

Research Article

Development of Single-Walled Carbon Nanotube-Based Biosensor for the Detection of *Staphylococcus aureus*

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The goal of this research is to develop a single-walled carbon nanotube- (SWCNT-) based biosensor to detect *Staphylococcus aureus*. The specificity of 11 bacteria and polyclonal anti-*Staphylococcus aureus* antibodies (pAbs) was determined using an indirect ELISA. The pAbs were immobilized onto sensor platform after the hybridization of 1-pyrenebutanoic acid succinimidyl ester (PBASE). The resistance difference (ΔR) was calculated using a potentiostat. The bacteria detected by the biosensor were observed using a scanning electron microscope (SEM). The optimum concentration of SWCNTs on the platform was determined to be 0.1 mg/mL. The binding of pAbs with *S. aureus* resulted in a significant increase in resistance value of the biosensor ($P < 0.05$). The SEM images confirmed the specific binding of *S. aureus* on the biosensor. The SWCNT-based biosensor was able to detect *S. aureus* with a limit of detection (LOD) of 4 log CFU/mL.

1. Introduction

Processed foods are monitored worldwide to protect people from infectious diseases primarily caused by foodborne pathogenic bacteria. Among the foodborne disease-causing bacteria involved in predominant foodborne disease outbreaks, *Staphylococcus aureus* is one of the leading causes of gastroenteritis resulting from the consumption of contaminated food [1]. Since 2010 in South Korea, approximately 1,180 people have been hospitalized from 66 outbreaks of *Staphylococcus* [2]. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food [3]. The major symptoms of staphylococcal enteritis include nausea, vomiting, severe abdominal cramps, diarrhea, sweating, and headache. Signs of toxicity, such as fever and hypotension, are rarely observed in cases of staphylococcal food poisoning.

Traditional detection methods for microorganisms such as plate counting agar (PCA) are arduous and time-consuming methods, requiring more than 24 h. In addition, it has been recognized that the culturing-based methods can

underestimate the pathogen numbers due to the limitations associated with culturing such as viable but nonculturable cells [4]. Polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), and enzyme-linked immunosorbent assay (ELISA) methods have improved both the speed and sensitivity of pathogen detection, compared with detection by the traditional culturing methods [5, 6]. However, PCR techniques often have high risk of false results owing to inhibition by components of the sample matrix and complicated pretreatment process such as extraction of the whole or a part of the pathogen DNA [5]. ELISA technique also requires extra-labeled antibodies that are costly and time-consuming steps [7].

Recently, nanowires, nanotubes, and nanospheres as donors of electrical responses have been studied for minimized nanostructures in a field of biosensors. Nanoscaled biosensor devices can support *in vivo* applications, high sensitivity, and low limit concentration of detection [8]. In addition, a number of researchers conducting a study for nanoscaled biosensors are trying to develop easy detection methods that are label-free, rapid, low-cost, and involving

multiplexed analysis. Single-walled carbon nanotubes (SWCNTs) are attractive materials to be used for nanoelectronics [9–11]. The electrical properties of SWCNTs are especially good for uses in advanced biological electronics and biosensors. SWCNTs and gold assembly onto silicon wafers enable researchers to affect the electrical response for high sensitivity of the biosensor.

There are several methods to immobilize the biomolecules onto SWCNTs. The covalent bonding methods onto SWCNTs using modification with chemical functional groups may cause problems concerning the electrical properties of SWCNTs [12, 13]. On the contrary, noncovalent bonding method using π - π stacking does not enable the transferring of chemical characteristics because it only utilizes physical forces to immobilize materials onto SWCNTs. Enzyme immobilization is also one of the most important processes for increasing the sensitivity and stability of the biosensor. The low activity of the immobilized enzyme is due to different local pH or electrostatic interactions at the matrix-enzyme interface, covalent linking to enzyme changing overall enzyme structure, and matrix induced confinement decreasing enzyme mobility for conformation changes during substrate catalysis [14–16].

In food industry, biosensor methods are receiving a great amount of attention due to the potential feasibility of practical on-site detection of foodborne pathogens as rapid detection methods. In addition, biosensor methods do not require a high level of operator expertise and complicated sample preparation steps [17]. The overall goal of this research is to develop and characterize a SWCNT-based biosensor to detect *Staphylococcus aureus*. The specific objectives of this research are to (1) investigate the binding specificity between the target *Staphylococcus aureus* and polyclonal anti-*Staphylococcus aureus* antibodies for biosensor applications; (2) develop a fabrication process for SWCNT-based biosensor; and (3) characterize the properties of SWCNT-based biosensor to detect *Staphylococcus aureus*.

