivity in a randomly oriented network of conductive elements, and the passage of current through the conductive network depends on the continuity of the network. It was shown that the electrical-conductivity fluctuates near the percolation threshold [21], which is defined mathematically as the critical value of the occupation probability such that infinite connectivity first occurs, so that the total resistance measured on the samples is due to the difference in contact between particles [22]. Electrical percolation occurs according to the mathematical definition when the

contact between the particles creates a continuous path that enables current to be conducted through the network of particles, and can be enhanced due to interconnection of three-dimensional pentacene islands in thin films [23], and the electrical percolation of polymer nanocomposites prepared with functionalized copper nanowires has also been studied [24].

Several electrical percolation and carbon nanotube-based conductivity detectors have been reviewed [25], and sensors that utilize electrical percolation for vapor [26] [27] and solvents [28] sensing were described. In such sensors, changes in electrical conductivity of the CNT network were attributed to swelling of the polymer matrix and/or conductive modification due to the solvent absorption.

For biosensing, the biological recognition element (ligand) is bound to the CNT forming a “gate” for a “biological semiconductor” and the conductivity of the network is influenced by the interaction ligand-target at the gate. The utilization of electrical percolation as a transistor gate in the context of biological semiconductors (BSCs) was described recently [18–20].

2. Technologies for fabrication of BSC biological sensor

2.1. Reagents and materials

2.1.1. Reagents

Single-walled carbon nanotubes (CNTs) were obtained from Carbon Solutions Inc (Riverside, CA). Poly(diallyldimethylammonium chloride) polymer (PDDA), BSA, lysozyme and anti-rabbit IgG were purchased from Sigma–Aldrich (St. Louis, MO). Staphylococcal enterotoxin B (SEB), rabbit anti-SEB affinity purified IgG, were purchased from Toxin Technology (Sarasota, FL). For enhanced chemiluminescence (ECL) detection, Immuno-Star HRP Chemiluminescence Kit was obtained from Bio-Rad (Hercules, CA). All other reagents were of analytical grade and de-ionized water was used throughout.

2.1.2. Materials for the fabrication of BSC

Clear 0.25 mm polycarbonate film and 1/8 inch acrylic were obtained from Piedmont Plastics (Beltsville, MD).

2.1.3. Electronic components

The resistance of the BSC was measured and recorded with a U1253A/001 digital multimeter (Agilent Technologies, Santa Clara CA). Six way valve from Cole-Parmer (Vernon Hills, IL), analog to digital/digital to analog converter (Multifunction I/O 779051-01, NI USB-6008) with 8 analog inputs (12-bit) and 2 analog outputs (12-bit) from National instruments (Austin, TX). Labview software from National instruments (Austin, TX). WPX-1 peristaltic pump from Welco (Japan), “Silver Liquid” (Electron Microscopy Sciences (Hatfield, PA) and Digital Multimeter from Agilent Technologies (Santa Clara CA).

2.1.4. Preparation equipment

Fisher (FS-14) Sonicator was obtained from Fisher Scientific (Pittsburgh, PA), Beckman mini centrifuge was obtained from Beckman (Fullerton, CA).

2.2. Fabrication of BSC biosensor

The BSC biosensor [20] shown in Fig. 2C was designed in Corel-Draw11 (Corel Corp. Ontario, Canada) and micro-machined in 1.5 mm acrylic using a computer controlled laser cutter Epilog Legend CO₂ 65 W cutter (Epilog, Golden, CO). Before engraving the common electrode for all sixteen BSCs (Fig. 2C-I), the connection well for the readout electrode (Fig. 2C-III) and cutting the slots

