Lab-on-a-chip for label free biological semiconductor analysis of Staphylococcal Enterotoxin B

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We describe a new lab-on-a-chip (LOC) which utilizes a biological semiconductor (BSC) transducer for label free analysis of Staphylococcal Enterotoxin B (SEB) (or other biological interactions) directly and electronically. BSCs are new transducers based on electrical percolation through a multi-layer carbon nanotube–antibody network. In BSCs the passage of current through the conductive network is dependent upon the continuity of the network. Molecular interactions within the network, such as binding of antigens to the antibodies, disrupt the network continuity causing increased resistance of the network. For the fabrication of a BSC based detector, we combined several elements: (1) BSC transducers for direct detection, (2) LOC for flow through continuous measurements, (3) a digital multimeter with computer connection for data logging, (4) pumps and valves for fluid delivery, and (5) a computer for fluid delivery control and data analysis. Polymer lamination technology was used for the fabrication of a four layer LOC for BSC detection, the BSC on the chip is fabricated by immobilizing pre-functionalized single-walled carbon nanotubes (SWNTs)–antibody complex directly on the PMMA surface of the LOC. SEB samples were loaded into the device using a peristaltic pump and the change in resistance resulting from antibody–antigen interactions was continuously monitored and recorded. Binding of SEB rapidly increases the BSC electrical resistance. SEB in buffer was assayed with limit of detection (LOD) of 5 ng mL^{-1} at a signal to baseline (S/B) ratio of 2. A secondary antibody was used to verify the presence of the SEB captured on the surface of the BSC and for signal amplification. The new LOC system permits rapid detection and semi-automated operation of BSCs. Such an approach may enable the development of multiple biological elements "Biological Central Processing Units (CPUs)" for parallel processing and sorting out automatically information on multiple analytes simultaneously. Such an approach has potential use for point-of-care medical and environmental testing.

1. Introduction

Lab-on-a-chip (LOC) for clinical diagnostics requires a transducer which converts biochemical interactions into a measurable signal. Two types of transducers are used for biodetection: (1) transducers for label free detection such as Surface Plasmon Resonance (SPR), piezoelectric, cantilever or field effect transistor (FET) and (2) transducers to detect labeled analytes, such as fluorescent or electrochemical transducers.

For LOC, transducers for label free detection have a great advantage because they reduce the number of steps needed for analysis, and therefore minimize the number of reagents and functions needed to be preformed on the chip (*e.g.*, incubation with secondary antibodies, labeling, washing, *etc.*). This simplifies the chip architecture and reduces cost, making the chip more attractive for clinical applications. Unlike current sandwich immunoassays which are three step tests (binding of target to primary antibody, binding of the labeled antibody, and enzymatic label detection), label free detection reduces the complexity of the assay to a single step: the binding of target to primary antibody.

Despite such advantages, label free transducers are not used widely as detectors for LOC analysis. Current transducers for label free detection SPR, piezoelectric or cantilever are too complex (*e.g.*, require complex optics, incorporation of mechanical elements, or require sophisticated fabrication capabilities) to be integrated into LOC or to be used for analysis of LOC assays. However, electrochemical detection[1] and contactless conductivity detection[2] were incorporated into LOC.

One promising transducer technology for biodetetection is based on nanomaterials. Single-walled carbon nanotubes (SWNTs) have the potential to enhance the sensitivity of biodetection because of their large surface area and electrical conductivity. SWNTs have been used for sandwich immunoassays detected optically with a labeled secondary antibody.[3–5] SWNTs were also used for label-free detection as gates for FETs,[6–8] which enabled accurate measurement of changes in electrical conductance in individual nanotubes. However, such a FET is normally fabricated on a chip from single SWNTs grown by chemical vapor

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deposition (CVD), which significantly complicates their fabrication. This also limits the number of channels in the device, rendering it impractical for LOC use.