2. Materials and Methods

2.1. Materials. A stock culture of *Staphylococcus aureus* KCTC 3881 was purchased from the Korea Research Institute Bioscience and Biotechnology (Daejeon, South Korea). The culture of *S. aureus* was maintained in nutrient broth (Difco Laboratories, Detroit, MI, USA) by incubating for 16 h at 37°C. The other bacteria including *Yersinia enterocolitica* ATCC23715, *Salmonella typhimurium* ATCC19586, *Klebsiella pneumoniae* ATCC13883, *E. coli* O157:H7, *Salmonella enteritidis* ATCC19586, *Shigella boydii* ATCC11190, *Pseudomonas aeruginosa* ATCC10145, *Listeria monocytogenes* ATCC19116, *Bacillus cereus* ATCC21768, and *Shigella sonnei* ATCC9290 were obtained from the Food Safety Laboratory at Kyungpook National University (Daegu, South Korea). The single-walled carbon nanotubes (SWCNTs) were purchased from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China) and the purity was greater than 95%. N,N-Dimethylformamide (DMF) was purchased from Daejung Inc. (Siheung, South Korea). 1-Pyrenebutanoic acid, succinimidyl ester (PBASE) was purchased from Thermo Fisher Scientific Inc. (Waltham,

MA, USA). A polyclonal anti-*Staphylococcus aureus* antibody (pAb) was purchased from Abcam Inc. (Cambridge, UK) and diluted to a concentration of 4 mg/mL with carbonate-bicarbonate buffer prior to use. Other reagents used in this research were of analytical grade.

2.2. Indirect ELISA for Binding Specificity of pAbs with Bacteria. The specificity of pAbs was compared with commercially available anti-*staphylococcus aureus* using an indirect ELISA following the procedures described in the previous study [18]. *S. aureus* was cultivated in nutrient broth (Difco Laboratories, Detroit, MI, USA), and the other bacteria were cultivated in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) in a shaking incubator at 37°C for 16 h. The grown and collected bacteria were washed three times with 10 mM PBS buffer (pH 7.4) by centrifugation at 5,000 rpm for 1 min at 4°C. An aliquot of 100 μ L anti-*Staphylococcus aureus* antibodies diluted ten thousandfold with PBS buffer was taken and incubated in each well of the microplate (SPL, Pocheon, South Korea). Then, the color development was measured using a microplate reader (Synergy HI hybrid reader, Seoul, South Korea) at 405 nm. The result was calculated using the following equation [19]:

$$\begin{aligned} & \text{Difference of absorbance} \\ &= \text{Absorbance at 405 nm after 30 min} \quad (1) \\ & - \text{Absorbance at 405 nm at 0 min.} \end{aligned}$$

2.3. Fabrication of Mask and Sensor Platform. A mask for the sensor platform was fabricated prior to manufacturing the biosensor platform. The pattern of gold deposition as electrodes on the platform was designed to achieve a maximum of six measurements at one-time treatment (Figure 1(a)). The distance between each gold deposition was approximately 0.1 cm. According to the mask designed, gold was deposited on the surface of a Cr-deposited silicon wafer using an electron-beam evaporator (SRN-110-1505-R2, Sorona Inc., Pyeongtaek, South Korea) under vacuum at 4.0×10^{-6} torr. The conductivity of Cr-deposited silicon wafer was tested before gold deposition to ensure that there was no electrical current on the surface. The conductivity of gold-deposited platform was also tested to confirm that the gold electrodes were working properly when the electrodes were connected by the electrical tester.

