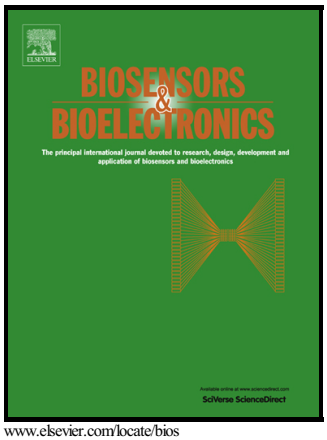

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PII: S0956-5663(15)30174-3
DOI: http://dx.doi.org/10.1016/j.bios.2015.06.007
Reference: BIOS7742

To appear in: *Biosensors and Bioelectronic*

Received date: 20 March 2015
Revised date: 27 May 2015
Accepted date: 3 June 2015

Cite this article as: Qi Chen, Jianhan Lin, Chengqi Gan, Yuhe Wang, Dan Wang, Yonghua Xiong, Weihua Lai, Yuntao Li and Maohua Wang, An ultrasensitive impedance biosensor based on immunomagnetic separation and urease catalysis for rapid detection of *listeria monocytogenes* using an immobilization-free interdigitated array microelectrode, *Biosensors and Bioelectronic,* http://dx.doi.org/10.1016/j.bios.2015.06.007

An ultrasensitive impedance biosensor based on immunomagnetic separation and urease catalysis for rapid detection of Listeria monocytogenes using an immobilization-free interdigitated array microelectrode

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ABSTRACT

In this study, we described a novel impedance biosensor combining immunomagnetic separation with urease catalysis for ultrasensitive detection of foodborne bacteria using Listeria monocytogenes as model and an immobilization-free microelectrode as detector. The monoclonal antibodies (MAbs) were immobilized on the surface of the magnetic nanoparticles (MNPs) with the diameter of 180 nm by biotin-streptavidin system for specifically and efficiently separating Listeria cells from sample background. The polyclonal antibodies (PAbs) and the urease were modified onto the surface of the gold nanoparticles (AuNPs) with the diameter of 20 nm and used to form the MNP-MAb-Listeria-PAb-AuNP-urease sandwich complexes. The urease in the complexes could catalyze the hydrolysis of the urea into ammonium carbonate, resulting in an increase in the ionic strength of the media, which was detected by the microelectrode. The magnetic separation efficiencies for Listeria monocytogenes at the concentrations ranging from 3.0×10^1 to 3.0×10^4 CFU/mL were over 95% for the pure cultures and over 85% for the spiked lettuce samples. The lower detection limit of this biosensor for Listeria monocytogenes was found to be 30 CFU/mL in both the pure cultures and the spiked lettuce samples. The microelectrode was demonstrated to be reusable for over 50 times with thorough cleaning by deionized water. This biosensor showed its potential

to provide a simple, low-cost and sensitive method for rapid screening of foodborne pathogens and could be extended for detection of other biological or chemical targets.

Key Words：Impedance biosensor, Urease catalysis, Ionic strength, Immunomagnetic separation, Listeria monocytogenes

1. Introduction

Recent outbreaks of foodborne diseases have drawn a great public attention globally. The US CDC estimated that major known pathogens and unspecified agents transmitted by foods resulted in an estimated 47.8 million illnesses, 127,839 hospitalizations, and 3,037 deaths each year in the United States. From the statistics of the Ministry of Health of China, pathogenic microorganisms were responsible for over 50% of foodborne illnesses since 2006. Foodborne pathogens has attributed to enormous economic loss and posed a huge threaten to public health (Gormley et al., 2011; Kozak et al., 2013; Larsen et al., 2014).

Listeria monocytogenes is one of the most dangerous foodborne pathogen and ubiquitous in the environment (Batz et al., 2012; Ferreira et al., 2014). It can survive at temperatures as low as 4 °C, at pH as low as 4.4, and at salt concentrations of up to 14% (Cole et al., 1990). The

infection sources of L. monocytogenes can be meal (Aureli et al., 2000), egg (Rivoal et al., 2013), poultry (Meyer et al., 2012), seafood (Gambarin et al., 2012), dairy product (El Marnissi et al., 2013) and vegetable (Sant'Ana et al., 2012), etc. Infection of L. monocytogenes may lead to listeriosis, which is one of the leading causes of death for foodborne illness, and the mortality rate is approximately 30% in high risk groups (Silk et al., 2014; Swaminathan and Gerner-Smidt., 2007).