More recently, a network of randomly distributed SWNTs was developed for direct detection using electrical percolation principles.[9,10] In this new type of transducer, a SWNTs–antibody complex forms a bio-nanocomposite network that functions as a "biological semiconductor" (BSC). A recognition element (*e.g.*, antibody) binds to a biological target (*e.g.*, antigen) and alters the electrical conductivity of the bio-nanocomposite network, which is then measured by a simple ohmmeter. BSCs can be easily integrated into LOCs, and thus can be used for on chip label free detection.

As a model system to demonstrate incorporation of BSC into LOC, we used the detection of Staphylococcal Enterotoxins (SEs). SEs are a group of twenty-one heat stable toxins implicated in foodborne diseases[11–15] and have also been implicated in other diseases such as rheumatoid arthritis,[16,17] atopic eczema[18–20] and toxic shock syndrome.[21] SEs are also recognized as potential bioweapons.[22–25]

Here we are presenting a LOC for real-time SE detection, which incorporates BSCs as transducers. The LOC consists of the following elements: (1) BSC transducers for direct detection, (2) LOC for flow through continuous measurements, (3) a digital multimeter with computer connection for data logging, (4) pumps and valves for fluid delivery and (5) a computer for fluid delivery control and data analysis. The real-time response of the assay is demonstrated for the detection of Staphylococcal Enterotoxin B.

2. Experimental

2.1 Reagents and materials

Reagents. Single-walled carbon nanotubes (SWNTs) 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) and rabbit anti-SEB affinity purified IgG were purchased from Toxin Technology (Sarasota, FL). All other reagents were of analytical grade and de-ionized water was used throughout.

Materials for the fabrication of LOC. Clear 0.25 mm polycarbonate film and 1/8 inch acrylic were obtained from Piedmont Plastics (Beltsville, MD).

Electronic components. 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).

Preparation equipment. Fisher (FS-14) sonicator was obtained from Fisher Scientific (Pittsburgh, PA) and Beckman mini centrifuge was obtained from Beckman (Fullerton, CA).

2.2 Preparation of the BSC

The SWNT solution was prepared as previously described.[3,4,10] The SWNTs (30 mg) 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 SWNTs 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). The positively charged polycation was adsorbed by dispersing the SWNTs in 50 mL of 1 mg mL^{-1} PDDA containing 0.5 M NaCl for 30 min followed by centrifugation (10 000 RPM in Beckman centrifuge for 15 minutes) and washed with 100 mL of water.

SWNT functionalization. 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^{-1} for 1 h at room temperature, so that the antibody was adsorbed onto the SWNT surface. After centrifugation (15 minutes) and washing extensively with water (10 mL), the modified SWNT was stored at 4 °C in pH 8.0 phosphate buffer at a concentration of approximately 1 mg mL^{-1} for no more than two weeks before use.

BSC fabrication. The SWNT–antibody complex (1 mg mL^{-1}) was applied to the chip surface by depositing pre-functionalized SWNTs with antibody to form a biological semiconductor layer into the PMMA–PC chip. 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.

2.3 Fabrication of LOC

The LOC was designed and fabricated as previously described.[26] The LOC was designed in CorelDraw11 (Corel Corp. Ontario, Canada) and micro-machined in 1/8 inch black acrylic using a computer controlled Epilog Legend CO2 65W laser cutter (Epilog, Golden, CO). Before cutting, both sides of the acrylic sheet were coated with 3 M 9770 adhesive transfer double sided tape (Piedmont Plastics, Beltsville, MD).

2.4 LOC measurements

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 the baseline (R_0), measured with the digital multimeter. Different concentrations of SEB samples in phosphate buffer are added to the chip at room temperature (25 °C) and the resistance measured again (R_1). The difference between the two readings ($R_1 - R_0$) is used as signal corresponding to different concentrations of SEB. The value obtained with concentration of 0 ng mL^{-1} SEB was defined as background. The ratio of the signal to background (S/B) was further used to quantify the SEB concentration. The resistance of the BSC was measured and recorded with a U1253A/001 digital multimeter (Agilent Technologies, Santa Clara, CA) connected to a laptop computer *via* USB port. The data generated are then imported

into Microsoft Excel (Microsoft, Redmond, WA) for further analysis.

