LWT - Food Science and Technology 132 (2020) 109230

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Dielectrophoresis-based microwire biosensor for rapid detection of *Escherichia coli* K-12 in ground beef

Won Choi[a], Yeo Wool Min[b], Kwang Yeon Lee[c], Soojin Jun[d], Hyeon Gyu Lee[b,*]

[a] Department of Landscape Architecture and Rural Systems Engineering and Research Institute of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 151-921, South Korea
[b] Department of Food and Nutrition, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, South Korea
[c] Research Institute of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 151-921, South Korea
[d] Department of Human Nutrition, Food, and Animal Sciences, 1955 East West Road, University of Hawaii, Honolulu, HI, 96822, USA

ARTICLE INFO

Keywords:
E. coli detection
Dielectrophoresis
Functionalized microwire biosensor
Nonspecific binding
Ground beef

ABSTRACT

By combining a functionalized microwire sensor with a dielectrophoresis (DEP) technique, a rapid and accurate detection method for bacterial pathogens was developed and demonstrated. The gold-tungsten microsensor (sensing diameter, 25 μm) was functionalized with specific *Escherichia coli* (*E. coli*) antibodies against surface antigens to capture *E. coli* bacterial cells from phosphate buffer saline (PBS) and ground beef. The DEP technique consisted of the application of a pin-type microwire and large base electrode under an alternating current (AC) electric field of 10 Vpp at a frequency of 3 MHz. Additionally, bovine serum albumin (BSA) was utilized as a blocking agent for the attachment of nonspecific proteins to the microwire surface to prevent false positive signals due to nonspecific binding. The resistance and fluorescent intensity (FI) demonstrated a linear relationship with the concentration of *E. coli* cells ranging from 10^3 to 10^7 CFU/mL. These results showed that DEP can be used as a reliable alternative for the detection of *E. coli* without a blocking effect minimizing the significant difference between PBS and ground beef.

1. Introduction

Foodborne diseases have received considerable attention due to their serious threat to public health and safety by microorganisms such as bacteria (Yang & Bashir, 2008). Traditionally, the methods for detection of foodborne pathogen exist such as qPCR assays. These conventional methods have own limitation including sensitivity, specificity, requirement of sample enrichment, sensitivity, and cost (Maraldo & Mutharasan, 2007). For example, a qPCR can be included as one of the most common detection methods for beef industries. qPCR methods have emerged as a strong competitor for bacteria identification with high sensitivity; however, commercial techniques are typically limited to 6–8 channels using published primers. The qPCR assay relies on the DNA extract, a series of reactions, which are performed in a thermal cycler (Kralik & Ricchi, 2017). qPCR requires a significant amount of DNA, which is often hindered with the ability to culture-specific bacteria. Therefore, developing methods for the detection of foodborne pathogenic bacteria is essential (Law, Ab Mutalib, Chan, & Lee, 2015; Zeng, Chen, Jiang, Xue, & Li, 2016; Zhao, Lin, Wang, & Oh, 2014). In particular, biosensor-based methods have been viewed as an attractive alternative method in this field (Yang, 2009). They offer several advantages including rapid detection time, cost-effectiveness, simple sample preparation, and sensitive and specific detection (Singh, Poshtiban, & Evoy, 2013; Suehiro, Hamada, Noutomi, Shutou, & Hara, 2003). There are many methods for improving sensor efficiency such as (1) improving antibody immobilization methods on the sensor surface, (2) improving electrode performance to enhance the sensitivity, (3) enhancing the detection signal, and (4) improving the dielectrophoresis (DEP) technique for concentrating samples (Heo & Susan, 2009; Kim et al., 2016). The immobilization method has been widely adapted as the key process for the composition of biosensors because the analytical performance can profoundly depend on the effectiveness of the antibody immobilization on the electrode surface (Kyprianou et al., 2009). In a previous study, when the width of the electrode bands was narrow, the sensitivity of the biosensor increased. On the other hand, the main interaction signal may be improved through case-specific amplification schemes, such as enzyme-labeled amplification, to strengthen the detection signal and achieve lower detection limits (He, Zang, Liu, He, & Lei, 2018; Liu et al., 2010).

DEP refers to the electrokinetic motion of neutral particles by

* Corresponding author.
E-mail address: hyeonlee@hanyang.ac.kr (H.G. Lee).

https://doi.org/10.1016/j.lwt.2020.109230
Received 13 November 2019; Received in revised form 19 February 2020; Accepted 3 March 2020
Available online 05 March 2020
0023-6438/