Early screening of foodborne pathogens in foods plays an important role in preventing and controlling the outbreaks of foodborne diseases. Currently available methods for the detection of foodborne pathogens mainly include plate colony counting (culture) (Bouguelia et al., 2013), polymerase chain reaction (PCR) (Kawasaki et al., 2011), enzyme-linked immune-sorbent assay (ELISA) (Galikowska et al., 2011) and immuno-chromatographic lateral flow assay (Strip) (Blažková et al., 2011). Culture is the gold standard method with high sensitivity, but requires long time (2~3 d). Conventional PCR or real-time PCR are fast and sensitive methods and recommended in many national standards, but need complex nucleic acid extraction procedures. ELISA is a fast method based on the antigen-antibody immunoreaction, but often lacks sufficient sensitivity. Strip is a very fast method and ready for in-field tests, but has low sensitivity and accuracy. These methods are either time-consuming, or requiring complex sample pretreatment procedures, or requiring

specialized laboratory facilities and skilled technicians, or insufficiently sensitive (Yeni et al., 2014; Zhao et al., 2014). Therefore, ultrasensitive, rapid, in-field and low-cost detection methods of foodborne pathogens are urgently needed to effectively prevent and control the outbreaks of foodborne diseases.

As an alternative, various biosensors, including Surface Plasmon Resonance (SPR) (Karoonuthaisiri et al., 2014), Quartz Crystal Microbalance(QCM)(Su and Li., 2005), optics (Massad-Ivanir et al., 2013) and electrochemistry (Safavieh et al., 2012), etc., have been developed for the detection of foodborne pathogens. Impedance biosensor is a typical electrochemical biosensor, generally relying on the measurement of the electrochemical impedance change on the interface of an electrode under an alternating-current potential with a direct-current bias. It is often featured with compact design, rapid detection, relatively low cost and easy integration. Interdigitated array microelectrodes, which are often modified with specific antibodies against target bacteria prior to detection, are commonly used in the development of impedance biosensors to capture the targets onto the microelectrodes and measure the impedance increase resulting from the increasing electron transfer resistance between the inter-digits of the microelectrode.

In our previous study, we developed an impedance biosensor based on a low-cost interdigitated array microelectrode and specific monoclonal

antibodies for rapid detection of avian influenza virus H5N1 in chicken swabs (Lin et al., 2015). The microelectrode was fabricated using photolithography and wet-etching process and modified with the anti-H5 antibodies for the detection of H5N1 virus. This developed biosensor was demonstrated to have a linear relationship between impedance change and logarithmic value of H5N1 viruses at the concentrations from 2^{-1} to 2^{4} HAU/50 uL and have a comparable accuracy with real-time reverse transcription PCR compared to gold standard viral isolation. However, this biosensor has two inherent drawbacks: (1) the immobilization of anti-H5 antibodies onto the surface of the microelectrode is time-consuming and complicated with unsatisfied stability, repeatability and regeneration ability, which is a key factor to limit its practical use; (2) the immunoreaction between the antibodies immobilized on the microelectrode and the targets suspended in the solution is a liquid-solid phase reaction, which mainly depends on the diffusion of the targets in the aqueous solution and has a low reaction rate, resulting in an inefficient binding and a great impact on the sensitivity of the biosensor (Yang., 2009). As such, new methods featured with immobilization-free and liquid-liquid phase reaction should facilitate the practical applications of this biosensor.

Enzymes are widely used as biological catalysts in the studies on biological detection since they can catalyze the transformation of

substrates with high efficiency and high specificity and thus significantly improve the sensitivity. Urease, which can catalyze the hydrolysis of urea into ammonium carbonate (see equation 1) (Fidaleo and Lavecchia, 2003; Vial et al., 2006) , is often employed in the development of enzyme catalysis related biosensors for the detection of urea in urine samples (Yang et al., 2007), Escherichia coli in vegetable foods (Ercole et al., 2003) and heavy metal ions in environmental samples (Rodriguez et al., 2004).