3. Results and discussion

To enable point-of-care label free immunoassay analysis of SEB with a biological semiconductor, a LOC was developed for applying electrical percolation based transducer. The overall system for real-time electrical percolation biosensing (Fig. 1A) includes three main elements: (1) LOC chip, (2) electronics and computer control system, and (3) fluid delivery system. The modular system (Fig. 1B) was designed to deliver fluid to the LOC chip (input) and to record the LOC data (output).

3.1 Electrical percolation-based LOC chip

The electrical percolation-based LOC consists of: (1) the "biological semiconductor" (BSC), which is a network of randomly distributed SWNTs–antibody complex that functions as the "gate" to control electrical current, (2) silver electrodes used as source and drain for the resistance measurement, and (3) the microfluidics fabricated from non-conductive material (PMMA) which are used as an insulator and base for fabrication of the semiconductor and the fluid delivery elements to the BSC.

The model of the BSC operation is shown in Fig. 2. The BSC (Fig. 2A) is a unipolar device, with the silver source and drain electrodes (I and III) painted on both sides of the printed SWNT–antibody complex gate (II) which is shown as an interconnected network of the BSC (black lines) with antibodies (crescents) which is connected to a power source *via* the electrodes. Binding of antigens (circles) to the network disrupts the network (non-contact SWNTs are shown in grey), thus increasing electrical resistance (Fig. 2B). A schematic of the PMMA circuit board with 16 BSCs in two rows is shown in Fig. 2C and a photo of the actual chip with the silver electrodes and the bio-nanocomposite is shown in Fig. 2D. In this configuration, each of the sixteen BSCs on the chip contains an individual source (right arrow) and all BSC have a common central drain (left arrow) in the middle of the chip. Each antibody bio-nanocomposite network gate is printed between the electrodes (middle arrow).

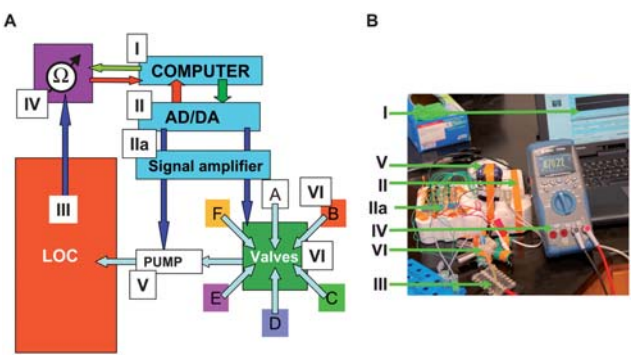

Fig. 1 Semi-automatic fluidics system for BSC: (A) schematic of the system and (B) the actual photo of the BSC LOC system. The system consists of: computer (I), analog to digital/digital to analog converter (II), signal amplifier (IIa), BSC chip (III), digital multimeter (IV), pump (V) and six-way valve (VI) with the valves marked as A, B, C, D, E and F.