2.4. Optimum Concentration of SWCNTs on the Sensor Platform. The SWCNT powder was suspended in DMF solution by sonicating for 120 min in a water-filled sonicator (UCP-02, Jeiotech, Daejeon, South Korea) at room temperature. The assembly of SWCNTs on the sensor platform was performed by drop-casting 5 μ L SWCNT solution onto the center of the surface of the gold-deposited biosensor platform to cover each space between the gold electrodes. The platform was then incubated for 15 min at 80°C in a drying oven to remove DMF residue and allowed to align SWCNTs between the gold electrodes. The resistance value (k Ω) of the SWCNT bundle plate was measured using a potentiostat (DY2013, EG Technology, Seoul, South Korea) to confirm the assembly

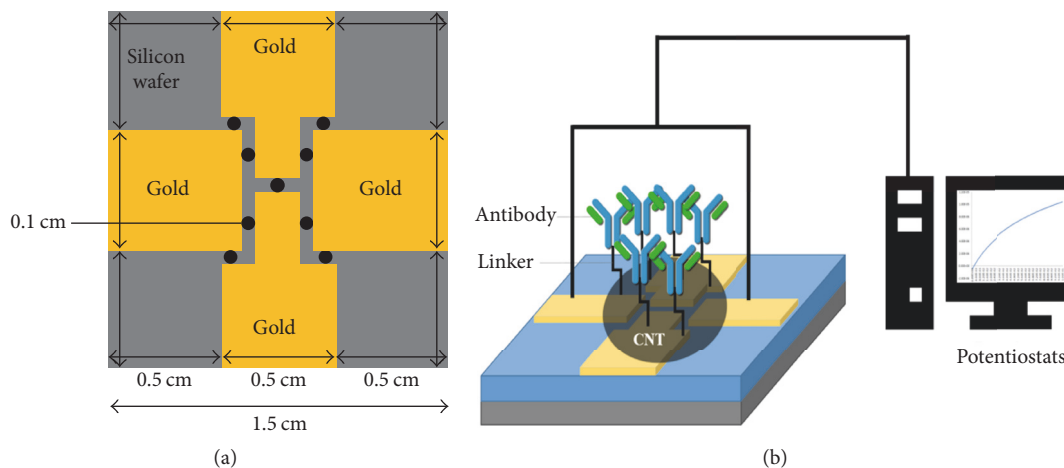


FIGURE 1: Platform design (a) and diagram of SWCNT-based biosensor (b).

of SWCNTs between the gold electrodes. The optimum concentration of SWCNTs was determined by applying the selected SWCNT-concentrations of 0.02, 0.1, 1, and 10 mg/mL onto the biosensor platform and washing the surface of the platform with 1 mL deionized (DI) water up to five times. The resistance value was measured after each washing step.

2.5. Fabrication of SWCNT-Based Biosensor. For the noncovalent functionalization of the SWCNT bundles, the biosensor platform assembled with SWCNTs was further incubated with 6 mM PBASE (9.8 mg PBASE in 5 mL DMF) as a linker for 2 h at room temperature followed by rinsing with pure DMF and DI water to remove excessive PBASE on the biosensor [20]. An anti-*Staphylococcus aureus* antibody at a concentration of 4 mg/mL was centrifuged at 12,000 rpm for 20 s and diluted ten thousandfold with carbonate-bicarbonate buffer (pH 9.3). The antibody was immobilized on the surface of SWCNT bundles by exposing the antibody to the PBASE linker overnight at 4°C, allowing the formation of covalent bonds with PBASE on the SWCNT bundles. The SWCNT-based biosensor immobilized with pAbs was then rinsed with PBS buffer (pH 7.4). Figure 1(b) illustrates the basic structure of the SWCNT-based biosensor immobilized with antibody. The resistance value (kΩ) of the antibody-immobilized biosensor was measured using a potentiostat (DY2013, EG Technology, Seoul, South Korea). The resistance measurements were conducted each step during the fabrication of SWCNT-based biosensor.

2.6. Detection of *S. aureus* Using an SWCNT-Based Biosensor. *S. aureus* cells were collected from the fully grown culture after washing 3 times with 10 mM PBS (pH 7.4) using a centrifuge at 5000 rpm for 5 min at 4°C. The collected bacterial populations were estimated by referring the optical density (OD) measured at 650 nm using a spectrophotometer (Uvmini 1240, Shimadzu, Kyoto, Japan) to a preconstructed standard curve for log CFU/mL.

