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Rapid detection of multiple foodborne pathogens using a nanoparticle-functionalized multi-junction biosensor

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ABSTRACT

Real-time identification of multiple bacterial pathogens in food is urgently needed to ensure food safety. Although rapid and sensitive detection methods offering simplicity, accuracy, and multiplexity are highly desirable for industrial food applications, the development of a biosensor that meets all criteria remains a challenge. In this study, a single walled carbon nanotube- (SWCNT) based multi-junction sensor was designed for potential multiplexed detection of foodborne pathogens. Gold tungsten wires (Ø: 50 μm) coated with polyethylenimine (PEI) and SWCNTs were aligned to form a 2×2 junction array, functionalized with streptavidin and biotinylated antibodies specific for *Escherichia coli* K-12 and *Staphylococcus aureus*. Electric current (I) measurements in response to target binding events in pure serial diluted samples of *E. coli* and *S. aureus* at each junction within the 2×2 array were monitored to create calibration curves. An inverse correlation between I signals and bacterial concentrations was observed. Changes in I (ΔI) were also calculated to reduce background noise and emphasize the SWCNT-based sensor's response to the biorecognition reactions between antibody and antigens. A linear regression was observed for both the *E. coli* and *S. aureus* functionalized array sensors, $R^2 = 0.978$ and $R^2 = 0.992$, in range of 10^2–10^5 CFU/mL. The calibration curves were used to evaluate the sensor's multiplexing capabilities to detect *E. coli* and *S. aureus* in 10 μL and 100 μL batch microbial cocktail samples.*I*signal responses exhibited similar measurement trends indicating that the developed SWCNT-based multi-junction biosensor has potential for sensitive, simple, and multiplexed applications.

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1. Introduction

Food safety monitoring is a key aspect within the food industry. The security and safety of our food depends on the ability to detect, identify and trace foodborne pathogens. However, minimizing the occurrence of microbial contamination remains a challenge due to the increase in production of minimally processed foods and the globalization of our food supply (Scallan et al., 2011). Potential biological threats to our health and economy emphasize the significance of foodborne pathogen detection methods. Despite greater biological understanding and technological advancements, current detection methods have significant drawbacks. Traditional plate counting, though accurate and affordable, is time-consuming and requires sample pre-enrichment. DNA amplification methods offer a faster detection time with good sensitivity, but are laborious and expensive; and magnetic-based approaches are applicable to complex food samples, but require lengthy sample preparation, costly reagents, and limited sensitivity (Kim et al., 2013). Furthermore, most methods involve the use of bench-top instruments in stationary laboratories operated by skilled personnel. Hence, development of an identification method that meets the requirements of miniaturization, cost-efficiency, and the ability for simultaneous detection of multiple analytes has become the key focus in the field of pathogen detection (Pedrero et al., 2009).

Biosensor technology offers promising solutions for portable, rapid and sensitive detection in food applications due to micro- and nano-fabrication techniques (Mello and Kubota, 2002). One of the advantages of applying micro- and nano-fabrication techniques into biosensors is the possibility of achieving multiplexed analysis within a shortened analysis time (Pedrero et al., 2009). Integration of nanomaterials into immunosensors has gained recognition for its ability to enhance bacterial detection. Engineered nanomaterials, such as magnetic nanoparticles (MNPs) (Varshney and Li, 2007; Ravindranath et al., 2009), carbon nanotubes (CNTs) (Chunglok et al., 2011; Zhao et al., 2011), nanorods (NRs) (Wang and Irudayaraj, 2008), quantum dots (QDs) (Zhao et al., 2009; Vinayaka and Thakur, 2010), and nanowires (NWs) (Wang et al.,

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2008) have been used in biosensor research as catalytic tools, immobilization platforms and optical labels to improve sensitivity, stability and response time. Amongst the many nanomaterials, single-walled carbon nanotubes (SWCNTs) are a key focus in molecule-based electrical circuits and biosensor research based on their electrical, structural, and mechanical properties. The unique electrical properties of SWCNTs stem from the electronic structure of the two-dimensional (2-D) graphene sheet composed of a single atomic layer of graphite with a honeycomb lattice of sp^2 bonded carbon atoms (McEuen et al., 2002). Enhanced sensing performance can be attributed to SWCNTs' size compatibility to biomolecules, as they have the smallest diameter of 1 nm (Chen et al., 2003; Cherukuri et al., 2004; Barone et al., 2005). SWCNTs provide maximum interaction with adjacent biomolecules, in that all carbon atoms are in direct contact with its environment (Allen et al., 2007; Maroto et al., 2007; Heller et al., 2008). Thereby, electrochemical reactivity can be amplified, even at minute environmental variations (Wang, 2005; Vashist et al., 2011). In addition, the low charge carrier density of SWCNTs is comparable to the surface charge density of proteins, which makes SWCNTs well suited for electronic detection of target biomolecules (Heller et al., 2006). Therefore, when binding events occur at the SWCNT interface, the charge accumulation or depletion in the 1-D nanostructure takes place in the "bulk" of the structure, creating large electrical property changes that can potentially enable the detection of a single molecule (Wanekaya et al., 2006).