$$NH_2CONH_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-} \quad (1)$$

In this study, we propose a novel impedance biosensor combining immunomagnetic separation based on the monoclonal antibodies modified magnetic nanoparticles with urease catalysis based on the polyclonal antibodies and urease modified gold nanoparticles for ultrasensitive detection of foodborne bacteria using the interdigitated array microelectrode, which is free of the immobilization of the antibodies against target bacteria, as detector and L. monocytogenes as model. As shown in Fig. 1, the magnetic nanoparticles (MNPs) coated with the monoclonal antibodies (MAbs) against Listeria were first used to specifically separate Listeria cells from sample background and concentrate them in a small volume of buffer solution. Then, the gold nanoparticles (AuNPs) modified by the anti-Listeria polyclonal antibodies (PAbs) and the urease were conjugated with the magnetic

Listeria cells (Listeria-MAb-MNP) to form the urease-AuNP-PAb-Listeria-MAb-MNP sandwich complexes. After successive washing with PBST (0.5% Tween-20 in PBS) and deionized water by magnetic separation to remove the redundant PAb and urease modified AuNPs and the conductive ions, the complexes were suspended in the urea in deionized water and the hydrolysis of the urea was catalyzed by the urease into ammonium carbonate resulting in an increase of the ionic strength of the media, which was detected by the microelectrode and analyzed by electrochemical impedance spectroscopy (EIS).

Fig. 1

2. Materials and Methods

2.1. Materials

Gold chloride tri-hydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate, streptavidin, biotin, urease (E.C.3.5.1.5, Type III, 15,000-50,000 units/g solid), urea (NH_2CONH_2), ammonium carbonate ($(NH_4)_2CO_3$), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 2-(N-morpholino) ethanesulfonic acid (MES), and phosphate buffered saline (PBS, 10 mM, pH 7.4) were purchased from Sigma Aldrich (St. Louis, MO, US). Bovine serum albumin (BSA) from EM Science (Gibbstown, NJ, US) was prepared in PBS for blocking.

Polyethylene glycol (PEG) 20,000 was purchased from Merck (Darmstadt, Germany) for blocking as well. Tween-20 was purchased from Amresco (Solon, OH, US) for washing. The magnetic nanoparticles were purchased from Allrun Nano (PM3-020, Shanghai, China) for immunomagnetic separation. The monoclonal and polyclonal antibodies against Listeria monocytogenes were developed by Nanchang University and obtained from Zodolabs Biotech (Nanchang, China) for immuno-reacting with Listeria cells. Other reagents were of analytical grade and purchased from Sinopharm Chemical (Shanghai, China). All the solutions were prepared with deionized water produced by Advantage A10 from Millipore (Billerica, MA, USA).

2.2. Preparation and Culture of Bacteria

Listeria monocytogenes (ATCC13932) used as target bacteria and Escherichia coli O157:H7 (ATCC43888) used as non-target bacteria were stored at -20 °C with 15% glycerol and were revived by streaking on Luria-Bertani (LB) agar plates. They were first cultured in LB medium (Aoboxing Biotech, Beijing, China) at 37 °C for 12-16 h with shaking at 180 rpm, respectively. Then, the cultures were 10-fold diluted with sterile PBS to obtain the bacteria at the concentrations of 10^1 to 10^4 CFU/mL, respectively.

For bacterial enumeration, the bacterial samples were serially diluted with sterile PBS and 100 μL of the diluents were surface plated on the LB

agar plates. The plates were then incubated at 37 ^{o}C for 22-24 h before the colonies were counted. The bacteria were enumerated in colony forming unit per milliliter (CFU/mL).

2.3. Modification of Magnetic Nanoparticle

The monodisperse magnetic nanoparticles (MNPs) with the diameter of 180 nm were functionalized with carboxyl group (~150 μM/g) and stored in deionized water at room temperature. The core of the MNPs is Fe_3O_4 with the Fe content of 10 mg/mL.