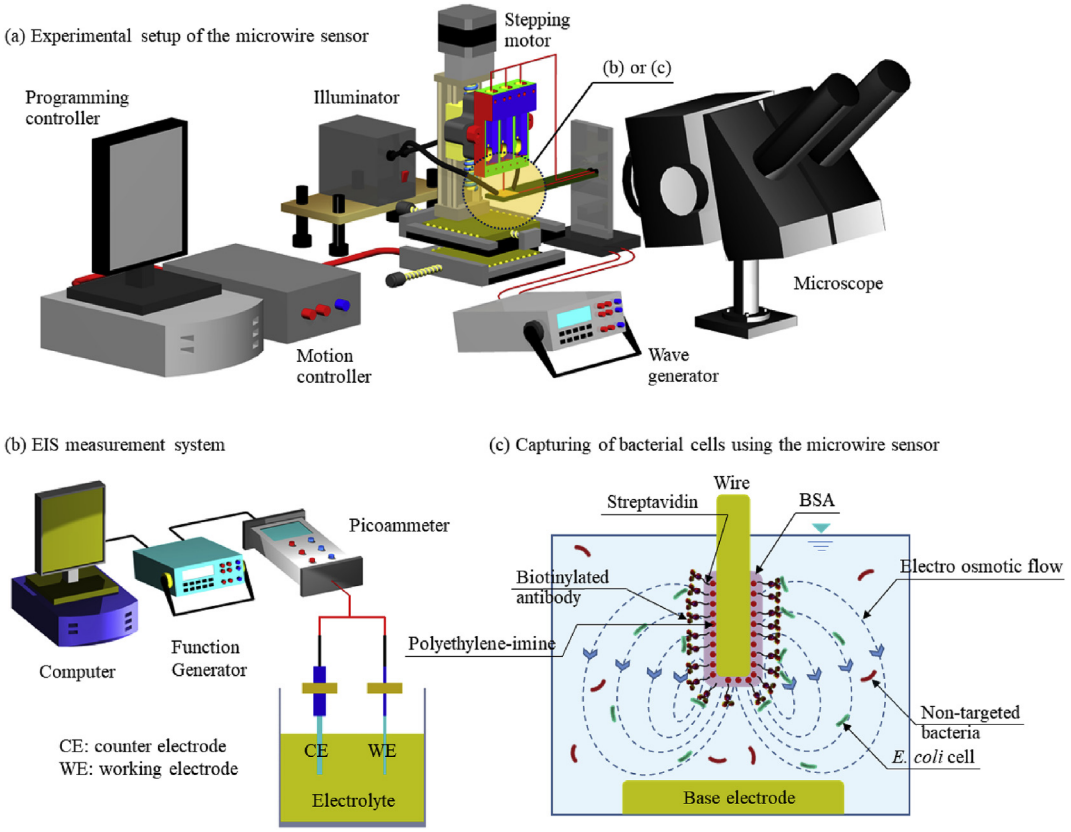

Fig. 1. Schematic diagram of experimental equipment system.

polarization effects in a nonuniform electric field. The DEP force (F_{DEP}) can be calculated according to the following equation (Huang, Wang, Tseng, & Fang, 2008; Lapizco-Encinas, Davalos, Simmons, Cummings, & Fintschenko, 2005):

$$F_{DEP} = 2\pi r^3 \varepsilon_m Re[F_{CM}]\nabla E^2 \quad (1)$$

where F_{DEP} is the DEP force (N), ε_m is the permittivity of the suspension medium (F/m), E is the electric field (V/m), r is the radius of the particle (m), and Re $[F_{CM}]$ is the real value of the Clausius-Mossotti (CM) factor, given by:

$$F_{CM} = \frac{\varepsilon_p^* + \varepsilon_m^*}{\varepsilon_p^* + \varepsilon_m^*} \quad (2)$$

where ε^*_P is the complex permittivity of the particle, and ε^*_m is the complex permittivity of the medium.

Depending on the relative polarizability of the particle and medium, the sign of the DEP force can be determined (Lu, Chee, Yamada, & Jun 2013). The DEP force has been integrated with biosensors to improve bacterial detection, such as through bacterial cell concentration in microfluidic chips (Gomez, Morisette, & Bashir, 2005; Li, Zheng, Akin, & Bashir, 2005; Morgan, Izquierdo, Bakewell, Green, & Ramos, 2001; Yang et al., 2006), separation of target cells and nontarget cells (Li & Bashir, 2002; Suehiro et al., 2003), and the enhancement of antibody capture efficiency to bacterial cells (Koo et al., 2009; Yang, 2009). Studies of the detection of pathogenic cells in real food samples using the DEP force have been documented (Kim et al., 2011; Lu & Jun 2012). Sensors were designed with functionalized microwires based on DEP and an antigen-antibody reaction for the rapid detection of foodborne pathogens. Top and bottom electrode configuration for generating DEP was used for capture the *E. coli* K12 cells from fresh produce (Kim et al., 2011) and orange juice (Lu & Jun 2012). The gold microwire with 25 μm in a diameter was used as a probe, and it was functionalized with monoclonal *E. coli* antibody on the surface of the microwire. The tip microwire sensor concentrates the analyte on its surface by attracting cells in the vicinity of the tip by AC electroosmotic flow and by the capture of cells on the high aspect ratio microtip by capillary action (Yeo, Liu, Chung, Liu, & Lee, 2009). These immunosensors were integrated with fluorescence and electrochemical impedance spectroscopy (EIS), and their sensing capability was explored in food systems such as orange juice and spinach (Kim et al., 2011; Lu et al., 2013; Lu & Jun 2012). However, these biosensors have some limitations, including high production cost and complexity for a portable biosensor. In addition, there have been few studies on microwire biosensors for the detection of foodborne pathogens in complex food systems that include proteins, lipids, and other macromolecules. Here, we describe a portable and simple EIS system based on a microwire and evaluate the biosensor for the rapid and specific detection of *E. coli* in ground beef samples. The aims of this study were to (1) characterize a rapid and precise biosensor for the detection of foodborne pathogens of *E. coli* in ground beef using the functionalized microwire attached with the DEP technique and (2) evaluate the sensing performance by calculating the resistance based on the circuit theory and comparing the FI values.

2. Materials and methods

2.1. Sensor preparation

An xyz stage (KAL4-0803; Kwonsys, Daejeon, South Korea) containing a probe guide panel was assembled on an aluminum board (300 × 450 mm) to control the accurate movement of the 25 μm diameter 7% gold-tungsten-coated wires (ESPI Metals, Ashland, OR, USA). A 2 × 2″ gold plate served as the bottom electrode, and a function generator (33120A; Agilent Technologies, Santa Clara, CA, USA) was utilized for the generation of sine waveforms. To observe the sensing wire in detail, an optical microscope (150× magnification) was placed on the board with a microscopy holder (03668; Edmund Optics Inc.,

Barrington, NJ, USA). A digital camcorder was used to monitor the experimental procedure. A fiber-optic Y-shaped dual light microscope illuminator (CS-A923WLED; OMAX, Irvine, CA, USA) was used to provide background light. A picoammeter (6485; Keithley, Cleveland, OH, USA) measured the wire current values through the electrolyte solution. The schematic design of the whole system is presented in Fig. 1. DEP is the translational motion imparted on uncharged particles as a result of polarization induced by non-uniform electric fields as shown in Fig. 1(c). This phenomenon can be used to move particles in inhomogeneous alternating current (AC) electric field. Any non-polar material will exhibit a certain degree of polarization when exposed to an electric field. Depending on the properties of the particle, electric dipoles are generated on opposing ends of the particle in response to an electric field, resulting in motions toward or away from the maximum electric field. Because the cells were rapidly concentrated at the end of the tip by DEP force, the force can improve the efficiency to capture the particles to compare to the absence of DEP. A droplet of bacterial sample (10 μL) was placed in a hemispheric concave (3 mm in diameter) on a gold plate as a bottom electrode. The microwire was dipped in the droplet at a velocity of 50 mm/min until the distance between the microwire tips and bottom electrode was as close as 1 mm. DEP was applied at 3 MHz and 20 V_{pp} for 2 min. Thereafter the microwire was withdrawn at a speed of 5 mm/min.