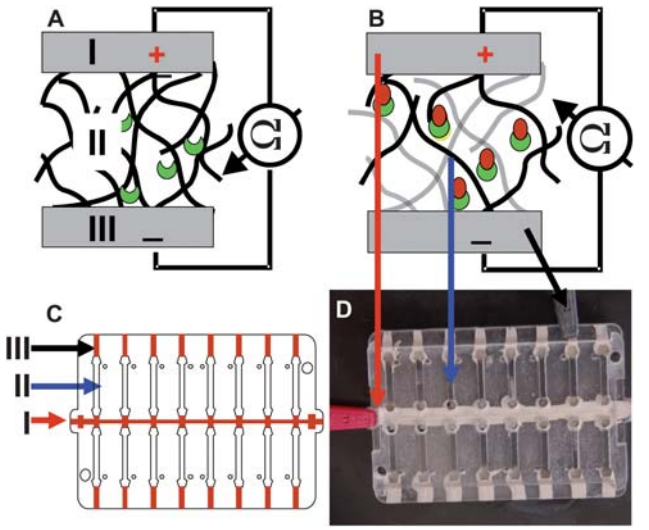

Fig. 2 Electrical percolation transducer. (A) Schematic of biological semiconductor (BSC) in low resistance mode (no antigen). (B) Schematic of BSC in high resistance mode (with antigen). (C) Schematic of the actual sensor and (D) a photo of the actual sensor (without the fluidics system). (A) BSC with the electrodes (I and III) connected to the + and − poles of the multimeter. SWNT–antibody bio-nanocomposite gate (III) where the SWNT interconnected network is shown as black lines with the antibodies is 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. (D) A photo of the actual chip with the silver common electrodes and the bio-nanocomposite. Each BSC contains an individual connection (right arrow) and all BSCs have a common central electrode (left arrow) in the middle of the chip, and an antibody bio-nanocomposite network printed between the electrodes (middle arrow).

In this configuration, the LOC functions as a "flow cell" in which the samples to be measured flow through with a small volume (~10 μL cells). To create the flow cell, a PMMA layer with holes for input fluid delivery for each of the 16 BSC (not shown) is placed on the top of the chip, another hole on the bottom is used for fluid input (from the pump) and the other for fluid output to the waste. To create the gate, anti-SEB primary antibodies are immobilized onto an SWNT surface through electrostatic adsorption. The antibody–nanotube mixture is then immobilized onto a polycarbonate surface which bonds to the main microfluidic chip to complete assembly of the LOC.

3.2 Fabrication of lab-on-a-chip for BSC based immunoassays

The laminated LOC system described here consists of sixteen independent flow cells in which the sample flows through the BSC channels and interacts with the BSC gate, which generates the signal.

The elements of the chip. Fig. 3 shows the functional elements of the LOC. The overall schematic of the sixteen channel LOC is shown in Fig. 3A. The main elements of the LOC are BSC channel specific electrodes (1), fluidic output ports for each BSC

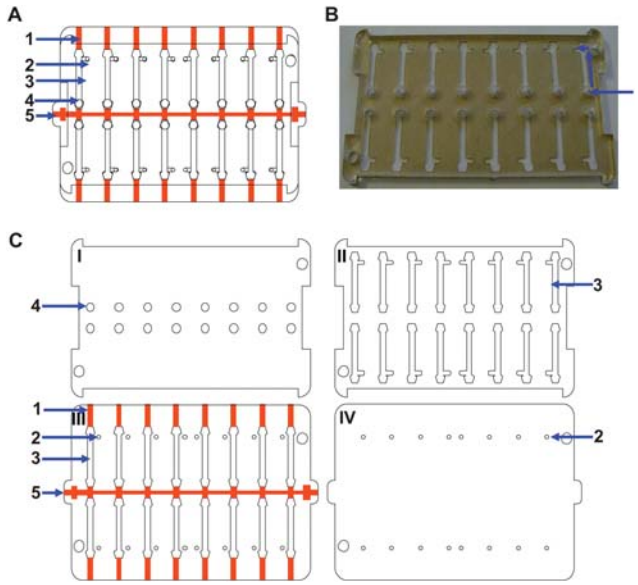

Fig. 3 Functional elements and the outline of layers for the four layers LOC. Functional elements of the LOC (A), an actual chip fluidics layer (B) and the outline of individual layers for the four layer LOC (C). (A) The functional elements of the LOC shown in (A) include: BSC channel specific electrodes (1), fluidic output ports for each BSC (2), the BSC gate with flow through channel (3), fluidic input ports for the flow cell of each BSC (4) and the common ground electrode for all BSC channels (5). The flow through path of the samples in an actual chip fluidics layer is shown in (B), where arrows show the fluidic path through the BSC. The outline of individual layers for the four layer LOC is shown in (C), the layers are numbered in order from the top layer down (I to IV) the functional elements in each layer are numbered as in (A).

(2), the BSC gate with flow through channel (3), fluidic input ports for each BSC (4) and the common ground electrode for all BSC channels (5). The flow through path of the samples in an actual chip is shown in Fig. 3-II, where arrows show the fluidic path through the BSC.