The anti-Listeria monocytogenes monoclonal antibodies (MAbs) were immobilized on the surface of magnetic nanoparticles for specific separation of Listeria cells by our previously-reported protocol with some modifications (Lin et al., 2015). Prior to use, 2 mg of the carboxylated MNPs were successively washed with 1 M HCl and deionized water using a magnetic separator with a max magnetic strength of ~1.1 T from Aibit Biotch (MS0206, Jiangyin, China) to capture the MNPs for 2 min. First, EDC·HCl (2 mg in 500 μL of deionized water) were freshly prepared and used to activate the carboxyl groups at room temperature for 10 min at 15 rpm. After washing with deionized water twice to remove redundant EDC, the MNPs were immediately suspended in 400 μL of PBS and 100 μL of streptavidin (1 mg/mL in PBS) and gently rotated at 15 rpm for 2 h. After the streptavidin modified MNPs were washed with PBS twice to remove surplus streptavidin, they were blocked with 1%

BSA for 45 min and washed with PBS for three times. Then, the MNPs were resuspended in 1.5 mL of PBS containing 1.33 mg of the biotinylated monoclonal antibodies against Listeria monocytogenes (~1.15 mg/mL) and incubated at 15 rpm for 45 min. After washing with PBS twice to remove surplus antibodies, the MAb modified MNPs were finally suspended in PBS with 1% BSA at a final concentration of 1 mg/mL (Fe content) and stored at 4 ^{o}C.

2.4. Synthesis and Modification of Gold Nanoparticle

The gold nanoparticles (AuNPs) with the diameter of ~20 nm were synthesized according to the citrate reduction method (Huang et al., 2015). Briefly, 100 mL of 0.01% $HAuCl_4 \cdot 3H_2O$ was first heated to boiling. Then, 1.5 mL of 1% sodium citrate was added while continuously stirring. After the mixture changed from red to purple, remain boiling for 10 min and the AuNPs were obtained and stored at 4 ^{o}C.

The anti-Listeria monocytogenes polyclonal antibodies (PAbs) and the urease were immobilized onto the surface of the AuNPs for binding the Listeria cells and catalyzing the hydrolysis of urea, respectively, using the reported protocol by Liu with some modifications (Liu et al., 2013). Briefly, the pH of the AuNPs was first adjusted to 7.0 with 0.2 M K_2CO_3. 5 μg of PAbs in 50 μL of deionized water was added drop-by-drop to 1 mL of AuNPs at room temperature followed by incubation for 5 min while gently stirring. Then, 10 μL of urease (10 mg/mL, in 10 mM MES)

was diluted by 40 μL of deionized water and added drop-by-drop into the AuNPs followed by gently stirring at room temperature for 1 h. After the AuNPs were blocked by 100 μL of the blocking solution containing 1% (w/v) PEG 20,000 for 30 min, 100 μL of 10% BSA was added drop-by-drop into the AuNPs for 30 min. The mixture was centrifuged at 6950 g for 15 min to remove surplus PAbs and urease, and the PAb and urease modified AuNPs were washed with deionized water and dissolved in 100 μL of deionized water and stored at 4 ℃.

2.5. Impedance Measurement

Impedance measurements were performed using the interdigitated array microelectrode fabricated at State Key Lab of Integrated Optoelectronics, Institute of Semiconductors, Chinese Academy of Science and an E4980A impedance analyzer with a frequency response range of 20 Hz - 2 MHz (Agilent, Santa Clara, CA, US). The microelectrode includes 25 pairs of digit electrodes with 15 μm digit width, 15 μm inter-digit spacing, and 3 mm digit length. The total area of the microelectrode was ~77 mm^2 with a working area of ~4.5 mm^2. For all the impedance measurements, a sinusoidal alternating potential with an amplitude of 5 mV, a direct-current bias of 0 V and a frequency range of 183 Hz - 500 kHz was applied on the microelectrode. The bode plots and an equivalent circuit were used to interpret the EIS data for better understanding.

2.6. Bacteria Detection

Bacteria detection was based on antigen-antibody binding and enzymatic reaction. 150 μg of the MAb modified MNPs were first incubated with 1 mL of Listeria monocytogenes with different concentrations (10^1 -10^4 CFU/mL) at 15 rpm for 45 min in a 2-mL sterile centrifuge tube, which was blocked by 1% BSA for 30 min prior to use. After magnetic separation for 2 min and washing with 500 μL of PBS twice, the Listeria cells captured by the MNPs were resuspended in a solution containing 195 μL of PBS and 5 μL of the PAb and urease modified AuNPs, followed by incubation at 15 rpm for 10 min to form the MNP-MAb-Listeria-PAb-AuNP-urease sandwich complexes. The complexes were sequentially washed with 200 μL of PBST and deionized water for three times to remove unbound PAb and urease modified AuNPs and residual conductive ions, respectively, and the complexes were resuspended in 200 μL of 1 mM urea. After incubation for 30 min, the complexes were magnetically separated for 2 min and 20 μL of the supernatant was transferred onto the microelectrode for impedance measurement. The impedance change at the characteristic frequency of 10 kHz was calculated as the difference between the impedance of the supernatant and that of deionized water for determining the amount of Listeria cells.