2.2. Materials

The 25-μm diameter 7% gold-tungsten-coated wire (Q11253) was ordered through ESPI Metals. Polyethyleneimine (PEI) solutions (P3143), streptavidin (S4762), and bovine serum albumin (BSA; A7906) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Rabbit polyclonal antibodies (B65003R) and biotinylated rabbit antibody (B65007R) were obtained from Meridian Life Science (Saco, ME, USA). Polydimethylsiloxane (PDMS; 0007590060) was purchased from Dow Corning (Midland, MI, USA). Potassium chloride (080M0091V), potassium hexacyanoferrate (II) trihydrate (110M0152V) and potassium hexacyanoferrate (III) (ACS reagent, ≥99.0%) (MKBQ4398V) for the electrolyte solution were obtained from Sigma-Aldrich Co. Ground beef was purchased from a local market (Seoul, South Korea), and the beef protein and fat content were analyzed by the Foundation of Agriculture Technology Commercialization and Transfer (FACT).

2.3. Bacterial preparation

Cultures of *E. coli* K-12 (KCCM 12515; Seoul, Korea) were transferred into tryptic soy broth (Becton Dickinson, MD, USA) and incubated at 37 °C for 24 h. The initial cell counts of the *E. coli* culture were counted using a serial dilution method.

2.4. Ground beef preparation

The preparation of the ground beef was conducted using the method by Varshney and Li (2009). Samples (25 g) of ground beef were weighed, and diluted with 225 mL of 0.1% peptone water in sterile stomacher bags. The mixture was homogenized and filtered by filter paper (40617102; ADVANTEC, Japan) to separate the beef particles. The filtered ground beef was inoculated with *E. coli* cultures ranging between 10^3-10^7 CFU/mL. We could not obtain consistent/credential readings from microbial concentrations of 10–100 CFU/mL. The limit of detection (LOD) was setup to be 1000 CFU/mL.

2.5. Functionalized wire

The microwires were cut into 25 mm in length, and washed by distilled water and 70% alcohol using the digital sonifier for 5 min each. The wires were placed on the automated xyz stage (Franklin Mechanical & Control Inc., Gilroy, CA) controlled by the COSMOS program (Franklin Mechanical & Control Inc., Gilroy, CA; Velmex, Inc., Bloomfield, NY) for the step-wise movements of wires. A computer was used to initiate the XYZ motor control program. Wires were immersed into glass vials containing 7 mL of 1% PEI solution for 5 min and withdrawn at a constant withdrawal velocity of 6 mm/min. PEI coated wires were baked at 175 °C for 1 h (Cairns, 2013). PEI has been utilized for the immobilization of proteins due to the intensity of binding between them (Mateo, Abian, Fernandez-Lafuente, & Guisan, 2000; Pessela et al., 2003, 2005; Schlauf, Assadollahi, Palkovits, Pointl, & Schalkhammer, 2015). Therefore, PEI was selected for immobilization of streptavidin, because PEI has a great positive charge, strongly binding with streptavidin which was negatively charged. Then, the wire was immersed in 3 μL of 0.01% streptavidin for 5 min and then withdrawn, and biotinylated antibodies were applied in the same manner for 5 min, resulting in streptavidin-linked biotinylated antibodies ready to capture bacterial cells. For the blocking treatment, the wire surface was washed with distilled water. To avoid nonspecific binding, 3 μL aliquot of bovine serum albumin (BSA) solution (1 mg/mL in phosphate buffer saline (PBS)) was placed on the wire surface coated with antibody. Finally, the electrode was washed with PBS and distilled water.

2.6. E. coli cell detection

A 5 μL of beef exudates and *E. coli* was deposited on the gold electrode. After the functionalized wire was placed in air for 2 min, an alternate current (AC) field was generated at 3 MHz and 10 Vpp. Then, the microwire tips were immersed in the sample at a depth of 400 μm at a rate of 200 μm/s. The tip remained into the solution for 2 min and then, the wires were pulled out from the solution at the rate of 8 μm/s.

2.7. Immunofluorescence method

To understand the effects of DEP and functionalization on the FI of *E. coli* cells captured on the wire in PBS at 1×10^8 CFU/mL, four cases such as plane wire without DEP, functionalized wire without DEP, plain wire with DEP, and functionalized wire with DEP were tested as follows: *E. coli* cells in the sample solution were captured on microwires and were bound to fluorescein isothiocyanate (FITC) labeled polyclonal *E. coli* antibodies. The wire was rinsed in water to remove nonspecific binding between the targeted cells and wire. Fluorescent images of the FITC antibodies were obtained using a real-time cell imaging system (DeltaVision system, Applied Precision, WA, USA). A 292 × 724 pixels area of the wire tip was cropped from each picture and their fluorescence intensities were measured by the Image J program (National Institutes of Health, Bethesda, MD).

2.8. Resistance measurement

To evaluate the sensitivity and specificity of the sensor, a resistance measurement of the developed sensor was conducted. This method is a simplification of the EIS measurement that transduces changes in interfacial properties between the electrode and the electrolyte. A key difference between EIS and the experiment is that the former applies a small amplitude sine wave perturbation to an electrochemical system throughout a wide range of frequencies, while the latter applies only a single frequency to the system without the need for a Nyquist plot, which is a widely used impedance plot to evaluate resistance. Additionally, the experiment only needs two electrodes to measure the impedance. Resistance measurements were carried out as followed. First, the electrolyte container was filled with a solution of 0.1 M KCl, 5 mM $K_4Fe(CN)_6$, and 5 mM $K_3Fe(CN)_6$. The platinum electrode (approximately 0.5 mm) (CHI 115; CH Instruments, Inc., Austin, TX, USA) was made for the counter electrode. To read the electrical current, a function generator to apply the voltage was attached to a picoammeter (Fig. 1(b)). The functionalized pure microwire which did not capture *E. coli* cells was soaked in a solution with 200 mV DC potential, and the

electron transfer resistance of the functionalized microwire was measured by the picoammeter. After 2 min of air-drying of the microwire captured *E. coli* cells using DEP and antigen-antibody interaction, the microwire was also immersed into the electrolyte solution at the same depth. The electron transfer resistance of the functionalized microwire detected *E. coli* cells was measured by the picoammeter. The change of electron transfers resistance (ΔRet) at the electrode interface with or without *E. coli* binding with the antibodies was calculated as:

ΔRet = Ret (antibody-bacteria) – Ret (antibody)

2.9. SEM imaging

E. coli cells captured on the functionalized wire tips were examined with a scanning electron microscope (SEM). Wires were deposited on the aluminum stub and were fixed using double-sided adhesive carbon-tape. A field emission scanning electron microscope (FESEM) (NOVA NANO SEM 450, Tokyo, Japan) was used for SEM analysis. The samples were viewed and photographed at a total magnification of 2000 × and 8000 × .