Aliquots of 100 μL stock solution of *S. aureus* culture were dropped onto the SWCNT-based biosensor and incubated for 30 min at room temperature to allow specific bindings

between the bacteria and antibody. The control biosensor was prepared by applying 100 μL of PBS buffer on the SWCNT-based biosensor immobilized with antibody instead of *S. aureus* culture. Linear sweep voltammetry was measured after incubation using a potentiostat (DY2013, EG Technology, Seoul, South Korea). The slopes of the current/voltage (I/V) curves between 0 and 0.1 V for each treatment were calculated using linear regression analysis with the resistance (R) values calculated by inverting the current/voltage value [21]. The resistance difference (ΔR) was then calculated using the following equation:

$$\Delta R = \frac{(R_1 - R_0)}{R_0}, \quad (2)$$

where R_0 is the resistance measured with linker using the biosensor and R_1 is the resistance measured with *Staphylococcus aureus* culture using the biosensor.

2.7. Scanning Electron Microscopy. The microstructures of the surface of the control and the *S. aureus*-detected SWCNT-based biosensor were observed using a scanning electron microscope (SEM) (SNE-4500M, SEC Cooperation, Suwon, South Korea). The biosensor specimens were coated with gold using a sputter-coater (MCM-100, SEC Cooperation, Suwon, South Korea) prior to SEM observation. The surfaces of the gold-coated sensor platforms were examined with an SEM under an electric voltage of 30 kV at a working distance of 3 mm with a 30,000x magnification.

2.8. Statistical Analysis. The potentiostat measurements were repeated six times. One-way analysis of variance (ANOVA) of the measurement was performed using SPSS software (version 11.5, SPSS Ins., Chicago, IL, USA) and the differences among means were analyzed using Duncan with the defined significance level of $P < 0.05$.

3. Results and Discussion

3.1. Binding Specificity of pAbs with Bacteria. Indirect ELISA was performed to demonstrate the specific binding of tested

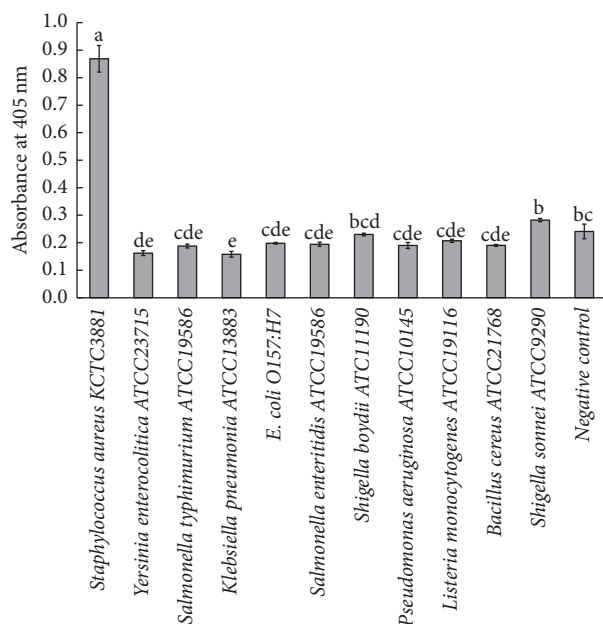


FIGURE 2: The specificity of custom prepared anti-*Staphylococcus aureus* polyclonal antibodies with selected bacterial strains determined by indirect ELISA. Error bars represent standard deviation ($n = 3$). Different superscript letters (a-e) represent significant differences among the samples at $P < 0.05$.

S. aureus with pAbs. The indirect ELISA method involved the primary antibodies without enzyme and the secondary antibodies (which reacted with primary antibodies) combined with enzymes. When the enzymes react with substrates that can produce a fluorescent compound, color reaction occurs between the substrates and the enzymes combined with secondary antibodies [18]. The concentration of the tested bacterial species was approximately 1×10^8 CFU/mL.

When the pAbs were diluted ten thousandfold and tested on the biosensor, the resistance of the biosensor was increased significantly from the preliminary experiment. Therefore, the ten thousand fold-dilution of pAbs was used for indirect ELISA. The specificity of the pAbs for the 11 tested bacteria was presented in Figure 2. The pAbs exhibited sufficient specificity with *Staphylococcus aureus* strains only, while the pAbs did not exhibit any specificity with other tested bacteria, evident from the absorbance values less than the cutoff value of 0.249 (mean of negative control + 0.008). Based on the specific binding of pAbs to *S. aureus* only confirmed by indirect ELISA, the pAbs were demonstrated as the suitable antibody for the development of biosensor to detect *S. aureus*.