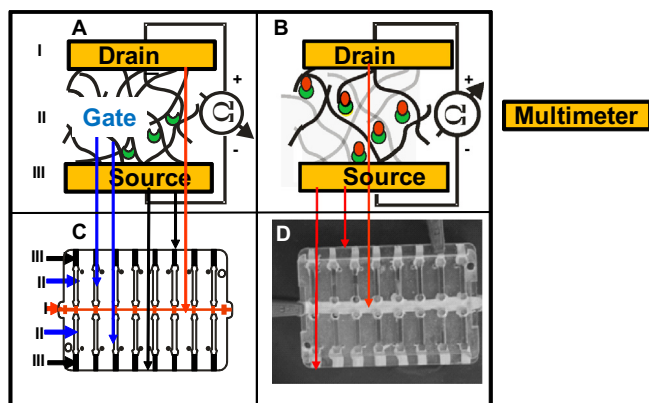


Fig. 2. Electrical percolation biological semiconductor (BSC). Electrical percolation transducer. (A) Schematic of Biological Semiconductor (BSC) in low resistance mode (no antigen). (B) Schematic of (BSC) in high resistance mode (with antigen). (C) Schematic of the actual sensor and (D) A photo of the actual sensor (without the fluidics system). (A) BSC with the electrodes (I and III) connected to the + and – poles of the multimeter. SWNT-antibody bio-nanocomposite gate (III) where the SWNT interconnected network shown as black lines with the antibodies shown as a half-moon shape; (B) Binding of the antigen (circles) to BSC results in disruption of the network (non-contacted CNTs are shown in grey) thus increasing the resistance; (C) A schematic of the PMMA circuit board with 16 BSCs in two rows with arrows pointing from the corresponding transistor elements in (A) and (B). Each BSC contains an individual connection (top arrow) and all BSC have a common central electrode (bottom arrow) in the middle of the chip, antibody bio-nanocomposite network printed between the electrode (middle arrow). (D) A photo of actual chip with the silver common and BSC electrodes (marked with arrows) and the bio-nanocomposite.

for the bio-nanocomposite material (Fig. 2C-II), the lower side of the PMMA sheet was coated with 3 M 9770 adhesive transfer double-sided tape (Piedmont Plastics, Beltsville, MD) and the polycarbonate film was immobilized directly on the PMMA. The bio-nanocomposite was bonded to the polycarbonate film and the electrodes were filled with silver conducting paste.

2.3. Carbon nanotube preparation

The single wall carbon nanotube solution was prepared as previously described [6,8,29]. The CNTs (30 mg/ml) were first shortened and oxidized by mixing with concentrated sulfuric acid and nitric acid mixture (3:1 v/v) and sonicating with a Fisher (FS-14) sonicator for 6 h followed by extensive washing in water (100 ml) until neutralized (pH 7.0). Then the CNT were dispersed in 100 ml 1 M NaOH solution for 5 min to achieve net negative charged carboxylic acid groups and washed with water (100 ml). A positively charged polycation (PDDA containing NaCl) was adsorbed by dispersing the CNT in 50 ml of 1 mg/mL PDDA containing 0.5 M NaCl for 30 min followed by centrifugation (10,000 rpm in Beckman centrifuge for 15 min) and washed with 100 ml of water.

2.4. CNT functionalization

A linker molecule to the carbon nanotube was used [30]. Poly(diallyldimethylammonium) chloride (PDDA) is positively charged and staphylococcal I enterotoxins (SEs) antibody is negatively charged, so the antibodies were electrostatically adsorbed onto carbon nanotube. The positively charged polycation was adsorbed by dispersing the CNT in 50 ml of 1 mg/mL PDDA containing 0.5 M NaCl for 30 min followed by centrifugation (10,000 rpm in Beckman centrifuge for 15 min) and washed with 100 ml of water.

2.5. CNT-antibody complex preparation

The CNT 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 CNT surface. After centrifugation (15 min) and washing extensively with water (10 ml), the modified CNT was stored at 4 °C in pH 8.0 phosphate buffer at a concentration of approximately 1 mg/mL for no more than 2 weeks before use.

2.6. BSC fabrication

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. The deposition process involved filling a pipette with the dispersed SWNT-antibody complex, then completely filling the 1 cm well. 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 amount of SWNTs that were deposited were directly related to their concentration times the volume of the well. The adhesion of the SWNT-antibody complex was found to be sufficient for immobilization on PMMA or polycarbonate necessary to resist removal during the pinding process. Optically, the dried SWNT-antibody complex appeared to provide fairly uniform coverage on the surfaces of the well.

2.7. BSC measurements

The CNT-antibody complex described above is immobilized directly on PMMA or polycarbonate. The range of resistance tolerances for a functional BSC is 30–100 Ω. Before applying SEB samples, either a buffer or the sample without the toxin was added to the chip to establish the resistance of the BSC at baseline measured with the digital multimeter. Different concentrations of SEB samples in phosphate buffer are added to the chip at room temperature (25 °C) and measured. The difference between the two readings is used as signal corresponding to different concentrations of SEB. The resistance of the BSC was measured and recorded with a U1253A/001 digital multimeter connected to a laptop computer via USB port. The data generated is then imported into Microsoft Excel (Microsoft, Redmond, WA) for further analysis.

2.8. Verification of BSC measurements

For the control experiment, after SEB binding the BSC was then blocked with 1% BSA in 15 µl buffer for 30 min. A HRP conjugated anti-rabbit IgG was added to the captured SEB and after 60 min incubation and washing, enhanced chemiluminescence (ECL) was achieved by adding 7 µL of ECL buffer (formed by mixing the two solutions from the chemiluminescent kit in a 1:1 volume ratio) into each well and the ECL intensity was measured immediately with a custom-built point-of-care CCD detector [7,8,31].