2.10. Statistical analysis

All experiments were repeated in at least triplicate. Statistical analyses were conducted with Statistical Package for the Social Science (SPSS, Version 21.0, IMB Corp., Armonk, NY, USA). The analysis of variance (ANOVA) followed by Duncan's multiple range test and Student's t-test were performed at $p < 0.05$.

3. Results and discussion

3.1. Fluorescent intensity (FI) value

In general, the fluorescent intensity (FI) value increases with the number of captured bacterial cells on the microwire. The effects of DEP and functionalization of FI values of captured *E. coli* cells are shown in Fig. 2. The plain wire without DEP treatment showed the lowest FI values. Compared to the FI value of the plain wire without DEP as control, the FI values of the functionalized wire without DEP and the plain wire with DEP were slightly higher, by 2% and 3%, respectively. However, the FI value of the functionalized wire with DEP was higher by 7% compared with that of the control. Similarly, Yang (2009) also increased the efficiency of antibody capture using DEP, which enhanced the close contact of the cells with the functionalized antibodies onto the chip surface and significantly improved the immune-capture efficiency. The *E. coli* cells were gathered around the tip-end of the microwire due to the circulation from the DEP force.

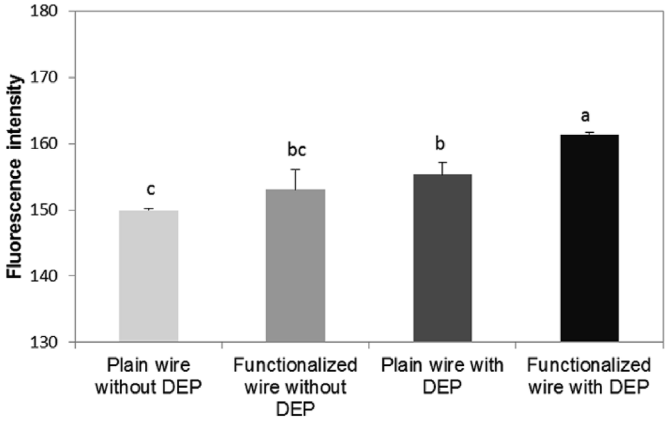

Fig. 2. Effects of DEP and functionalization on the FI of *E. coli* cells captured on the wire in PBS at 1 × 10^8 CFU/mL.
[a-c] Means with different letters are significantly different at $p < 0.05$.

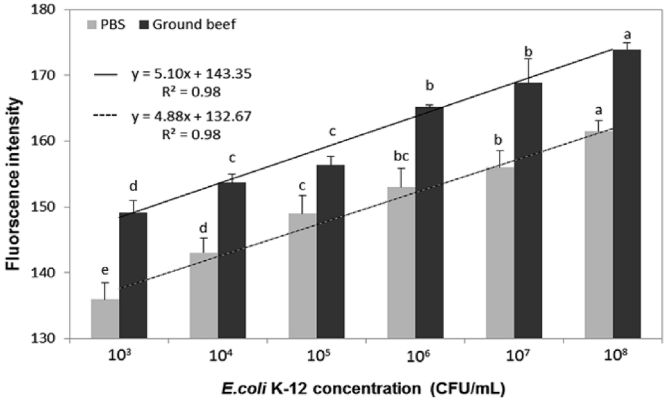

Fig. 3. FI values of different concentrations of *E. coli* cells from PBS and ground beef.
[a-e] Means with different letters are significantly different at $p < 0.05$.

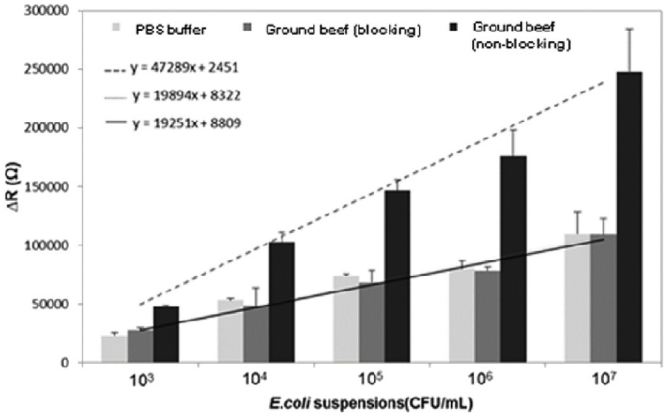

Fig. 4. Change in the electron transfer resistance of *E. coli* cells captured from PBS and ground beef with and without blocking agent. The cell concentrations vary from 10^3 to 10^7 CFU/mL.

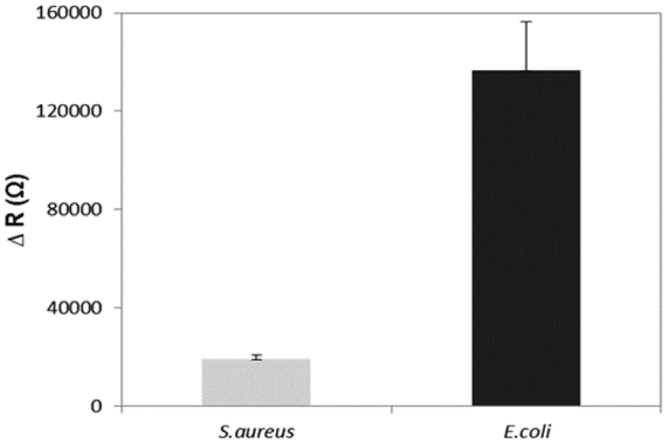

Fig. 5. Sensing specificity of the functionalized microwire biosensor for the detection of *E. coli* and *S. aureus* at 10^8 CFU/mL.