3.2. Optimum Concentration of SWCNTs on Sensor Platform.

In general, CNT possesses a great potential for the applications to solid-state devices due to its small size as well as its superior electronic properties [22]. The immobilization of SWCNTs onto the sensor platform was conducted and confirmed by determining the resistance values at the selected concentrations of SWCNTs (Table 1). As the immobilized SWCNT-concentrations increased, the resistance values of

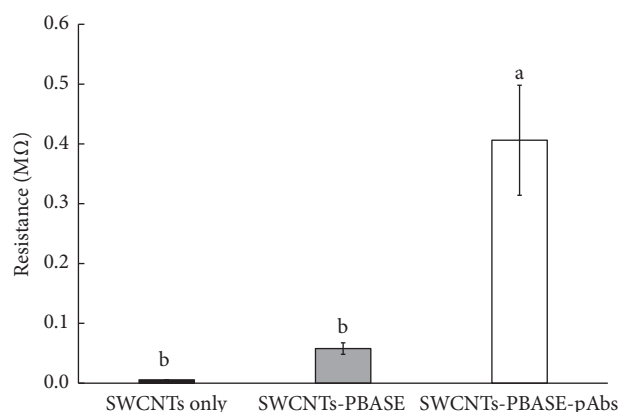


FIGURE 3: Resistance after immobilization of SWCNTs, 1-pyrenebutanoic acid, succinimidyl ester (PBASE), and anti-*Staphylococcus aureus* polyclonal antibodies (PABS) to the sensor platform. Error bars represent standard deviation ($n = 3$). Different letters (a-b) represent significant differences among the samples at $P < 0.05$.

the sensor platform decreased. This result was in good agreement with the result of Wang and Musameh [23] in that the resistance values decreased as CNT concentrations increased. In the concentration ranges of 1 to 10 mg/mL, the resistance values were very small. On the contrary, the resistance values at 0.02 mg/mL of SWCNTs were the highest among the tested concentrations; however, the resistance values were fluctuated with the washing steps. The fluctuation of resistance values might be attributed to the unstable immobilization of SWCNTs on the surface of the platform at 0.02 mg/mL-SWCNT concentration. The concentration of SWCNTs at 0.1 mg/mL exhibited a reasonable range of resistance value among the tested concentrations and more importantly maintained stable resistance values even after 5-time washing steps. Therefore, the optimum concentration of SWCNTs for immobilization on the sensor surface was determined as 0.1 mg/mL in this study.

3.3. Immobilization of Antibody on SWCNT-Based Biosensor.

The immobilization of antibody onto the CNT-immobilized sensor platform requires a linker between the CNT surface and antibody [21]. Many biological species can be non-covalently adsorbed on the CNT surfaces through hydrophobic, π - π stacking, and/or electrostatic interactions [23]. 1-Pyrenebutanoic acid, succinimidyl ester is widely used as a linker for CNTs [24]. The hydrophobic pyrenyl group of PBASE can be irreversibly adsorbed into the hydrophobic sidewall of a CNT through a π - π stacking interaction [25].

As presented in Figure 3, the binding of PBASE with SWCNT surface did not significantly alter the resistance value of the SWCNT-based sensor platform. The succinimidyl ester groups on the other end of 1-pyrenebutanoic react with primary and secondary amines on the surface of the antibody for nucleophilic substitution in the presence of DMF solvent. Antibodies consist of two regions: the variable region and the constant region (stem of the Y). It is believed that the variable region of the antibody is defined for antigen binding, and the constant region reacts with effective cells or molecules [5].

TABLE 1: Resistance of SWCNT immobilized onto sensor platform.

Concentration of SWCNTs (mg/mL)	No washing	Resistance (K Ω) after each washing				
		1st washing	2nd washing	3rd washing	4th washing	5th washing
0.02	233.4 \pm 38.8 ^c	328.5 \pm 57.4 ^{bc}	362.1 \pm 56.7 ^{bc}	406.8 \pm 90.2 ^b	340.1 \pm 62.1 ^{bc}	497.6 \pm 98.9 ^a
0.1	4.30 \pm 0.82 ^a	4.70 \pm 0.97 ^a	4.92 \pm 1.07 ^a	4.86 \pm 1.00 ^a	4.86 \pm 1.03 ^a	4.77 \pm 1.27 ^a
1	0.03 \pm 0.001 ^a	0.03 \pm 0.001 ^a	0.03 \pm 0.002 ^a	0.03 \pm 0.002 ^a	0.03 \pm 0.002 ^a	0.03 \pm 0.002 ^a
10	0.02 \pm 0.004 ^a	0.03 \pm 0.008 ^a	0.06 \pm 0.018 ^a	0.07 \pm 0.02 ^a	0.07 \pm 0.021 ^a	0.07 \pm 0.023 ^a

Different superscript letters (a–c) represent significant differences among the samples at $P < 0.05$.