3. Results and Discussions

3.1. Concept proof based on EIS analysis of urea and ammonium carbonate

This novel impedance biosensor was based on urease's catalyzing the hydrolysis of urea into ammonium carbonate. Therefore, the impedance change was verified to be related with the concentration of ammonium carbonate and remain unchanged or less changed at the presence of urea using the microelectrode. Triplicate tests were conducted for each concentration of ammonium carbonate and urea, which were prepared in deionized water. Fig. 2(a) shows that the impedance of ammonium carbonate in deionized water decreases while the concentration of ammonium carbonate increases, indicating that the impedance change is related with the concentration of ammonium carbonate ranging from 10 nM to 1 mM. Fig. 2 (b) shows that EIS of different concentrations (1 μM to 10 mM) of urea in deionized water basically overlaps that of deionized water, verifying that urea is of very poor conductivity. To further investigate the effect of the concentration of ammonium carbonate on the impedance change, the impedance measured at the characteristic frequency of 10 kHz were selected and compared to the deionized water. Fig. 2(c) shows that a linear relationship between the impedance change (△Z) at the characteristic frequency of 10 kHz and the concentration of ammonium carbonate (C) ranging from 10 nM to 1 mM is obtained and can be described by $\triangle Z=9407.4 \log (C) +77288$ (R^2 =

0.96). Fig. 2(d) shows that the impedance values of the urea at the concentrations of 1 μM-10 mM at the frequency of 10 kHz are close to that of deionized water with a relative standard deviation of 2.2%, indicating that redundant urea in the media does not have impact on the impedance change.

Fig. 2

3.2. AuNP Characterization based on TEM imaging

The synthesis of the AuNPs and the modification of the anti-Listeria antibodies and the urease onto the AuNPs are important to the development of this biosensor. TEM imaging was used to evaluate the synthesis of the AuNPs. As shown in Fig. S1(a) in the supplementary material, the AuNPs are observed with an averaged diameter of ~20 nm. Besides, the ultraviolet absorption analysis was used to confirm the modification of the PAbs and the urease. Fig. S1(b) shows that the spectrum of the bare AuNPs exhibits a characteristic plasmon absorption peak at 520 nm and the peak shifts to 525 nm for the PAb and urease modified AuNPs, indicating that the PAbs and the urease have been successfully coated on the surface of the AuNPs. For further confirm that the PAbs were successfully coated on the surface of the AuNPs, the Listeria cells were incubated with the bare AuNPs blocked by BSA and the PAb and urease modified AuNPs for 45 min, respectively, and

analyzed by TEM imaging. Fig. S1(c) and (d) show that seldom BSA blocked AuNPs are conjugated with Listeria cells and hundreds of the PAb and urease modified AuNPs are conjugated with one Listeria cell.

3.3. Immunomagnetic separation of Listeria monocytogenes using the MAb modified MNPs

The separation efficiency (SE) of Listeria using the MAb modified MNPs directly affects the sensitivity of this biosensor. SE was defined as the percentage of the magnetically separated bacteria compared to the total bacteria in the positive control and calculated by equation (2):

$$SE\ (\%) = N_{ms}/N_{pc} \times 100\% \quad (2)$$

where, N_{ms} is the number of the magnetically separated bacteria cells and N_{pc} is the number of the positive control. Triplicate tests were conducted for each concentration of Listeria ranging from 3.0×10^1 to 3.0×10^4 CFU/mL in both the pure cultures and the spiked lettuce samples. As shown in Fig. 3, the SEs of Listeria at all the concentrations are over 95% in the pure cultures and over 85% in the spiked lettuce samples, while the SEs of non-target bacteria (E. coli O157:H7) at the concentration of 2.6×10^4 CFU/mL in the pure culture are less than 3%, indicating that the immunomagnetic separation has a high separation efficiency and a good specificity. More specificity tests on this monoclonal antibody against Listeria monocytogenes have been done and reported by the previously-published paper (Huang et al., 2015).