The FI values of *E. coli* cells detected on a microwire in PBS and ground beef are shown in Fig. 3. The FI values increased linearly over the 10^3–10^8 CFU/mL range. As the *E. coli* cell concentration increased, the FI values increased, with a high correlation ($R^2 = 0.983$ in PBS and 0.9817 in ground beef), demonstrating that *E. coli* concentrations can be quantified by the developed sensor. The experimentation determined that the lowest concentration of bacterial suspension at which the

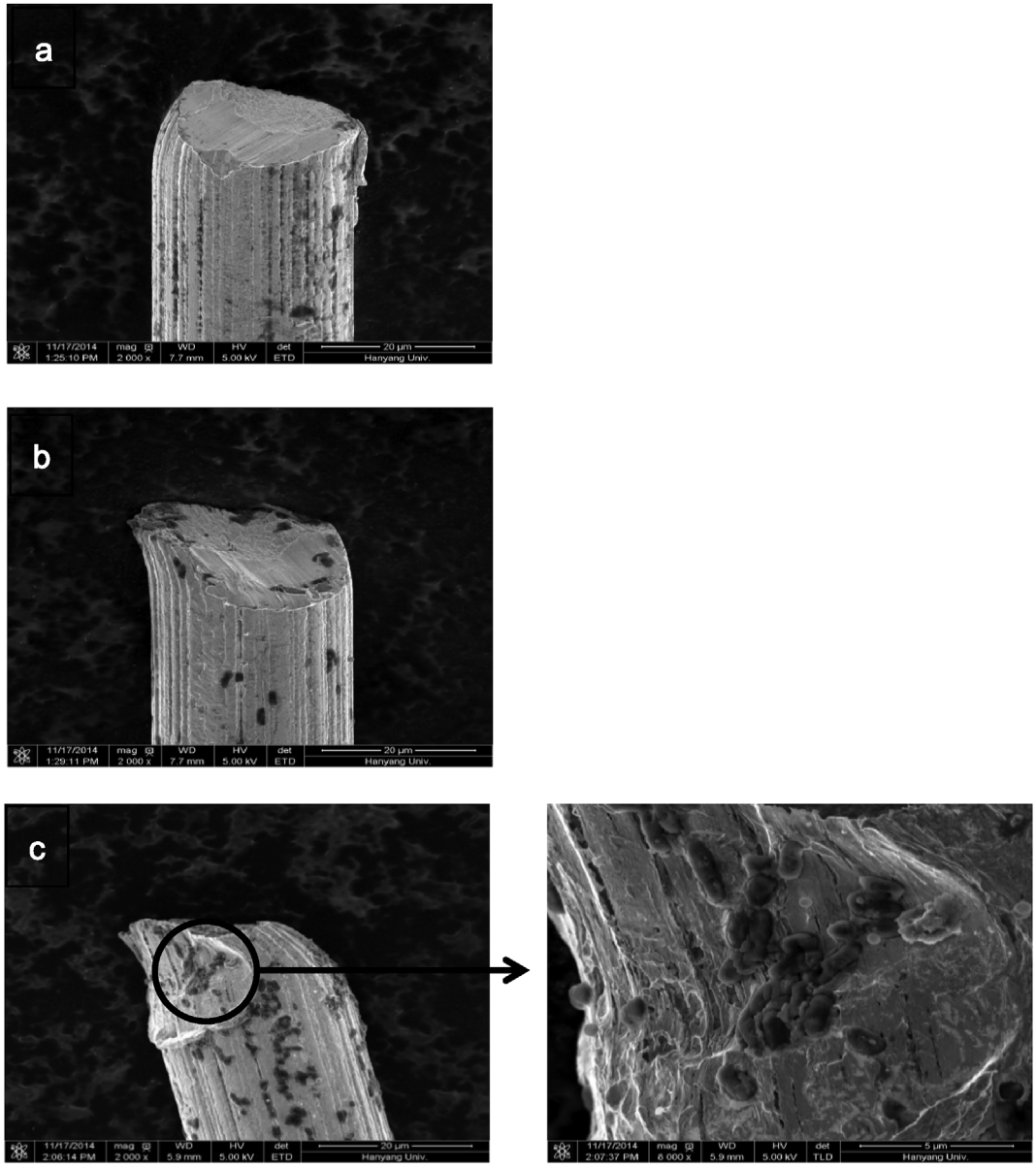

Fig. 6. FESEM images of microwire-detected *E. coli* cells from PBS. (a) Plain wire. (b) Plain wire with detected *E. coli,* and (c) Functionalized wire (without blocking agent) with *E. coli* detected using DEP force.

sensor was able to detect a signal was 1×10^3 CFU/mL and that the number of CFU could be estimated as $(1 \times 10^3$ CFU/mL) × (5 μL) = 5 CFU. Thus, the detection limit of the developed sensor was determined to be 5 bacterial cells per wire in a sample concentration (1000 CFU/mL). Lu and Jun (2012) found no significant difference in the FI values of *E. coli* values between orange juice and a control. Kim et al. (2011) also concluded that spinach had no adverse effects on the capturing of *E. coli* cells due to the size selectivity of the capillary action on the surface and distinct permittivity variation (Yeo et al., 2009).

We did, however, find a difference in FI values between PBS and ground beef. This finding indicates that the proteins in the ground beef might have affected the FI values of the *E. coli* cells captured on the wire. In ground beef, nonspecific antibody staining could cause non-immunological binding to the microwire because the ground beef contains different macro and micro nutrients (e.g., moisture, protein, fat, ash, iron, zinc, copper, and magnesium). This complex sample in this study, which contained 20% protein and 6% fat, may have compromised the ability of the sensor to detect pathogens due to the nonspecific adsorption of the sensor by the food components (Maraldo & Mutharasan, 2007).

3.2. Resistance values of E. coli cells from PBS and ground beef

The electron transfer resistance with different concentrations ranged from 10^3-10^7 CFU/mL of *E. coli* diluted in PBS and ground beef, as shown in Fig. 4. The results show a linear correlation between the resistance and concentration of *E. coli*, and there were significant differences between each concentration ($p < 0.05$). If cells attach on an electrode surface, they effectively decrease the electrode area, which the current reaches and thereby increases the interface impedance. The density, growth, and long-term behavior of cells on the electrodes were shown to change the impedance of the sensor (Yang & Bashir, 2008). Ruan, Yang, and Li (2002) also reported that the electron transfer (R_{et}) increased following an increase in cell concentration; however, the change in the electron transfer resistance from ground beef increased rapidly relative to that of PBS. This observation could be due to other food components such as proteins or fats attached to the microwire in