LOC fabrication. A layered fabrication approach is employed for the LOC consisting of a four layer (Fig. 3C), 3D fluidic structure constructed with a rigid 3.2 mm PMMA (acrylic) core laminated with additional layers of thin polycarbonate (PC) film bonded with adhesive. The PMMA core (Fig. 3, layer #III) provides rigidity to the assembly and space for the SWNT–antibody gate fabrication. Layer #I is also constructed from acrylic, which provides anchor points for fluid input. The thin PC laminating layers #II and #IV are used for fabrication of fluidics channels, as well as encapsulation of the chip, reducing user exposure to toxic analytes.

For LOC production, PC channel layers are laminated with adhesive and then cut by a CO_2 laser, machining both the adhesive and the PC simultaneously. After removing the protective layer after the cut, the chip is assembled, layer-by-layer, by aligning them with the adjoining layer and applying pressure. The layer order is such that every other layer is a polymer coated with double layer adhesive.

The layers in the design shown in Fig. 3B correspond to their order in the completed chip, with layer #I on top. All layers have alignment holes for easier assembly in addition to their functional elements, labeled using the numbering scheme introduced in Fig. 3A as follows:

Layer #I: the top layer, made from PMMA, seals the LOC's upper fluidic channels while enabling loading of samples by providing an inlet connection for the peristaltic pump (Fig. 3C-4).

Layer #II: the second layer, made from PC, forms the fluidic channels of the BSC flow cell (Fig. 3C-3). For fabrication and assembly, this layer is constructed with adhesive on both sides.

Layer #III: this core layer is made from PMMA, which provides rigidity to the assembly, an engraved surface for the electrode assembly (Fig. 3C, 1 and 5), as well as high volume for the SWNT–antibody gate fabrication and for the sample flow through (Fig. 3C-3). In addition this layer provides an outlet for the sample (Fig. 3C-2) and serves as an anchor point for electrical connectors. This layer carries adhesive on the bottom.

Layer #IV: the forth layer is made from PC and forms a bottom seal for the core layer and provides an outlet for the fluid from the BSC channels (Fig. 3C-2).

The lamination fabrication method used here employs laser machining, which is very rapid (takes only minutes) and ideal for LOC prototyping, unlike PDMS prototyping which requires multi-step fabrication which may be tedious and time consuming for multi-channel 3D devices.

3.3 LOC computer control and fluid delivery systems

While the LOC performs the actual measurements, a support system is needed for fluid delivery to the LOC, electronic measurements, control and data analysis. Fig. 1A shows the schematic of the system and Fig. 1B shows the actual system.

Software for LOC control. The computer control of LOC 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 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 be 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. Future additions include conditional operation.

Hardware for LOC data analysis, computer control and fluid delivery. The components of the LOC data analysis, computer control and fluid delivery 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 LOC, 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 LOC.

(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 LOC.

(VI) A six way valve enabling switching the fluids (samples, buffers, antibodies) delivered to the LOC.

The configuration shown in Fig. 1 is capable of automatic delivery for up to six different fluids to the LOC. The multimeter is connected to the LOC 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 are plotted in real time on the computer and are saved for further analysis.

3.4 Electrical percolation based LOC for continuous real-time monitoring of SEB

Unlike indirect detection assays which require the binding of secondary label antibody and assaying the label with techniques such as ELISA, 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^{-1} SEB was injected *via* the LOC serving as a flow cell (marked in arrow in Fig. 4-a). The resistive response of the BSC was immediate, with the signal increasing rapidly upon adding the toxin. Such measurements can be preformed in a few minutes compared to several hours needed for the conventional ELISA assays.