3. Electrical percolation based biosensor for staphylococcal enterotoxins

As a model system to demonstrate BSC, the detection of staphylococcal enterotoxins (SEs) was used. SEs are a group of twenty-one heat stable toxins implicated in foodborne diseases resulting from consumption of contaminated foods [32–36]. BSC-based assays for SEs (and for other microbial toxins) offer advantages such as speed and high-throughput.

3.1. Functional elements of BSC for immunological detection

To demonstrate BSC detection, a detector with the bio-nano-composite material was fabricated by depositing pre-functionalized SWNTs with biological ligands to form a biological semiconductor (BSC) layer. A schematic of a sensor using the electrical percolation BSC is shown in Fig. 2. In the low resistance mode (Fig. 2A), the SWNT-antibody network of the BSC (black lines) is shown with no antigens bound to antibodies (half-moon shape). In the high resistance mode (Fig. 2B), binding of antigens (circles) results in disruption of the network thus increasing electrical resistance. Non-contacting SWNTs are shown in grey in Fig. 2B.

A simplified prototype of the BSC sensor is shown in Fig. 2C. The BSC is a unipolar device, with two electrodes painted with silver contact paste on both sides of the printed SWNT-antibody bio-nanocomposite. Several BSCs can be easily fabricated in a row on the same surface. At the circuit level, each BSC contains a connection well (Fig. 2C-III) for the silver electrode, a channel for the bio-nanocomposite (Fig. 2C-II) and a channel for the common silver electrode (Fig. 2C-I) to all eight BSCs in a single row. The assembled chip is shown in Fig. 2D.

The SWNTs are functionalized with rabbit anti-SEB IgG. A previously developed CNT functionalization scheme is employed for binding the SWNTs with the antibodies [7,8,31]. The bio-nanocomposite is then immobilized by drying it directly on the surface of either Poly(methyl methacrylate) (PMMA) or polycarbonate wells (Fig. 2D) fabricated by laser micromachining [7,8,31].

The electrical percolation BSC is operated simply by measuring the electrical resistance between the silver paste electrodes (Fig. 2D-I and III). Binding of the specific antigen to the antibody disrupts the network (Fig. 2B) and increases the resistance. The amount of binding of the specific antigen to the antibody controls the overall resistance of the electrical percolation BSC network, which is measured by an ohmmeter via each BSC electrode and the common electrode.

A circuit board with sixteen electrical percolation BSCs was used in a conventional immunodetection assay by allowing binding of SEB to the antibody gate and washing off unbound material. The measurement value was calculated as the difference between the initial reading recorded (R_0) with no SEB and the reading with SEB (R_1). The difference between the two readings ($R_1 - R_0$) was measured as the signal, and is normalized by R_0 to obtain the signal-to-baseline ratio. The use of a common electrode simplifies fabrication but it introduces a constant difference of resistance among the 16 BSC (based on their position relative to the measuring point). In our case, since we are measuring the difference

between the two readings ($R_1 - R_0$), this constant difference will have no effect.

3.2. SWNT-antibody bio-nanocomposite percolation

The percolation of the SWNT-antibody network was established using various concentrations, v , of SWNT immobilized onto a PMMA surface without (Fig. 3A-a) and with anti-SEB antibody (Fig. 3A-b). Their resistance, Ω , was measured to determine the percolation threshold, v_p , using a conventional power law equation from percolation theory (see form of equation in Fig. 3A and arrow pointing to data fit) with a baseline resistance, σ_0 . Using the power law fit, it was possible to determine that the percolation threshold for the SWNT-antibody network is between 0.2 and 0.3 mg/mL, and does not change significantly after antibody immobilization in Fig. 2A. The rate of change in resistance is directly related to the power-law exponent, n , which was 8 and the power-law coefficient, a , which was 5×10^{-6} as determined from the fit of the power-law to the data in the figure.

There are three characteristic regimes in SWNT concentration associated with these values: (1) between ~ 0.2 and 0.5 mg/mL the percolation threshold is characterized by a steep change (approximately four orders of magnitude) in resistance due to the onset of percolation, (2) between ~ 0.5 and 1 mg/mL the change levels off and the increase is approximately one order of magnitude, (3) over ~ 1 mg/mL the resistance levels off and does not change significantly with higher concentrations of SWNT resulting in nearly complete percolation. Over the entire range, the total change in resistance is approximately five orders of magnitude. The percolation threshold of the SWNT-antibody bio-nanocomposite network also indicates that its typical resistance (Fig. 3A-b) will be higher than the resistance that is attributed to the SWNT only (Fig. 3A-a), presumably due to the contacts between the antibody and the functionalized SWNT.

