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A microwire sensor for rapid detection of *Escherichia coli* K-12 in fresh produce

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ABSTRACT

A rapid immunofluorescence method for foodborne pathogens in food systems using microwire sensor coupled with high frequency alternating current was developed. The method was intended to enrich and quantify *E. coli* cells internalized in baby spinach leaves. The targeted bacterial cells in the sample solution were captured on microwires in a diameter of 25 μm, and were bound to fluorescein isothiocyanate (FITC) labeled polyclonal *E. coli* antibodies. Fluorescent images of the FITC antibodies were obtained using a fluorescence microscope equipped with a charge-coupled-device (CCD) camera, and the fluorescent intensity (FI) was quantified through image processing. The capture of *E. coli* K-12 in PBS buffer was optimized when the electric field was generated at the frequency of 3 MHz and 20 Vpp with bacterial concentration of 10^7 CFU/mL. The detection limit of our sensing device was determined to be 10^3 CFU/mL. Field emission scanning electron microscopy (FESEM) was used to validate and visualize *E. coli* cells captured on the tip surface. The sensitivity and specificity of the developed sensor has been successfully validated by testing *E. coli* internalized in baby spinach leaves. The immunofluorescence detection has been completed within 15 min. Moreover, it was found that the enrichment process of *E. coli* cells using the dielectrophoretic force was rarely affected by food particles, which proved the sensing selectivity. The developed sensor is expected to meet the steady demand for a simple, rapid, highly sensitive detection approach to quantify the targeted microbes in food systems.

Industrial Relevance: There has been an increase in the number of foodborne illnesses linked to the consumption of fresh and minimally processed fruits and vegetables. Some E.coli strains such as E.coli O 157:H7, can cause a variety of diseases, including diarrhea, urinary tract infections, respiratory diseases, meningitis and more. In general, consumers wash the fresh produces under cold running tap water to remove any lingering dirt on the surface of the produces before eating or preparing. However, how do the consumers know if there is any possible pathogen hiding inside of the fresh produce after rinsing? It was reported from many researchers that, the E.coli internalization, which may occur when fresh produces intake E. coli containing water or manure from the soil, would be a main cause of the foodborne illness outbreak. To ensure the safety of drinking water, E.coli concentration cannot be higher than 1 CFU/mL. How can we detect such a low level of E.coli in an easy yet efficient way? To our knowledge, none of the traditional detecting approaches such as cultural based method, polymerase chain reaction(PCR), surface plasmon resonance (SPR) biosensor, and Latex Agglutination, has performed perfectly. Hence, a rapid and accurate technique for detecting foodborne pathogens in fresh produce is urgently needed in order to secure the food safety.

To overcome this issue, a simple detection method for foodborne pathogens in food systems using the microwire sensor coupled with high frequency alternative current was developed. The sensitivity and specificity of the developed sensor have been successfully validated by testing with E. coli internalized inside baby spinach leaves. It was found that spinach particles rarely affect the performance of our sensing device, which shows a promising prospect of its application in food industries.

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1. Introduction

There has been an increase in the number of foodborne illnesses linked to the consumption of fresh and minimally processed fruits and vegetables (Beuchat, 2002; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004; Tauxe, Kruse, Hedberg, Potter, Madden, & Wachsmuth,

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1997). In general, consumers wash the fresh produces under cold running tap water to remove any lingering dirt on the surface of the produces before eating or preparing. However, the consumers are still at the risk of being infected by potential pathogens that may exist inside the fresh produce after rinsing. It was reported that the *E. coli* internalization, which may occur when fresh produces were in contact with *E. coli* containing water or manure from the soil, would be a main cause of the foodborne illness outbreak (Ingham et al., 2004; Song, Stine, Choi, & Gerba, 2006; Wachtel, Whitehand, & Mandrell, 2002). In 2006, 276 people became ill with *E. coli* O157:H7 associated with the consumption of fresh spinach and shredded lettuce (USFDA, 2006). These outbreaks were considered unusual because of high rates of hospitalization and hemolytic uremic syndrome compared with previous outbreaks (Thorpe, Ritchie, & Acheson, 2002). Moreover, the incidence of foodborne outbreaks associated with leafy greens increased by 39% from 1996 to 2005, while leafy green consumption increased by only 9% (Herman, Ayers, & Lynch, 2008). Hence, in order to improve food security level and prevent people from being infected by contaminated food, a rapid and accurate technique to detect foodborne pathogens such as *E. coli* O157:H7 is urgently needed.

Several conventional techniques have been used to detect pathogenic microbes: polymerase chain reaction (PCR) (Lau et al., 2010; Shi, Wu, Cai & Shang, 2010), surface plasmon resonance (SPR) (Kumbhat, Sharma, Gehlot, Solanki & Joshi, 2010), enzyme-linked immunosorbent assay (ELISA) (Kerkaert, Mestdagh, & Meulenaer, 2010), piezoelectric biosensors (Chen, Wu, Chuang, & Lin, 2008; Mao, Yang, Su, & Li, 2006) and amperometric biosensors (Palchetti & Mascini, 2008). However, none of the above detection methods fully satisfies the detection performance criteria which include sensing specificity, detection limit, detection rate, and associated cost. For instance, PCR is relatively sensitive but requires precision instruments, proficient manipulation and a long running period. Moreover, the detection limit of ELISA (10^5 CFU/mL) (Wang, Ye, Si & Ying, 2011) is insufficient to the targeted pathogens in the food sample even though it can quantify rapidly and precisely the targeted bacteria with relatively low cost.