3.4 EIS analysis using the equivalent circuit

For interpreting the EIS data, an equivalent circuit was used to simulate the impedance data. As shown in Fig. 4(a), the equivalent circuit consists of solution resistance (R_s), double layer capacitor (C_{dl}) and dielectric capacitor (C_{di}). R_s represents the conductivity of the bulk electrolyte solution; C_{dl} represents the effect of ionic species on the capacitance near the surface of the electrode; and C_{di} represents the dielectric characteristics of the electrolyte solution. Fig. 4(b) shows the measured impedance data and the equivalent circuit fitted impedance data for the impedance measurement of 3.0×10^3 CFU/mL of Listeria monocytogenes using the microelectrode. To verify the fitting of the equivalent circuit, the measured impedance data with the frequency of 183 Hz-500 kHz were used as input data to the impedance simulation software ZSimpWin for simulation. The errors of R_s, C_{dl} and C_{di} were 0.65%, 2.44% and 1.01%, respectively. In the impedance spectra, there are three regions corresponding to the three components in the equivalent circuit. In the low frequency region from 183 Hz to 1 kHz, the middle frequency region from 1 kHz to 50 kHz and the high frequency region from 50 kHz to 500 kHz, the impedance of the microelectrode was dominated by the double layer capacitor, the solution resistance and the

dielectric capacitor, respectively.

3.5. Detection of Listeria monocytogenes in the pure cultures and spiked lettuce samples

The impedance (ion strength) of the catalyzed supernatant is dependent on the amount of the urease conjugated with the Listeria cells. The impedance spectra for different concentrations of Listeria in the pure cultures and spiked lettuce samples were recorded as bode plots and shown in Fig. 5(a) and (b). It can be seen that the impedance decreases while the concentration of Listeria increases. Triplicate tests of Listeria at the concentrations of 3.0×10^1 - 3.0×10^4 CFU/mL were conducted using this impedance biosensor. TEM imaging was used to check the forming of the MNP-MAb-Listeria-PAb-AuNP-urease complexes (see Fig. 5c). The frequency range of 1 kHz - 50 kHz in the impedance spectra was observed to have obvious changes in the detection of Listeria. The impedance change at the characteristic frequency of 10 kHz was plotted for different concentrations of Listeria. Linear relationships between the impedance change ($\triangle Z$) and the concentration of Listeria (C) were obtained for both the pure cultures and the spiked lettuce samples and described as $\triangle Z = 2782.0 \log(C) + 5575.5$ ($R^2 = 0.93$ for pure culture) and $\triangle Z = 2635.2 \log(C) + 8110.2$ ($R^2 = 0.96$ for spiked lettuce sample),

respectively. Besides, the average relative standard deviations of the impedance change in the parallel detection of different concentrations of Listeria were 5.4% for the pure cultures 2.5% and for the spiked lettuce samples, respectively, indicating that this biosensor had a good reproducibility.

Fig. 5

To further verify that the solution resistance (R_s) dominates the impedance change of the microelectrode, the components of the equivalent circuit were obtained using the software ZSimpWin. Table 1 shows that when the concentration of Listeria increases from 3.0×10^1 CFU/mL to 3.0×10^4 CFU/mL, the solution resistance decreases from 34.7 kΩ to 14.9 kΩ, however both the double layer capacitor and the dielectric capacitor only have a slight change.

Table 1

The lower detection limit of this biosensor was determined by three times of standard deviation of control. PBS was used as control and 10 tests of control were conducted with impedance change of 6295 ± 793 Ω for the pure cultures and 12671 ± 731 Ω for the spiked lettuce samples. Therefore, the detection limit of this biosensor was 30 CFU/mL. Compared to some reported Listeria monocytogenes detection methods,

such as QCM, SPR, optics and piezoelectricity (see Table S1 in the supplementary material), this biosensor showed a higher sensitivity. This was mainly due to the following reasons: (1) the separation efficiency of Listeria using the MAb modified MNPs was high (over 95% for the pure cultures and over 85% for the spiked lettuce samples); (2) urease catalysis significantly amplified the detection signal and the product of urease catalyzing the hydrolysis of urea is ammonium carbonate, which is a strong electrolyte and sensitive to the conductivity of the solution; (3) the PAbs were employed to provide more antigen binding sites and as a result more urease were obtained in the sandwich complexes; (4) the interdigitated array microelectrode with a high sensitivity of the electrochemical impedance was used for the measurements of the impedance change of the media.