3.3. BSC-based analysis of SEB

To show the specificity of the BSC response, various amounts of SEB (from 0.1 to 100 ng/mL) in buffer were added to the chip with 1 mg/mL of SWNT (Fig. 4a). The resistance increased proportionally to the amount of SEB. Non-specific antigens were used to study the BSC leak rate, which is the change in resistance with non-specific binding and is an indication of the specificity and the selectivity of BSC actuation. Various non-specific antigens were used, including a smaller molecular weight (14 kDa) protein, lysozyme (Fig. 4b), and a higher molecular weight (150 kDa) protein, human IgG (Fig. 4c). As shown in Fig. 4, the level of non-specific binding in

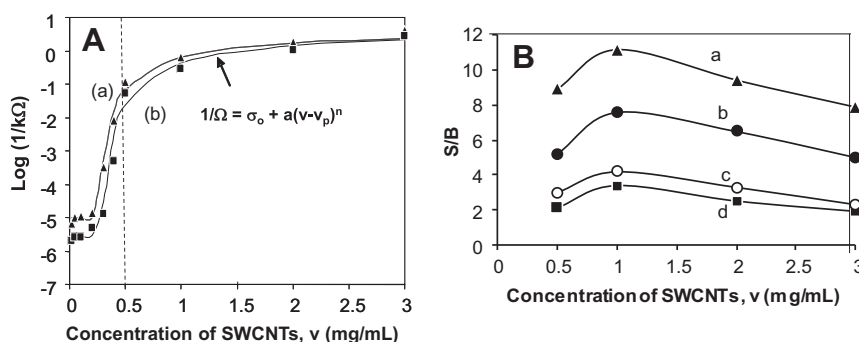


Fig. 3. Percolation of the SWNT bio-nanocomposite. (A) The measured resistance of various concentrations, v , of SWNTs immobilized onto a PMMA surface without (a-triangle) and with (b-rectangle) anti-SEB antibody. (B) The effect of SWNT concentration on BSC response to SEB binding. Various concentrations of SWNT were immobilized with anti-SEB antibody and reacted with SEB: (a) 100 ng/ml SEB, (b) 10 ng/ml, (c) 1 ng/ml and (d) 0.5 ng/ml. For all SEB concentrations, the highest S/B was detected at the point of nearly complete percolation corresponding to 1 mg/ml SWNT.

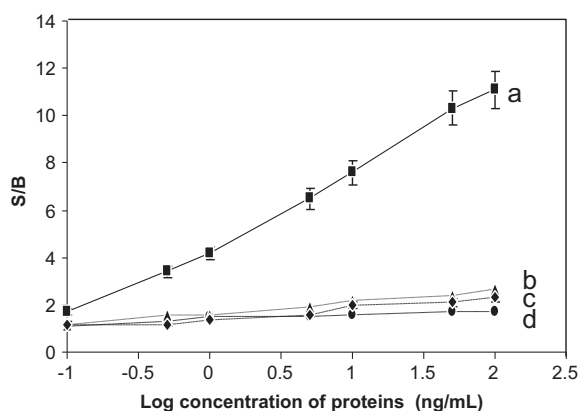


Fig. 4. Electrical characteristics of SEB actuation of BSC. Various concentrations of SEB (0, 0.1, 1, 5, 10, and 50 ng/mL) were applied to the sensor composed of 1 mg/mL SWNT with immobilized anti-SEB IgG (a-rectangle). Nonspecific binding of similar concentrations of lysozyme (b-diamond) and human IgG (c-triangle) to the chip is shown, along with SWNT without antibody (d-circle). Error bar = SD ($n = 8$).

these semiconductors is relatively small regardless of concentration, which is similar to the S/B for SEB concentrations when there is no antibody on the SWNTs (Fig. 4d).

To determine the limit of detection (LOD) for SEB, the S/B ratio from eight replicates of various concentrations of SEB was compared to buffer. A *t*-test demonstrated that at 1 ng/mL, the S/B ratio is significantly different ($p < 0.00017$) from the value using buffer only. Thus, the current configuration has a LOD of 1 ng/mL for SEB. In contrast, the ELISA LOD, using sandwich assays combined with optical detection for SE, ranges from 0.5 to 2 ng/g of food [37–42].

To confirm that the percolation of the SWNT-antibody and the antibody gate mechanisms shown in Fig. 2 depend on SEB binding, an independent measurement of bound SEB to the SWNTs bio-nanocomposite was carried out using a sandwich immunoassay detected by enhanced chemiluminescence (ECL). As shown in Fig. 5A, the intensity of the signal from the captured SEB on the BSC chip is proportional to the amount of SEB. Quantitative analysis of the data (Fig. 5B) suggests a high correlation between the amount of SEB and the ECL signal and that there is a very high correlation ($R^2 = 0.9942$) between the electrical measurements (Fig. 4a) and the ECL measurements (Fig. 5A). The linear regression is also highly significant ($p < 0.0056$), suggesting that the anti-SEB antibody on the BSC chip did indeed capture SEB, and that the direct electrical measurements are in agreement with the indirect sandwich immunoassay detected by ECL.