The antibody immobilization on the biosensor was achieved by reacting amino groups of the constant regions of the antibody with the succinimidyl ester of the linker to form covalent amide bonds.

The immobilization of antibody through PBASE significantly increased the resistance value of the biosensor platform ($P < 0.05$) (Figure 3). García-Aljaro et al. [21] reported that the increase in resistance of the biosensor, that is, the decrease in current, was due to the accumulation of negative charge from the antibodies. Other researchers reported similar results with functionalization of antibodies onto the CNTs by measuring electric current changes. Villamizar et al. [26] reported that the functionalization steps of anti-*Salmonella* antibodies decreased the electrical current of a typical CNT field effect transistor. The electrical current of the device decreased after the absorption of anti-*Salmonella* due to the charge transfer process in which the antibodies provide electrons to the CNTs [27]. These electrons are provided by the amid groups with amino acid residues, which results in a decrease in the electrical current and a shift in the threshold voltage of the CNTs.

3.4. Detection Response of SWCNT-Based Biosensor. In general, microbial metabolism usually results in an increase in both conductance and capacitance, causing a decrease in impedance [24]. In addition, the specific binding of bacteria with antibody can cause an increase in resistance and can be monitored by the detector such as potentiostats. The specific interaction between the microorganism and antibody hinders the current flow resulting in a significant increase in the resistance value step by step [25].

Figure 4 presents the detection responses of the SWCNT-based biosensor with *S. aureus* measured during each step of the fabrication of the biosensor. There were no significant differences in resistance values between the PBS buffer and bacterial culture, when the biosensor platforms were immobilized with SWCNTs only or SWCNTs and PBASE. However, there was a significant difference in resistance between PBS and bacterial culture after the sensor platform was immobilized with antibody ($P < 0.05$). The significant increase in the resistance value was speculated from the specific interaction between the antibody and *S. aureus*. This result indicated that *S. aureus* cells did not react with the SWCNT-based sensor platforms without the immobilization of antibodies. The result was in good agreement with the results of García-Aljaro et al. [21], in that the resistance values were increased as the steps of preparation of the biosensor were performed.

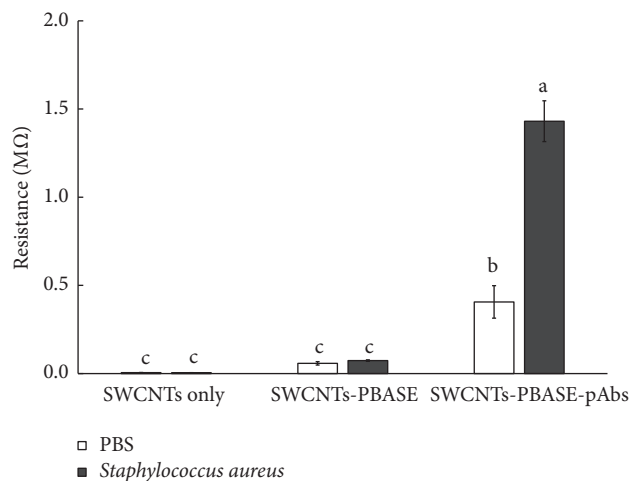


FIGURE 4: Resistance of SWCNT-based biosensor reacted with PBS buffer and *Staphylococcus aureus* culture. Error bars represent standard deviation ($n = 3$). Different letters (a–c) represent significant differences among the samples at $P < 0.05$. PBASE and pAbs stand for 1-pyrenebutanoic acid, succinimidyl ester, and anti-*Staphylococcus aureus* polyclonal antibodies, respectively.