3.5. Regeneration of the interdigitated array microelectrode

The microelectrode was used free of anti-Listeria antibody immobilization for the measurements of the impedance change of the media. Therefore, the microelectrode only suffered negligible impacts on the surficial properties during the impedance measurements. The microelectrode was demonstrated to be reusable for over 50 times after a simple cleaning method by washing with deionized water for three times and drying by nitrogen flow. The impedance spectra of the microelectrode in the measurements of deionized water were recorded for comparison of

the impedance change. The relative standard deviation of 20 tests of deionized water at the frequency of 10 kHz was ~4.9%. There is no obvious change of the impedance spectra of deionized water, when the microelectrode was thoroughly cleaned.

4. Conclusions

The present study showed a novel impedance biosensor combining immunomagnetic separation and urease catalysis for rapid detection of Listeria monocytogenes using an antibody immobilization-free interdigitated array microelectrode. The immunomagnetic separation with anti-Listeria monoclonal antibodies modified magnetic nanoparticles was used to specifically separate Listeria cells with a high separation efficiency of over 85%. The anti-Listeria polyclonal antibodies and the urease were modified on the gold nanoparticle and used to efficiently catalyze the hydrolysis of urea into ammonium carbonate. The interdigitated array microelectrode was used for sensitive measurement of the ion strength change and could be reused for over 50 times. This novel biosensor was able to detect as low as 30 cells of Listeria in both the pure cultures and the spiked lettuce samples. This biosensor might be easily extended for the detection of other foodborne pathogens and biological targets by changing the antibodies and it is potential to integrate with Lab-on-a-chip technology to develop a sensitive, rapid and low-cost method for the detection of biological or chemical targets.

Acknowledgments

This study was supported in part by Special Fund for Agro-scientific Research in the Public Interest (No. 201303045), Chinese Universities Scientific Fund (No. 2014RC013) and National Natural Science Foundation of China (No. 31271863). The authors thank Dr. Yingchun Fu for English edition and correction.

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Figure captions

Fig. 1. The principle of the impedance biosensor based on immunomagnetic separation and urease catalysis.

Fig. 2. (a) EIS of ammonium carbonate in deionized water with the concentrations of 10 nM-1 mM; (b) EIS of urea in deionized water with the concentrations of 1 μM-10 mM; (c) Linear relationship between the impedance change at the frequency of 10 kHz and the concentration of ammonium carbonate ranging from 10 nM to 1 mM; (d) Impedance of the urea with the concentrations of 1 μM-10 mM at the frequency of 10 kHz.

Fig. 3. (a) Separation efficiency of Listeria monocytogenes at the concentrations of 3.0×10^1 - 3.0×10^4 CFU/mL in the pure cultures and the spiked lettuce samples; (b) Separation efficiency of Listeria monocytogenes at the concentration of 3.0×10^4 CFU/mL and E. coli O157:H7 at the concentration of 2.6×10^4 CFU/mL in the pure cultures.

Fig. 4. (a) The equivalent circuit for fitting of the impedance data, (b) Bode plots of the impedance spectra of the measured and simulated data in the frequency range of 183 Hz to 500 kHz in the detection of 3.0×10^3 CFU/mL of Listeria monocytogenes.

Fig. 5. (a) Bode plots of the EIS of Listeria monocytogenes at the concentrations of 3.0×10^1 - 3.0×10^4 CFU/mL in the pure cultures; (b) Bode plots of the EIS of Listeria monocytogenes at the concentrations of 3.0×10^1 - 3.0×10^4 CFU/mL in the spiked lettuce samples; (c) TEM image of the MNP-Listeria-AuNP complex; (d) Linear relationship between the impedance change and the concentration of Listeria in the pure cultures and the spiked lettuce samples.

Table 1 Simulated value of the components in the equivalent circuit for the detection of different concentrations of Listeria monocytogenes.