Our interpretation of the data is that antigen binding leads to rearrangement of the SWNT-antibody network, resulting in physical depletion of electron carriers in the bulk of the SWNT-antibody bio-nanocomposite through changes in contact between the SWNTs. Such contacts are analogous to the physical edge of the conduction band. We suggest that being at this point, the antibody gate mechanism initiated by binding the antigen to the antibody shifts the complex closer to the band gap, which is an energy range where statistically few electron states exist so fewer electrons can jump between SWNTs. This is analogous to decreasing an electric field in a classical semiconductor, and therefore increases the electrical resistance of the SWNT-antibody network. The percolated SWNT-antibody network can therefore be considered the “conduction band”, and the number of electrons in the conduction band (i.e., the band gap) is physically determined by the number of SWNT-antibody complexes in the conduction band rather than by the conventional electronic band gap at the surface of the SWNT that is responsible for electrochemical detection principles.

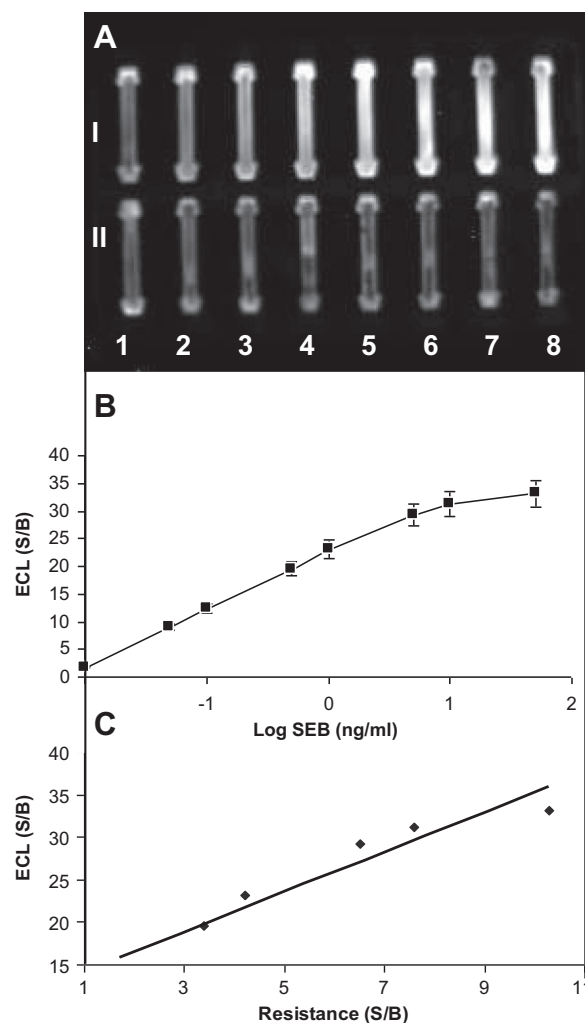


Fig. 5. Sandwich immunoassay of captured SEB on electrical percolation BSC detected using ECL. Different concentrations of captured SEB incubated with Horseradish Peroxidase (HRP) conjugated anti-SEB IgG and assayed with ECL: (A) ECL intensity measured with a custom-built Point-of-Care CCD detector after exposure time of 20 min at SEB concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 ng/mL. (B) Plot of SEB concentration vs. ECL signal. (C) Correlation between the S/B of ECL and resistance measurements at various SEB concentrations.

3.4. BSC computer controlled BSC analysis

While the BSC performs the actual measurements, a support system is needed for fluid delivery to the BSC, electronic measurements, control and data analysis. Fig. 6A shows the schematic of the system and Fig. 6B shows the actual system.

The computer control of BSC is performed using National Instruments Labview control software in conjunction with a USB-6008 multifunction ADC/DAC. Along with supporting power circuitry, which provides the proper voltage and current for the different devices, the software system is responsible for controlling two pumps, as well as the six-way valve.