To measure the specificity of the signal, non-specific antigens were used as controls to measure change in resistance resulting from non-specific binding. An excess of such non-specific antigens were measured in separate BSCs including 1 μg mL^{-1} BSA (Fig. 4-b), 1 μg mL^{-1} lysozyme (Fig. 4-c) and 1 μg mL^{-1} IgG (Fig. 4-d) were applied to the BSA. All of these non-specific antigens resulted in a low signal, which demonstrates the specificity of the BSC for SEB. In this flow cell LOC, samples can be continuously monitored while passing through the chip for the

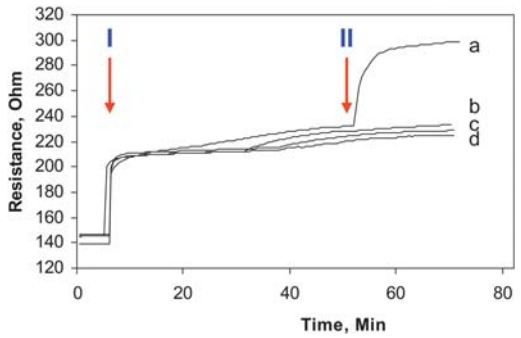

Fig. 4 Real time resistance response of the BSC to the addition of various analytes, various analytes were injected to the LOC through the peristaltic pump and the change of resistance was measured by ohmmeter connected *via* USB port to a computer for data analysis. (a) 50 ng mL^{-1} SEB; (b) 1 μg mL^{-1} BSA; (c) 1 μg mL^{-1} lysozyme; and (d) 1 μg mL^{-1} anti rabbit IgG.

presence of a specific antigen. There are many biomedical applications for such a capability including blood 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. 5) 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. 5. The addition of 100 ng mL^{-1} SEB (curve A) or 1 μg mL^{-1} 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 μg mL^{-1} of anti SEB secondary antibody at around 26 min (IV) increased

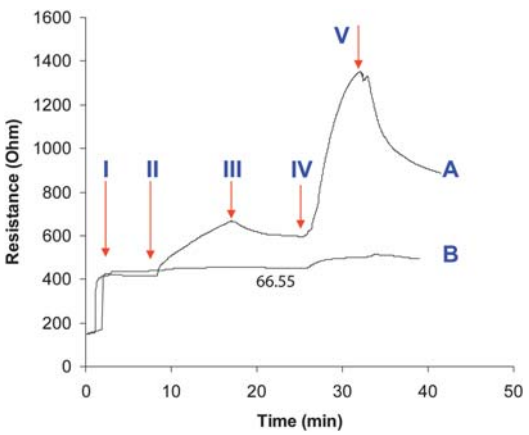

Fig. 5 Resistance response for binding of a second antibody to the captured antigen on the BSC: (I) the response after addition of buffer; (II) the addition of 100 ng mL^{-1} SEB (curve A) or 1 μg mL^{-1} lysozyme (curve B) around 10 min; (III) the addition of washing buffer at around 17 min for both samples; (IV) the addition of 1 μg mL^{-1} secondary antibody at around 26 min for both samples; and (V) the addition of washing buffer at around 35 min for both samples.

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 disrupts the CNT network and increases resistance. Although for direct detection a secondary antibody is not needed, the indirect mode of detection using a 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.

To measure the response of the LOC to SEB, a serial dilution in the range 5–100 ng mL^{-1} of SEB was measured (Fig. 6). The signal to noise ratio of the assay was calculated as the ratio between the values of the measurement to the value at T_0. The SEB limit of detection is at a concentration of 5 ng mL^{-1} which generates a signal to baseline (S/B) ratio of approximately 2 and the signal increased with the increased concentration level of SEB.

Regarding the device reproducibility, we analyzed the data from this device (Fig. 6A) and data obtained from a similar BSC-device used for SEB detection in the none LOC configuration.[27] As shown in Fig. 6B there is high correlation between the values of the two devices ($r^2 = 0.9982$) suggesting high reproducibility of the device.

SEs have been traditionally assayed immunologically using Enzyme-Linked Immunosorbent assays (ELISA),[28] which generally use labeled antibodies and optical detection and takes hours to complete. The BSC transducer in the LOC enables very rapid detection without a labeled secondary antibody. Many detection assays (*e.g.* food testing) involve sample preparation steps which concentrate the sample and increase assay's sensitivity. In our previous work, to detect SEB in food samples, a partial sample purification with carboxymethylcellulose (CM) was used to lower the assay background[3–5] and to increase sensitivity. Incorporation of a CM sample purification to the LOC is likely to improve the sensitivity of BSC based detection by 5–10 fold.