Dielectrophoresis (DEP) is the translational motion of neutral particle caused by polarization effects in a non-uniform electric field, and the DEP force can be given by (Huang, Wang, Tseng, & Fang, 2008),

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

where F refers to the DEP force, r is the particle radius, E is non-uniform electric field, $_m$ is permittivity of medium, and Re[F_{CM}] is the real value of F_{CM}, which is the Clausius–Mossotti (CM) factor, given by

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

where ε_p^* is the complex permittivity of the particle, ε_m^* is the complex permittivity of the medium. Therefore, depending upon the relative permittivities of particle and medium, the sign of DEP force can be determined. For example, if particles' permittivity is greater than medium permittivity, the real value of F_{CM} becomes positive thus the positive DEP force will move the particle toward the maximum region of the electric field strength.

The DEP force has been studied for microscale and nanoscale particle manipulation (Harrower & Oliver, 2006; Yeo, Chung, Liu, & Lee, 2009). DEP has also been applied to filtration and detection of biological cells such as *E. coli* and *Mycobacterium tuberculosis* (Lagally, Lee, & Soh, 2005; Lapizco-Encinas, Simmons, Cummings, & Fintschenko, 2004; Yang, 2009). However, none of the studies has been reported for the detection of the pathogenic cells in real food samples using the DEP force. In this study, a rapid detection device to integrate the DEP and capillary forces was designed and fabricated to capture *E. coli* cells in fresh produce leaves. Furthermore, bioaffinity reaction with fluorescent antibodies was used to quantify bacterial cells captured on the microwire.

2. Methodology

2.1. Sensor design

A 18″×18″ English bench plate (Edmund Optics Inc, Barrington, NJ), and xyz stage (Franklin Mechanical & Control Inc., Gilroy, CA) were installed to manipulate 7% gold–tungsten coated wires in 25 μm diameter (ESPI Metals, Ashland, OR) with the probe guide panel (Fig. 1(a)). A silver coated copper wire (250 μm in diameter, OK industries, Tuckahoe, NY) was coiled using a wrapping tool to form ring-shaped electrode in a 1.5 mm diameter, and was held by a micromanipulator (Model MM572R; Micromanipulator Company Inc., Carson City, NV). Optical microscope (40× magnification) was installed on the bench plate with Techspec microscopy holder (Edmund Optics Inc, Barrington, NJ) consisting of two of three-screw adjustable ring mount in 53 mm outer diameter, two of 4″ length steel post (1/4–20 stud), and two of 2″ post holder assembly. Fiber optic Y-shape dual light microscope illuminator (Microscope World, Carlsbad, CA) was equipped as background light source.

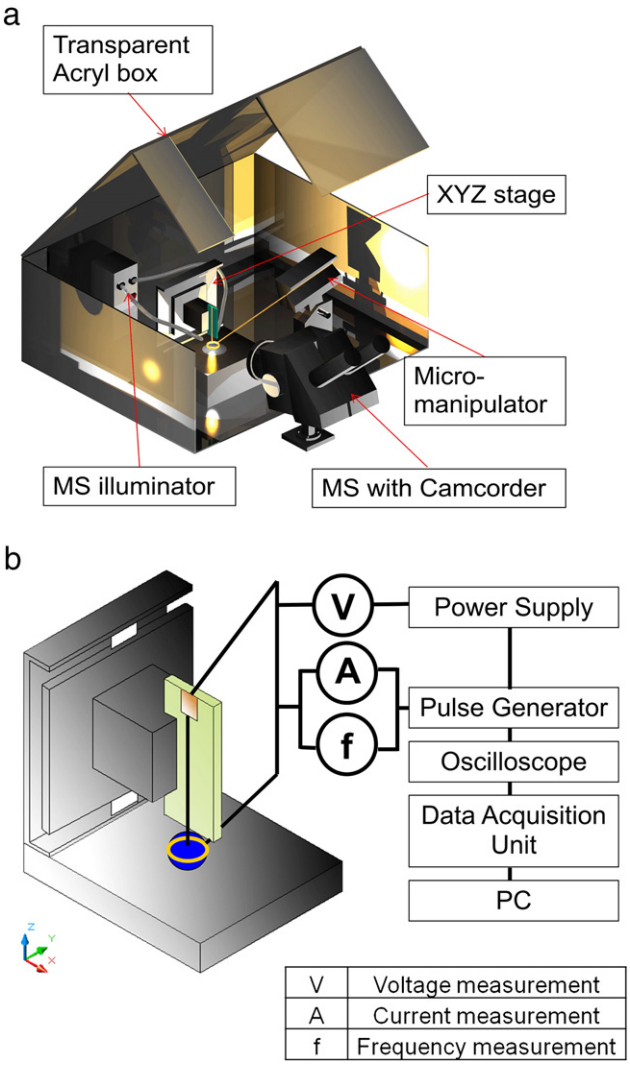

Fig. 1. Schematic diagrams of (a) a microwire sensing unit, and (b) power supply and measurement equipments.