The system's pumps can be programmed to deliver various fluids (by activating the various ports of the valve) and control the flow rate of each individual pump by utilizing pulse width modulation (PWM) with the constant speed pumps. Each pump can be independently programmed to work at different speeds. The speed of each pump is determined by two user-defined variables: (a) the frequency of each pump period, and (b) the duration of each flow pulse relative to the frequency. Higher frequencies allow for a smoother overall flow rate at the cost of more micropulsations. The duration of the fluid pulse is the variable that

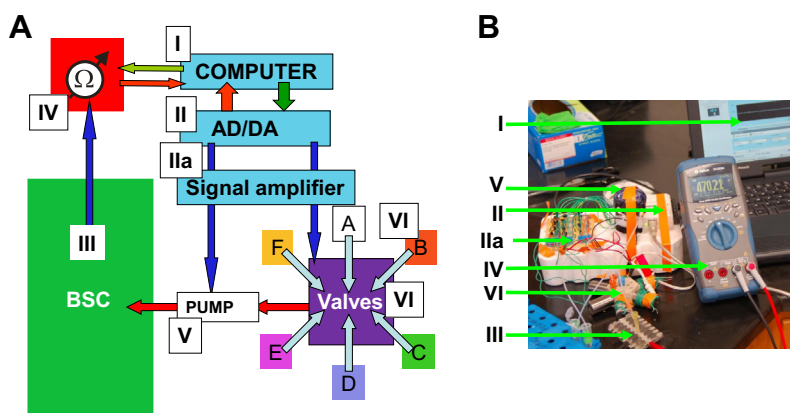


Fig. 6. Automation of BSC detection. (A) Schematic of the system and B the actual photo of the BSC BSC system. The system consists of: Computer (I), analog to digital/digital to analog converter (II), signal amplifier (IIa), BSC chip (III), digital multimeter (IV), pump (V) and six-way valve (VI) with the valves marked as A–F.

actually controls the flow rate, and is measured as a percentage of each pulse. Percentage duration of 100 percent is the equivalent of continuous, full speed fluid pumping and zero percent is effectively off regardless of frequency setting.

The six-way valve is designed to provide one or more fluidic connections for fluid flow to an input, and is used in conjunction with the pumps. Each fluid change requires some extra time in which fluid currently present in the pump tubing needs to be pumped out before new fluid delivery can occur. To do this, the pump is temporarily stopped while switching to a different valve, then restarted after the switch is complete.

The software is designed to run continuously, and is capable of timed operation, although it was not used in this experiment. The controlling functions for each valve and pump are independent of another, and can be started and stopped by the user as long as power is supplied. It is also possible to include conditional operation if necessary.

3.5. Hardware for computer controlled BSC data analysis and fluid delivery

The components of the BDC data analysis, computer control and fluid delivery shown in Fig. 6 are:

- (I) A notebook computer for measurement analysis and the fluidics system control for pumps and valves.
- (II) 12 Channel analog to digital/digital to analog converter (ADC/DAC) which is the interface between the BSC, the pump, the valve and the computer. (IIa) A signal amplifier to amplify the AD/DA signal for operating the pump and the valve.
- (III) The BSC.
- (IV) Digital multimeter for measurement and data transfer to the computer.
- (V) Peristaltic pump for fluid delivery, moving fluids from fluid reservoirs through the valve and to the chip. In this configuration only one pump is used, however a second pump also controlled by the computer is attached to provide fluid for two BSC.
- (VI) A six way valve enabling switching the fluids (samples, buffers, antibodies) delivered to the BSC.

The configuration shown in Fig. 6 is capable of automatic delivery for up to six different fluids to the BSC. The multimeter is connected to the BSC by two electrodes for measuring resistance and is connected to the computer via USB port for data transmission and

for the operation of the device. The data is plotted in real time on the computer and is saved for further analysis.

3.6. Electrical percolation based BSC for real time continuous direct detection of SEB

Unlike indirect detection assays which requires the binding of secondary label antibody and assaying the label, direct biosensors detect analytes directly, enabling simple and rapid detection. To demonstrate the BSC response, the baseline (R_0) was established by adding buffer to the chip, which significantly increased the signal. The baseline was measured, then a solution of 50 ng/mL SEB was injected via the BSC serving as a flow cell (marked in arrow in Fig. 7a). The resistive response of the BSC was immediate, with the signal increasing rapidly upon adding the toxin. Such measurements can be performed in few minutes compared to several hours needed for the conventional ELISA assays.