3.5. SEM Images of *S. aureus* Cells Captured on the SWCNT-Based Biosensor. The SEM microstructures of *S. aureus* on the SWCNT sensor platform are presented in Figure 4. The SWCNT-based biosensor that reacted with PBS buffer (control sensor) exhibited a typical thread-tangled shape of SWCNTs on the sensor platform surface under the SEM (Figure 5(a)). When the biosensor was applied with *S. aureus* cell culture, the SEM image revealed that the *S. aureus* was captured onto the surface of the SWCNT-based biosensor (Figure 5(b)). Villamizar et al. [26] also reported that SEM confirmed the number of *Salmonella* cells attached to the CNTs was proportional to the concentration of *Salmonella* in solution.

The SEM images demonstrated that the SWCNT-based biosensor could capture *S. aureus* cells, resulting in a significant increase in the resistance value of the biosensor as presented in Table 2. The biosensor immobilized with PBASE only was not able to capture *S. aureus*, exhibiting no significant resistance difference between the PBS buffer and bacterial cell culture. However, when the antibody was immobilized onto PBASE, the resistance difference was significantly different between the biosensors ($P < 0.05$). García-Aljaro et al. [21] reported the resistance increased after functionalizing

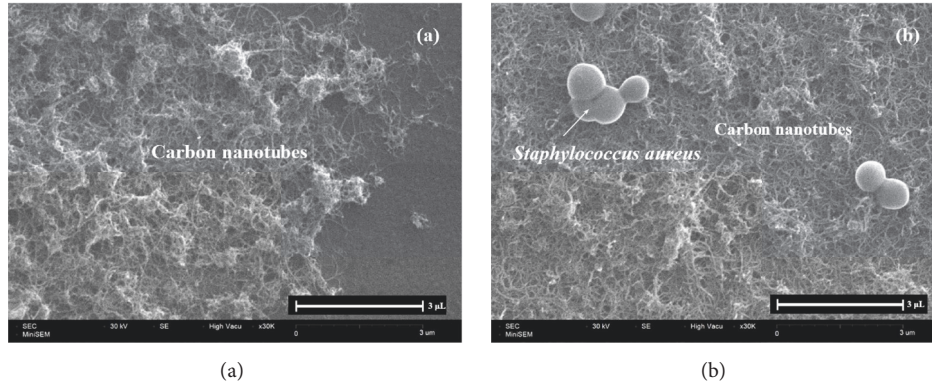


FIGURE 5: Scanning electron microscopic images of the detected *Staphylococcus aureus* by control sensor (a) and antibody-immobilized biosensor (b).

TABLE 2: Effect of immobilization steps on the resistance difference of SWCNT-based biosensor.

Immobilization step	Resistance difference (ΔR)
PBASE only	0.02 ± 0.06
PBASE and pAbs	9.78 ± 0.47

PBASE and pAbs stand for 1-pyrenebutanoic acid, succinimidyl ester and anti-*Staphylococcus aureus* polyclonal antibodies, respectively.

the biosensor with specific antibody for *E. coli* O157:H7 and concluded that the resistance increase was due to the specific binding of antibody with the bacteria on the CNT-based sensor.

3.6. Limit of Detection of SWCNT-Based Biosensor. The SWCNT-based biosensor immobilized with pAbs was exposed to increasing concentrations of *S. aureus* culture. As bacterial concentrations increased, the resistance differences also increased up to the bacterial concentration of 8 log CFU/mL. This is a typical characteristic of the functionalized CNT-based biosensor because the binding of the antibody with bacteria results in an increase in the resistance of the biosensor [21, 26]. The linear regression of the resistance differences with selected concentrations of bacterial culture is presented in Figure 6. The resistance difference of the SWCNT-based biosensor increased as the concentration of *S. aureus* cells increased, while the control sensor did not exhibit any difference in resistance. The regression coefficient (R^2) of the SWCNT-based biosensor was determined to be 0.9634. At the bacterial concentration of 4 log CFU/mL, the resistance difference began to differ significantly between the measurement biosensor and the control biosensor ($P < 0.05$). Therefore, the limit of detection (LOD) of SWCNT-based biosensor was determined to be the concentration of 10^4 CFU/mL. Villamizar et al. [26] reported that the biosensor response was a linear function of the logarithm of the bacterial concentrations between 3 and 7 log CFU/mL ($R^2 = 0.93$). Pyun et al. [28] also reported that the limit of detection was determined to be 3.0×10^5 CFU/mL,