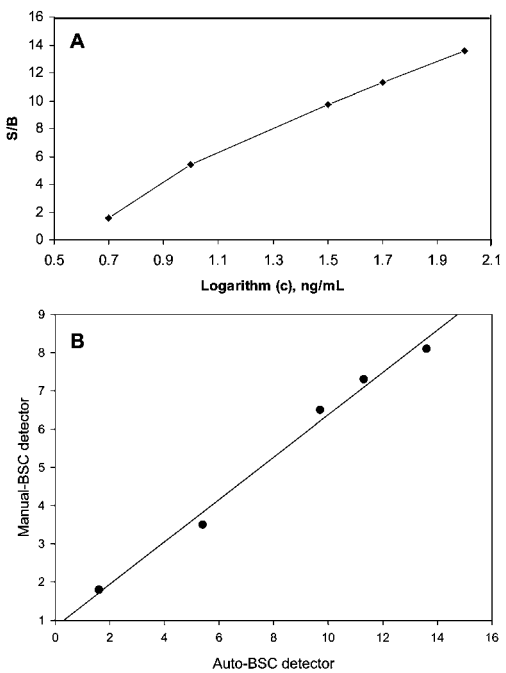

Fig. 6 LOC detection of SEB. (A) Various concentrations of SEB (in the range 5–100 ng ml^{-1}) were injected to BSC and the response was measured electronically. The signal to noise ratio (S/B) of the assay was calculated as the ratio between the values of the measurement to the value of the control (T0). (B) Regression analysis of the data in (A) (auto-BSC detector) and data from manual-BSC detector.

4. Conclusions

Lab-on-a-chip (LOC) for clinical diagnostics requires a transducer which converts biochemical interactions into measurable signal. The integration of a biological semiconductor (BSC) transducer based on electrical percolation principles using a SWNT–antibody complex for label free analysis into LOC enables rapid (few minutes) immunological detection of Staphylococcal Enterotoxin B (SEB) directly and electronically. The flow cell LOC enables continuous real-time monitoring of the sample while passing through the chip, and is capable of specific detection of SE. This feature can be used for monitoring the presence of analytes in the sample for biomedical, food safety, chemical and industrial applications. A similar approach can be used for detection of other biological interactions. The limit of detection is 5 ng mL^{-1} which is in the range (1–10 ng mL^{-1}) of SPR real time detection of SEB[29,30] and is in the range of ELISA the most common method for detection of SEs with sensitivity of 1.56 ng mL^{-1} for colorimetric ELISA.[31] Moreover, many detection assays for SEB utilize sample preparation to improve sensitivity, the addition of sample preparation was shown to improve the binding of the antibody increased the sensitivity of ELISA assay.[3–5] However, sample preparation, which can be added to our assay, to improve sensitivity will slow the assay and is not suitable for the rapid detection focus of this assay.

One of the most attractive features of the approach described here is its relative simplicity in terms of LOC fabrication, BSC integration and assay measurement. For LOC fabrication we used lamination fabrication technology which enables rapid prototyping and fabrication. Integration of BSCs is also relatively simple using screen-printing or any other depositing technologies and the simplicity of measurements (multimeter) makes this technology attractive for various clinical applications.

The new LOC system described here also includes pump and computer control which allows semi-automated operation of BSCs. Although only sixteen BSCs were used in the LOC described here, numerous BSCs can be fabricated on the same chip enabling multi-analyte detection with the ultimate goal of fabricating miniaturized "Biological Central Processing Units (CPUs)" with multiple biological elements capable of processing and sorting out information on multiple analytes simultaneously. In combination with fluid delivery systems, microfluidics and computer algorithms, it may be possible to automatically perform multi-analyte detection and make decisions analogous to the way a silicon chip processes digital information to make calculations, which is useful for many biomedical and clinical applications including direct biodetection of microbial pathogens and their toxins, cancer cardiovascular or kidney rapid

diagnostics. Such an approach has potential use for point-of-care medical and environmental testing.

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