To measure the specificity of the signal, as controls non-specific antigens were used to measure change in resistance resulting from non-specific binding, an access of such non-specific antigens were measured in separate BSCs including 1 μ g/mL BSA (Fig. 7b), 1 μ g/mL lysozyme (Fig. 7c) and 1 μ g/mL IgG (Fig. 7d) were applied to the BSA. All of these nonspecific antigens resulted in low signal, which demonstrates the specificity of the BSC for SEB. In this flow cell BSC, samples can be continuously monitored while passing through the chip for the presence of a specific antigen. There are many biomedical applications for such a capability including blood

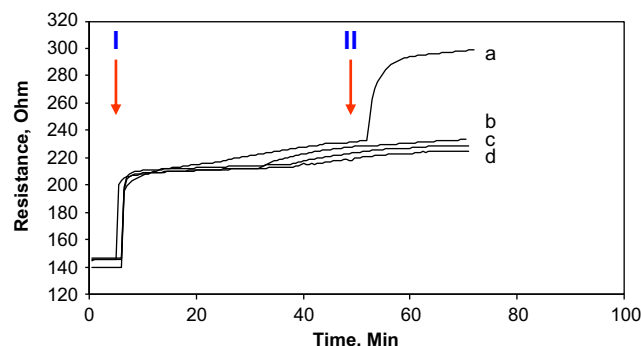


Fig. 7. Real time resistance response of the BSC to the addition of various analytes, various analytes were injected to the BSC through the peristaltic pump and the change of resistance was measured by Ohm meter connected via USB port to a computer for data analysis. (a) 50 ng/mL SEB; (b), 1 μ g/mL BSA; (c) 1 μ g/mL lysozyme; (d) 1 μ g/mL anti rabbit IgG.

and body fluid measurements. In addition, such a flow system can be used for food safety continuous analysis, for monitoring fermenters, and for chemical and industrial applications.

To demonstrate that SEB is actually bound to the BSC, a second anti SEB antibody was injected into the flow cell to “label” the captured SEB on the BSC (Fig. 8) in a “sandwich assay”. In this experiment the resistive response of the BSC was measured in different steps marked with arrows. The addition of buffer (I) resulted in increase in resistance shown in Fig. 8. The addition of 100 ng/mL SEB (curve A) or 1 μ g/mL Lysozyme (curve B) resulted in increased signal (II) around 10 min for the SEB but not for the Lysozyme. A washing step with buffer at around 17 min for both analytes (III) washed unbound material and reduced the none specific signal. The addition of 1 μ g/mL of anti SEB secondary antibody at around 26 min (IV) increased the signal in the BSC with SEB which demonstrates that SEB is indeed bound to the BSC. Additional washing with buffer at around 35 min (V) removed unbound material and decreased the signal.

The binding of the second antibody to the captured antigen further disrupt the CNT network and increase resistance. Although for direct detection a secondary antibody is not needed, the indirect mode of detection using second antibody can be used to verify that the signal from the direct detection is specific. This may be needed to increase confidence in the results in clinical and food safety assays. In addition, the secondary can be used to amplify the signal of the direct detection, especially in cases of low signal or questionable measurement results.

4. Proposed detection mechanism for BSC

While the BSC mechanism is not well known, it has been hypothesized [20] that using a nanomaterial with a biological recognition element in a multilayer 3-D interconnected network (Fig. 2A) enables the number of contacts within the network to be varied by molecular interactions (e.g. antibody-antigen binding) which changes the resistance of the network (Fig. 2B). This change can be measured to determine the number of interactions and hence the concentration of the analyte. Such a mechanism is distinctly different from that of the FETs used in biosensing. In FETs, the mobility of electrons within a single nanotube is dependent on surface interactions (Fig. 1). In contrast, this model assigns

changes in electrical conductivity of the network to the number of contacts of the elements within the network, which is more of a “bulk” based measurement versus the “surface” based measurement of for FETs. Molecular interactions disrupt the network continuity, resulting in an increased resistance. The use of electrical percolation for specific direct electronic gating requires a recognition element to bind with the biological target. Recognition elements can be ligands such as antibodies, DNA, receptors, aptamers, or hormones that control the electrical conductivity of the bio-nanocomposite containing the nanomaterial and recognition element.

Our model suggests that using percolation principles, it will be possible to characterize changes in the connectivity of elements within the SWNT network by modeling electrical percolation as the bulk flow of electrons through a randomly distributed network of conducting elements. In such a network, sites (vertices) or bonds (edges) are established by randomly placing resistors in a 3-D vector space with a statistically independent probability (p) of making contacts. At a critical threshold (p_c), long-range connectivity within the vector space first appears (known as the “percolation threshold”) [43]. Beyond this threshold, the conducting elements increase precipitously and there is an onset of a sharp and very significant increase in the electrical conductivity of the material [44]. Therefore, it is characteristic of the minimal concentration of conductive filler required to form a randomly distributed network that spans the whole material system. Furthermore, mechanical contact is not a necessary condition, since there can be a slight separation with electron flow across elements due to “tunneling”, which results in a gradual change in contact resistance rather than an abrupt “binary” change. Thus, as previously stated, the concentration of conductive filler correlating to the percolation threshold will be affected not by the mobility of electrons within the filler, but rather by the characteristics that control the number of contacts and the contact resistance between filler elements. Thus, the principles governing the percolation threshold are not “electrochemical”, but rather “electrophysical” (e.g., morphology, scale, and orientation of the filler).