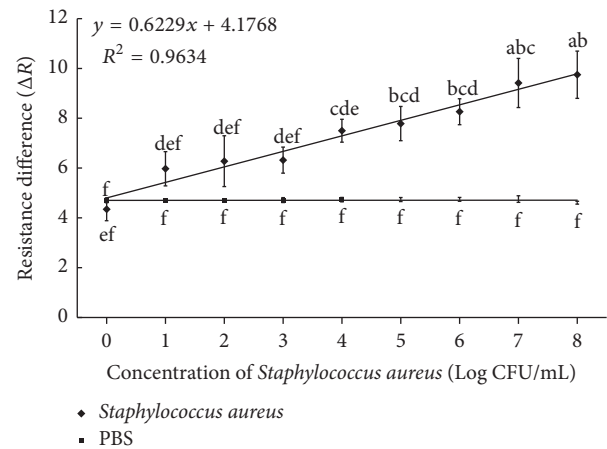


FIGURE 6: Resistance difference of SWCNT-based biosensor reacted with the selected concentration of *Staphylococcus aureus* from 10^0 to 10^8 CFU/mL. Error bars represent standard deviation ($n = 3$). Different superscript letters (a–f) represent significant differences among the samples at $P < 0.05$.

when a flexural plate wave transducer was used for the detection of *E. coli*.

The SWCNT-based biosensor developed in this study was not able to detect *S. aureus* less than 10^4 CFU/mL. Therefore, when a small amount of bacteria is present in food, it is necessary to perform an enrichment process to increase the bacterial number to a detectable level. A future study should be conducted in a way to increase the sensitivity of the biosensor by decreasing the LOD of the biosensor. Nonetheless, the SWCNT-based biosensor in this research may possess advantages compared with other techniques such as its miniaturized and simple structure, rapid detection less than 30 min, and easy operation at room temperature.

4. Conclusions

This research demonstrated that the SWCNT-based biosensor could be useful for the detection of foodborne pathogens

such as *S. aureus*. The indirect ELISA confirmed that the anti-*S. aureus* polyclonal antibodies were specific enough to bind to the target *S. aureus* cells among the tested bacteria. The gold electrodes fabricated on the silicon wafer were assembled with single-walled carbon nanotubes. The optimum concentration of assembled SWCNTs was determined to be 0.1 mg/mL. 1-Pyrenebutanoic acid, succinimidyl ester was selected as a linker to bind between SWCNTs and pAbs. As PBASE and pAbs were immobilized on the biosensor platform, the resistance of the SWCNT-based biosensor increased. The captured *S. aureus* on the SWCNT-based biosensor was also viewed using a scanning electron microscope. As bacterial number increased, the resistance decreased. The SWCNT-based biosensors developed in this study was able to detect *S. aureus* with a concentration as low as 10^4 CFU/mL. Therefore, the limit of detection of the biosensor was determined to be 4 log CFU/mL.

Additional Points

Practical Applications. Recently, biosensor methods are receiving a great amount of attention because of the potential feasibility for detection of foodborne pathogens. A single-walled carbon nanotube- (SWCNT-) based biosensor was developed and characterized to detect *Staphylococcus aureus*, one of the major food pathogens. The research demonstrated that the developed SWCNT-based biosensor could detect *S. aureus* specifically with a detection limit of 10^4 CFU/mL. This biosensor method was relatively simple, handy, and easy to operate. Therefore, the developed biosensor can be practically applied to detect food pathogens on-site in food industry.

Disclosure

Part of this research was presented as a poster at the Korean Society of Food Preservation (2015 Annual Meeting and Symposium).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. The support by Basic Science Research Program through the National Research Foundation (NRF) of Korea will not lead to any conflicts of interest regarding this publication.

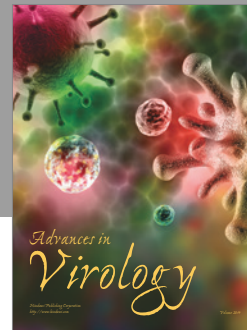
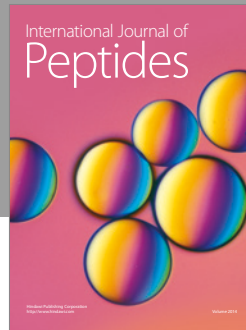
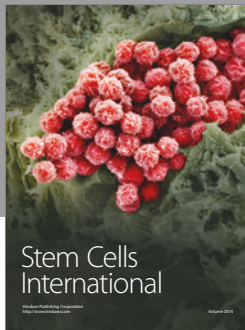
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