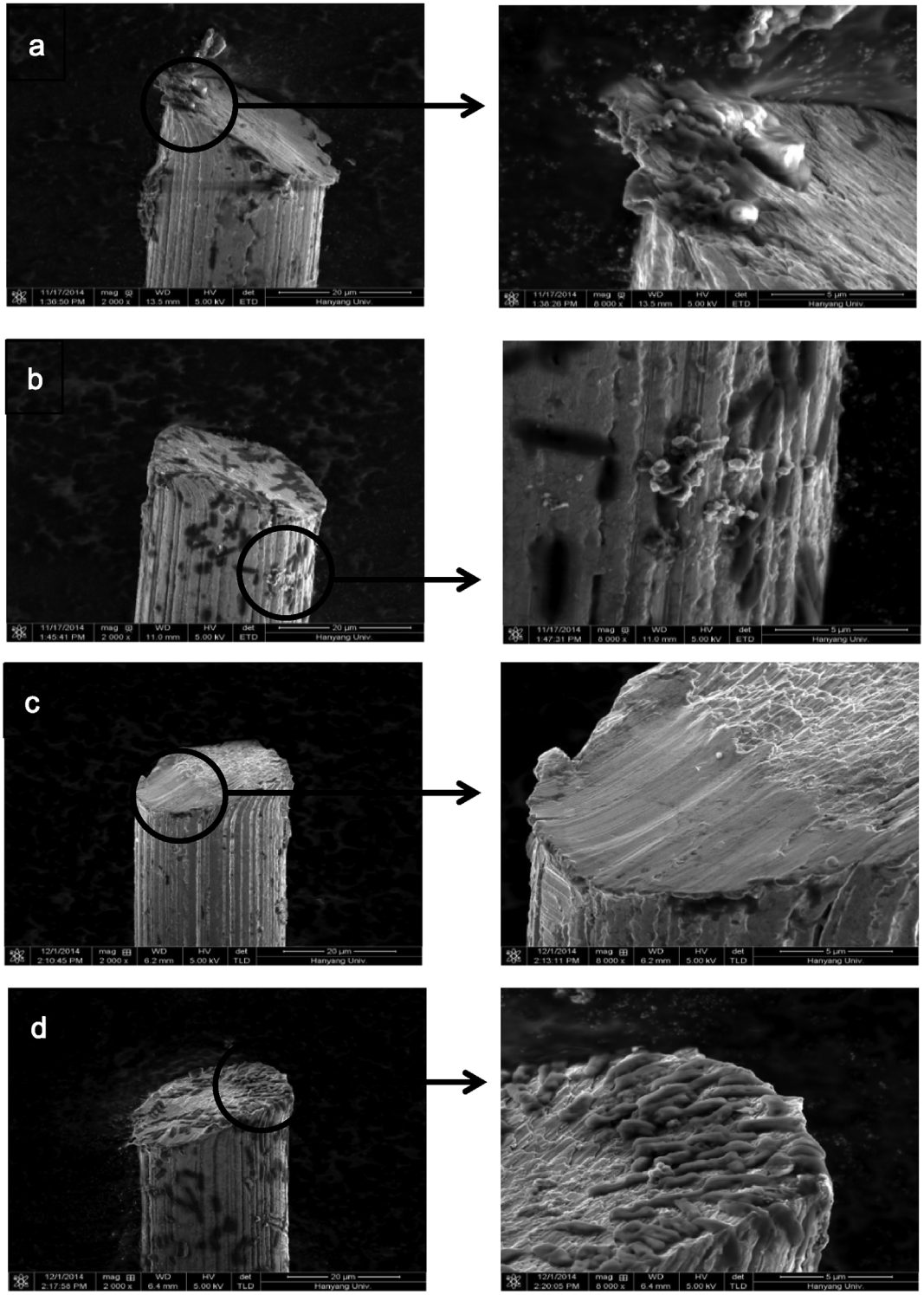

Fig. 7. FESEM images of functionalized microwire-detected *E. coli* cells from ground beef with DEP (a) without blocking agent from an uninoculated sample, (b) without blocking agent from an inoculated sample, (c) with blocking agent from an uninoculated sample, and (d) with blocking agent from an inoculated sample.

the ground beef samples, causing a false positive signal and reduction in sensor sensitivity (Daniels & Pourmand, 2007; Shen et al., 2012). Because the impedance sensor needs to be very sensitive to characterize the properties of binding materials, the surface of the sensor could be bound with nonspecific binding substances (Varshney & Li, 2009). Therefore, eliminating nonspecific binding is important for capturing the target bacteria and improving the sensitivity of detection (Kim, Moon, & Morgan, 2013). Vidal, Bonel, Ezquerra, Duato, and Castillo (2012) also addressed problems of nonspecific adsorption on the remaining active sites. The unspecific adsorption onto unblocked streptavidin sites will produce a higher current.

Bacterial cells can be bound to immobilized antibodies via the bioaffinity reaction but it may also be attached to the non-functionalized area. The latter can be target bacteria or non-target bacteria. In either case, it affects sensor's accuracy and sensitivity. These non-specific binding can be minimized by filling the unoccupied sites with a

blocking agent. BSA is widely used for non-specific binding blocker with 0.1–3% of solutions (Shen et al., 2012). Non-target bacteria will be unbound to BSA coating area as well as antibody layer. To solve this problem, BSA was used as a blocking agent. BSA is a serum albumin protein and one of the most commonly used blocking reagents in biological detection due to its stability and low cost (Shen et al., 2012). The effect of BSA on the change in resistance from ground beef is presented in Fig. 4. Additionally, after the blocking treatment, the electrical current data in the ground beef showed a linear relationship ($R^2 = 0.975$) between the change in resistance and concentration of *E. coli*. Regarding the electron transfer resistance, there was no significant difference between PBS and ground beef ($p > 0.05$). Therefore, by applying this BSA treatment, only cells are selectively collected and detected in the food-based mixtures.

3.3. Resistance values of E. coli cells and Staphylococcus aureus

The specificity of *E. coli* relative to that of *S. aureus* at 10^8 CFU/mL as a negative control for a functionalized microwire sensor was compared as seen in Fig. 5, *S. aureus* as a negative control significantly decreased the electron transfer resistance of the wire compared to that of the wire with captured *E. coli* cells on the electrode surface ($p < 0.05$). The result clearly showed that the functionalized microwire sensor had a high selectivity for *E. coli*. Lu et al. (2013) also studied a modified microwire sensor immobilized with antibodies for *E. coli* versus *S. aureus,* which indicated the sensing specificity to *E. coli* K-12.