SWCNTs have been integrated into electrochemical immunosensors in field effect transistor (FET) designs (Besteman et al., 2003; Boussaad et al., 2003; Artyukhin et al., 2006) and for electrode surface modification as a means to improve electron transfer rates and working surface area (Okuno et al., 2007; Zhao et al., 2011). Studies have also used nanotubes to construct molecular junctions based on its ability to control the energy gap of electrons (Forzani et al., 2004; Aguilar et al., 2005; Maruccio et al., 2007). However, the intricate sensor designs and elaborate fabrication processes prevent SWCNT biosensors from evolving into practical sensing tools for industrial applications. A SWCNT-based biosensor with simple fabrication and minimal sensing procedures will offer an important step towards development of sensitive and selective bio-recognition devices for multiplexed foodborne pathogen detection.

In our previous study, the change in electrical current measurements of a SWCNT functionalized microwire single junction biosensor was monitored to detect *E. coli* at 10^2 CFU/mL with a detection time of less than 5 min (Yamada et al., 2014). Based on research findings, the objective was to incorporate SWCNTs into a multi-junction biosensing device for potential as a rapid sensing unit for portable multiplexed applications. The biosensor operates by optimizing a junction array with a bio-nano modified recognition platform to convert molecular binding events between target antigens and antibodies at each junction into measurable electrical signals used for pathogen identification and quantification.

2. Materials and methods

2.1. Materials

7% gold-tungsten plated wire (Ø: 50 μm) was manufactured from ESPI Metals (Ashland, OR). Polydimethylsiloxane (PDMS; Sylgard 184 silicone elastomer curing agent and base) was ordered through Dow Corning (Midland, MI). Copper clad printed circuit boards ($160.78 \times 114.30 \times 1.52$ mm^3) and etchant solutions were purchased from Radio Shack (Honolulu, HI). SWCNTs (>95% purity, Ø: 15 ± 5 nm, 1–5 μm lengths) were supplied from NanoLab, Inc. (Waltham, MA). Alcohol (95%), BD Bacto peptone, BBL trypticase soy broth and Difco plate count agar were procured from VWR (West Chester, PA). N,N-dimethylformamide (DMF), polyethylenimine (PEI, branched, average $M_w \sim 25{,}000$) and streptavidin from *Streptomyces avidinii* (affinity purified, lyophilized from 10 mM potassium phosphate, ≥13 U/mg protein) were purchased from Sigma Aldrich (St. Louis, MO). Biotinylated polyclonal *E. coli* (4 mg/mL) and *S. aureus* (4 mg/mL) antibodies and OXOID MacConkey agar were purchased from Thermo Fisher Scientific (Waltham, MA).

2.2. Instrumentation

Microwire sanitization and SWCNT dispersion were performed using a digital sonifier (450, Branson, Danbury, CT). An automated XYZ stage (Franklin Mechanical & Control Inc., Gilroy, CA) controlled by the COSMOS program (Velmex, Inc., Bloomfield, NY), furnace (Thermolyne, Thermo Scientific, Waltham, MA), and solder kit (Radio Shack, Honolulu, HI) were used during SWCNT coating and wire assembly. A desktop 3-D printer (TAZ 4, Lulz Bot, Loveland, CO) was used to fabricate the sensing device housing. Circuitry parts (Radio Shack, Honolul, HI) and picoammeter (6485, Keithley, Cleveland, Ohio) were integrated into the sensing system.

2.3. SWCNT coating technique

SWCNT networks can be formed by several approaches including spin coating and spray coating (Jang et al., 2008). Though spin coating is a simple method used to form SWCNT networks, it is limited to small planar areas and large amounts of SWCNT colloid solution are lost. In addition, spray coating, which is applicable to large surface areas, is not useful for obtaining uniform networks. To overcome these difficulties, the dip-coating method was used to coat the microwire electrodes.

SWCNTs were dispersed in DMF at a concentration of 0.1 g/L by sonicating the solution for 6 h (Rouse et al., 2004). After the initial 6 h of sonication, the dispersion was further sonicated for 2 h before use each day. Prior to SWCNT coating, microwire electrodes were cut to a length of 31 mm and sonicated in DI water, followed by 70% alcohol for 5 min each. Sanitized wires were dried in a furnace at 175 °C for 10 min and mounted onto the automated XYZ stage for step-wise surface modification. A computer was used to initiate the XYZ motor control program. Wires were immersed into glass vials containing 9 mL of 1% PEI solution for 5 min and withdrawn at a constant withdrawal velocity (ν_w) of 6 mm/min. PEI coated wires were baked at 175 °C for 1 h (Cairns, 2013). Subsequently, wires were re-mounted onto the XYZ stage and immersed into the SWCNT-DMF suspension for 5 min. The PEI coated wires were withdrawn at the same constant velocity ($\nu_w = 6$ mm/min) to generate a high capillary force and large influx of SWCNT colloids onto the wire (Jang et al., 2008). Dip coating into the SWCNT-DMF solution was repeated to achieve two dip coats, approximately 0.84 μm thick.

2.4. Device fabrication

Disposable sensor chips were fabricated as a base for the multi-junction arrays. Copper clad printed circuit (PC) boards were cut ($26 \times 26 \times 1.5$ mm^3), etched and cured with PDMS to form electrode connector pads and a PDMS sample well on each sensing chip (Fig. 1(a)). Four SWCNT coated wires were orthogonally placed and soldered to the connector pads to create a 2×2 junction array.

A multiplexing circuit, composed of a basic stamp 2 module (Parallax Inc.) with 20 MHz CPU speed and 32 bytes RAM, relay switches and a 1 V power source housed in a 3D printed box ($120 \times 92 \times 52$ mm), was connected to a picoammeter and

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