5. Technological potential of BSC

Unlike field-effect transistor (FET) based sensors, which rely on an electric field at the surface of the SWNTs to control conductivity, the response of the electrical percolation BSC can be attributed to the number of contacts of CNTs within the network. Since the number of contacts can be varied by molecular interactions (i.e., by antibody-antigen binding), changes in the resistance of the network can be used to determine the number of interactions and hence the concentration of the analyte.

One of the most attractive features of electrical percolation BSCs based on SWNTs is the simplicity of the preparation (screen printing). In contrast, FETs are often fabricated using on-chip chemical vapor deposition (CVD), and require a high-tech infrastructure (clean rooms) for microfabrication of solid-state semiconductor components. Furthermore, unlike FETs, which are constructed with SWNTs as a single wire or sub-monolayer network, BSC do not need to be oriented, so they can be easily fabricated and functionalized in bulk. Electrical percolation BSCs can simply be printed on any non-conductive material to create biosensors capable of detecting a variety of molecules. Selectivity is achieved by printing different specific biological “gates” (such as antibodies, DNA, receptors, or aptamers) which takes full advantage of the natural selectivity of these biological molecules. Moreover, electrical percolation BSC production can be readily scaled to perform multi-analyte detection, unlike single CNT devices that are challenging to fabricate and functionalize.

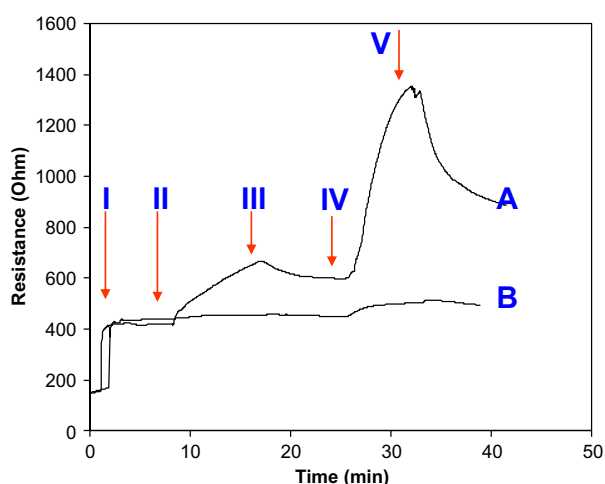


Fig. 8. Resistance response for binding of a second antibody to the captured antigen on the BSC. (I), the response after addition of buffer; (II) the addition of 100 ng/mL SEB (curve A) or 1 μ g/mL Lysozyme (curve B) around 10 min; (III) the addition of washing buffer at around 17 min for both samples; (IV) the addition of 1 μ g/mL secondary antibody at around 26 min for both samples; (V) the addition of washing buffer at around 35 min for both samples.

Having simple biosensor technology may permit wider use of biosensors. Existing technologies are relatively complex, have limited capability for multi-analyte detection, and are costly. The BSC described here overcomes each of these limitations. BSCs are very simple to fabricate and to operate, and are capable of multi-analyte detection. Using BSCs, it may be possible to fabricate miniaturized “biological central processing units (CPUs)” with multiple biological elements, capable of processing and sorting out information on multiple analytes simultaneously. By combining them with computer algorithms, it is possible to automatically perform multi-analyte detection and make decisions analogous to the way a silicon chip processes digital information to make decisions important for direct biodetection of multiple microbial pathogens and their toxins, as well as numerous cancer biomarkers, cardiovascular, or kidney biomarkers. Therefore, a “Biological CPU” could be a transformational technology, providing users with immediate decision-making capability that is useful for many biomedical applications including regulation and actuation of implantable biomedical devices, such as insulin pumps, cardiac assist devices or other theranostics.

In conclusion, we have demonstrated the fabrication of a novel biological semiconductor (BSC) based on electrical percolation through a multi-layer 3-D carbon nanotube-antibody network which can measure biological interactions directly and electronically. In this system, molecular interactions, such as binding of antigens to the antibodies, disrupt the network continuity causing increased resistance of the network. Unlike FET based sensors with oriented SWNTs synthesized on-chip, electrical percolation based biosensors rely on the bulk conductivity of network. Therefore, various ligands can be easily used to functionalize pre-made SWNTs gates in bulk, and the pre-made gates can be simply printed or deposited on non-conductive materials. These factors make BSC a practical approach for direct electronic sensing. The use of BSCs for direct electronic measurements of biological agents has currently been demonstrated using staphylococcal enterotoxin B (SEB) as a model system.

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