3.4. SEM images of E. coli cells captured on the microwire

Fig. 6 and Fig. 7 show FESEM pictures of the *E. coli* cells captured on the microwire (*E. coli* concentration of 1×10^3 CFU/mL in PBS and ground beef). The plain wire without any treatment and without any cells attached to the wire is shown in Fig. 6(a). The plain wire immersed in the *E. coli* cell solution is presented in Fig. 6(b). A few cells captured on the wire without functionalization and DEP force are shown, which may have been due to the sensing deficiency. A large number of bacterial cells were captured on the functionalized wire as shown in Fig. 6(c), indicating improvement in the sensing deficiency after modification. A significant difference was observed between Fig. 6(b) and (c), which confirm the presence of microbes captured onto the functionalized microwire with DEP force in PBS. Additionally, the DEP force assisted by an AC field notably increased the sensitivity. Similarly, Lu and Jun (2012) showed improved specificity and sensitivity of the detection of *E. coli* cells and polystyrene beads in a functionalized microwire, which may be due to different DEP forces based on differences in the size and properties of the object.

The evaluation of the functionalized microwire with DEP force in ground beef is presented in Fig. 7. In Fig. 7(a), to test for the microwire specificity, the microwire was placed in ground beef that did not contain *E. coli* cells; however, nonspecific substances considered to be beef protein were found to be attached to the wire in Fig. 7(a). Many *E. coli* cells and a few nonspecific substances captured on the functionalized microwire with applied DEP force are shown in Fig. 7(b). After blocking treatment with BSA, however, the results showed a significant improvement in the sensing specificity of the sensor. The microwire immersed in the ground beef lacking inoculation after functionalization and blocking treatments is presented in Fig. 7(c). In Fig. 7(c), nonspecific binding cannot be observed on the wire surface, indicating that BSA fully covered the nonspecific binding sites. The functionalized microwire with blocking detected *E. coli* cells in ground beef as demonstrated in Fig. 7(d). Many *E. coli* cells were captured onto the functionalized wire.

4. Conclusion

In conclusion, the functionalized microwire sensor was developed under DEP conditions based on the combination of PEI-streptavidin-antibodies. The biosensor allows for the accurate detection of *E. coli* in exudate filtered from ground beef to remove particles. The performance of the developed sensor was characterized in terms of FI and the change in resistance on the wire surface with captured bacterial cells. The changes in resistance and FI in PBS and ground beef indicated a linear correlation with increasing *E. coli* concentration in the range of 10^3–10^7 CFU/mL. Additionally, using the developed sensor, *E. coli* cells could be detected in the range of 10^3–10^7 CFU/mL within 25 min 25 min is an average detection time in the lab scale when the sensing procedure was exercised by skilled labors. The estimated detection time is expected to be much faster than other conventional techniques (i.e. PCR) due to no enrichment process required. This technique was successfully experimentally tested using a complex food system, ground beef, which is expected to have great potential for the rapid, specific, and accurate detection of other pathogenic bacteria. Future study is needed to optimize the system parameters to minimize false positive values and validate the detection protocols.

CRediT authorship contribution statement

Yeo Wool Min: Methodology, Data curation, Funding acquisition. **Kwang Yeon Lee:** Data curation, Formal analysis. **Soojin Jun:** Writing - review & editing. **Hyeon Gyu Lee:** Supervision.

Declaration of competing interest

All authors declare there are no conflicts of interest.

Acknowledgement

This work was supported by Creative-Pioneering Researchers Program through Seoul National University (SNU).

References

Cairns, A. (2013). *Optimization study of polyethyleneimine surface coating for microtip DNA purification*A thesis of Master of Science. University of Washington.

Daniels, J. S., & Pourmand, N. (2007). Label-Free impedance biosensors: Opportunities and challenges. *Electroanalysis, 19*, 1239–1257.

Gomez-Sjoberg, R., Morisette, D. T., & Bashir, R. (2005). Impedance microbiology-on-a-chip: Microfluidic bioprocessor for rapid detection of bacterial metabolism. *Journal of Microelectromechanical Systems, 14*, 829–838.

Heo, J., & Hua, S. Z. (2009). An overview of recent strategies in pathogen sensing. *Sensors, 9*, 4483–4502.

He, Z., Zang, S., Liu, Y., He, Y., & Lei, H. (2018). A multi-walled carbon nanotubes-poly(l-lysine) modified enantioselective immunosensor for ofloxacin by using multi-enzyme-labeled gold nanoflower as signal enhancer. *Biosensors and Bioelectronics, 73*, 85–92.

Huang, J.-T., Wang, G.-C., Tseng, K.-M., & Fang, S.-B. (2008). A chip for catching, separating, and transporting bio-particles with dielectrophoresis. *Journal of Industrial Microbiology & Biotechnology, 35*, 1551–1557.

Kim, H. J., Kim, J., Yoo, Y. K., Lee, J. H., Park, J. H., & Hwang, K. S. (2016). Sensitivity improvement of an electrical sensor achieved by control of biomolecules based on the negative dielectrophoretic force. *Biosensors and Bioelectronics, 85*, 977–985.

Kim, S., Lu, L., Chung, J.-H., Lee, K., Li, Y., & Jun, S. (2011). A microwire sensor for rapid detection of *Escherichia coli* K-12 in fresh produce. *Innovative Food Science & Emerging Technologies, 12*, 617–622.

Kim, G., Moon, J.-H., & Morgan, M. (2013). Multivariate data analysis of impedimetric biosensor responses from Salmonella typhimurium. *Analytical Methods, 5*, 4074–4080.

Koo, O. K., Liu, Y., Shuaib, S., Bhattacharya, S., Ladisch, M. R., Bashir, R., et al. (2009). Targeted capture of pathogenic bacteria using a mammalian cell receptor coupled with dielectrophoresis on a biochip. *Analytical Chemistry, 81*, 3094–3101.

Kralik, P., & Ricchi, M. (2017). A basic guide to real time PCR in microbial diagnostics: Definitions, parameters, and everything. *Frontiers in Microbiology, 8*(108), https://doi.org/10.3389/fmicb.2017.00108.

Kyprianou, D., Guerreiro, A. R., Chianella, I., Piletska, E. V., Fowler, S. A., Karim, K., et al. (2009). New reactive polymer for protein immobilisation on sensor surfaces. *Biosensors and Bioelectronics, 24*, 1365–1371.

Lapizco-Encinas, B. H., Davalos, R. V., Simmons, B. A., Cummings, E. B., & Fintschenko, Y. (2005). An insulator-based (electrodeless) dielectrophoretic concentrator for microbes in water. *Journal of Microbiological Methods, 62*, 317–326.

Law, J. W.-F., Ab Mutalib, N.-S., Chan, K.-G., & Lee, L.-H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and

limitations. *Frontiers in Microbiology, 5*(770), https://doi.org/10.3389/fmicb.2014.00770.

Li, H., & Bashir, R. (2002). Dielectrophoretic separation and manipulation of live and heat-treated cells of Listeria on microfabricated devices with interdigitated electrodes. *Sensors and Actuators B: Chemical, 86*, 215–221.

Liu, M., Jia, C., Huang, Y., Lou, X., Yao, S., Jin, Q., et al. (2010). Highly sensitive protein detection using enzyme-labeled gold nanoparticleprobes. *The Analyst, 135*, 327–331.

Li, H., Zheng, Y., Akin, D., & Bashir, R. (2005). Characterization and modeling of a microfluidic dielectrophoresis filter for biological species. *Journal of Microelectromechanical Systems, 14*, 103–112.

Lu, L., Chee, G., Yamada, K., & Jun, S. (2013). Electrochemical impedance spectroscopic technique with a functionalized microwire sensor for rapid detection of foodborne-pathogens. *Biosensors and Bioelectronics, 42*, 492–495.

Lu, L., & Jun, S. (2012). Evaluation of a microwire sensor functionalized to detect Escherichia coli bacterial cells. *Biosensors and Bioelectronics, 36*, 257–261.

Maraldo, D., & Mutharasan, R. (2007a). 10-minutes assay for detecting *Escherichia coli* O157:H7 in ground beef samples using piezoelectric-excited millimeter-size cantilever sensors. *Journal of Food Protection, 70*, 1670–1677.

Maraldo, D., & Mutharasan, R. (2007b). Preparation-free method for detecting Escherichia coli O157: H7 in the presence of spinach, spring lettuce mix, and ground beef particulates. *Journal of Food Protection, 70*, 2651–2655.

Mateo, C., Abian, O., Fernandez-Lafuente, R., & Guisan, J. M. (2000). Reversible enzyme immobilization via a very strong and nondistorting ionic adsorption on support—polyethylenimine composites. *Biotechnology and Bioengineering, 68*(1), 98–105.

Morgan, H., Izquierdo, A. G., Bakewell, D., Green, N. G., & Ramos, A. (2001). The dielectrophoretic and travelling wave forces generated by interdigitated electrode arrays: Analytical solution using fourier series. *Journal of Physics D: Applied Physics, 34*, 1553.

Pessela, B. C. C., Betancor, L., Lopez-Gallego, F., Torres, R., Dellamora-Ortiz, G. M., Alonso-Morales, N., et al. (2005). Increasing the binding strength of proteins to PEI coated supports by immobilizing at high ionic strength. *Enzyme and Microbial Technology, 37*(3), 295–299.

Pessela, B. C. C., Fernández-Lafuente, R., Fuentes, M., Vián, A., García, J. L., Carrascosa, A. V., et al. (2003). Reversible immobilization of a thermophilic β-galactosidase via ionic adsorption on PEI-coated Sepabeads. *Enzyme and Microbial Technology, 32*(3–4), 369–374.

Ruan, C., Yang, L., & Li, Y. (2002). Immunobiosensor chips for detection of Escherichia coli O157: H7 using electrochemical impedance spectroscopy. *Analytical Chemistry, 74*, 4814–4820.

Schlauf, M., Assadollahi, S., Palkovits, R., Pointl, P., & Schalkhammer, T. G. M. (2015). Immobilization techniques and integrated signal enhancement for POC nanocolor microfluidic devices. *Journal of Nanomaterials, 2015*. https://doi.org/10.1155/2015/386794 386794.

Shen, W., Li, S., Park, M.-K., Zhang, Z., Cheng, Z., Petrenko, V. A., et al. (2012). Blocking agent optimization for nonspecific binding on phage based magnetoelastic biosensors. *Journal of the Electrochemical Society, 159*, B818–B823.

Singh, A., Poshtiban, S., & Evoy, S. (2013). Recent advances in bacteriophage based biosensors for food-borne pathogen detection. *Sensors, 13*, 1763–1786.

Suehiro, J., Hamada, R., Noutomi, D., Shutou, M., & Hara, M. (2003). Selective detection of viable bacteria using dielectrophoretic impedance measurement method. *Journal of Electrostatics, 57*, 157–168.

Varshney, M., & Li, Y. (2009). Interdigitated array microelectrodes based impedance biosensors for detection of bacterial cells. *Biosensors and Bioelectronics, 24*, 2951–2960.

Vidal, J. C., Bonel, L., Ezquerra, A., Duato, P., & Castillo, J. R. (2012). An electrochemical immunosensor for ochratoxin A determination in wines based on a monoclonal antibody and paramagnetic microbeads. *Analytical Bioanalytical Chemistry, 403*, 1585–1593.

Yang, L. (2009). Dielectrophoresis assisted immuno-capture and detection of foodborne pathogenic bacteria in biochips. *Talanta, 80*, 551–558.

Yang, L., Banada, P. P., Chatni, M. R., Lim, K. S., Bhunia, A. K., Ladisch, M., et al. (2006). A multifunctional micro-fluidic system for dielectrophoretic concentration coupled with immuno-capture of low numbers of Listeria monocytogenes. *Lab on a Chip, 6*, 896–905.

Yang, L., & Bashir, R. (2008). Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnology Advances, 26*, 135–150.

Yeo, W.-H., Liu, S., Chung, J.-H., Liu, Y., & Lee, K.-H. (2009). Rapid detection of Mycobacterium tuberculosis cells by using microtip-based immunoassay. *Analytical Bioanalytical Chemistry, 393*, 1593–1600.

Zeng, D., Chen, Z., Jiang, Y., Xue, F., & Li, B. (2016). Advances and Challenges in viability detection of foodborne pathogens. *Frontiers in Microbiology, 7*(1833), https://doi.org/10.3389/fmicb.2016.01833.

Zhao, X., Lin, C.-W., Wang, J., & Oh, D. H. (2014). Advances in rapid detection methods for foodborne pathogens. *Journal of Microbiology and Biotechnology, 24*(3), 297–312.