Listeria Concentration (CFU/mL)	R_s (Ω)	Error (%)	C_{dl} (F)	Error (%)	C_{di} (F)	Error (%)
Control	4.33×10^{4}	0.36	2.17×10^{-8}	2.37	7.05×10^{-11}	0.42
3.0×10^{1}	3.47×10^{4}	0.44	2.23×10^{-8}	2.56	7.03×10^{-11}	0.56
3.0×10^{2}	3.06×10^{4}	0.46	1.93×10^{-8}	2.21	7.02×10^{-11}	0.61
3.0×10^{3}	2.03×10^{4}	0.66	1.82×10^{-8}	2.44	7.07×10^{-11}	1.01
3.0×10^{4}	1.49×10^{4}	0.61	2.08×10^{-8}	2.10	7.02×10^{-11}	1.05

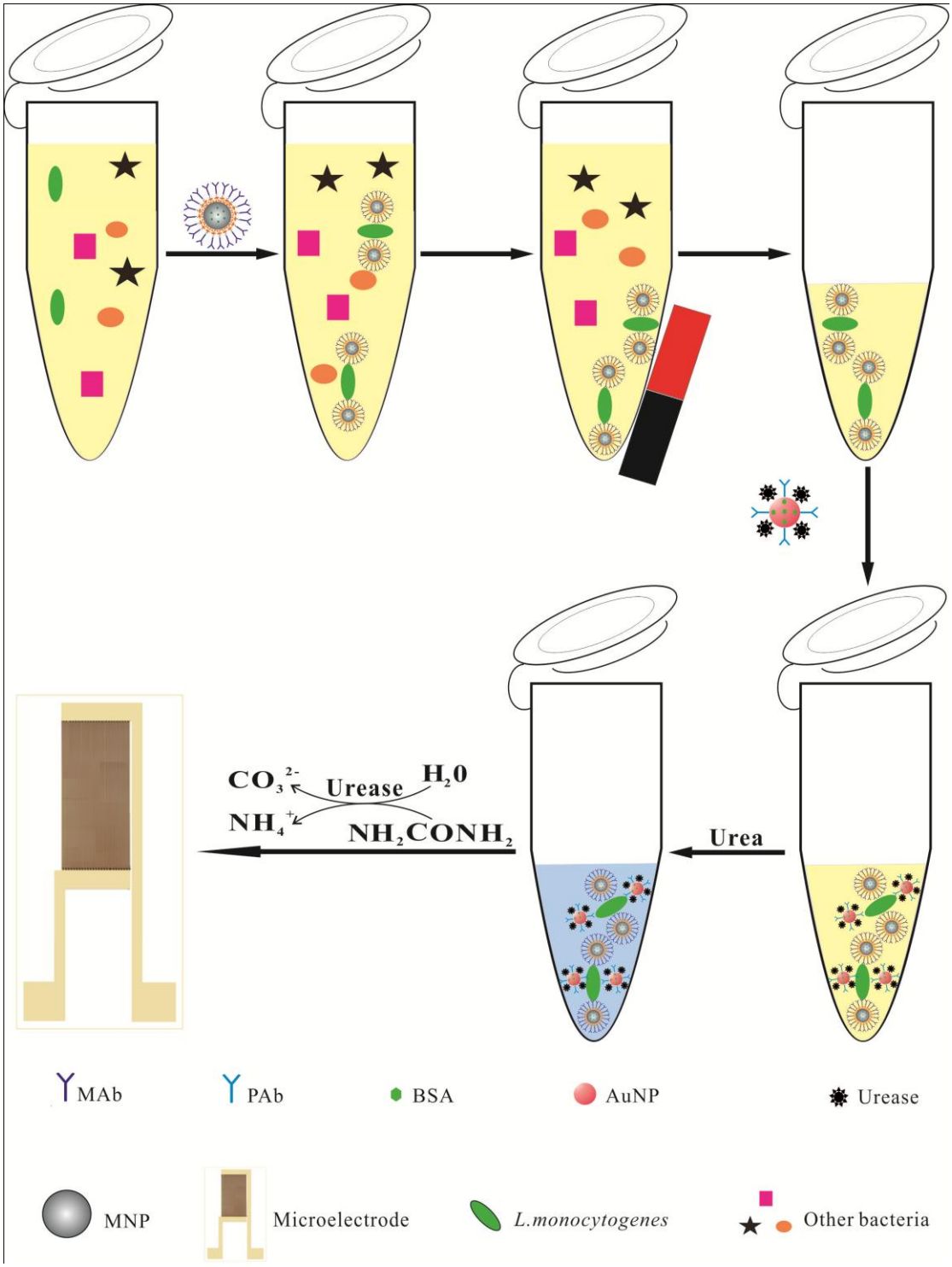
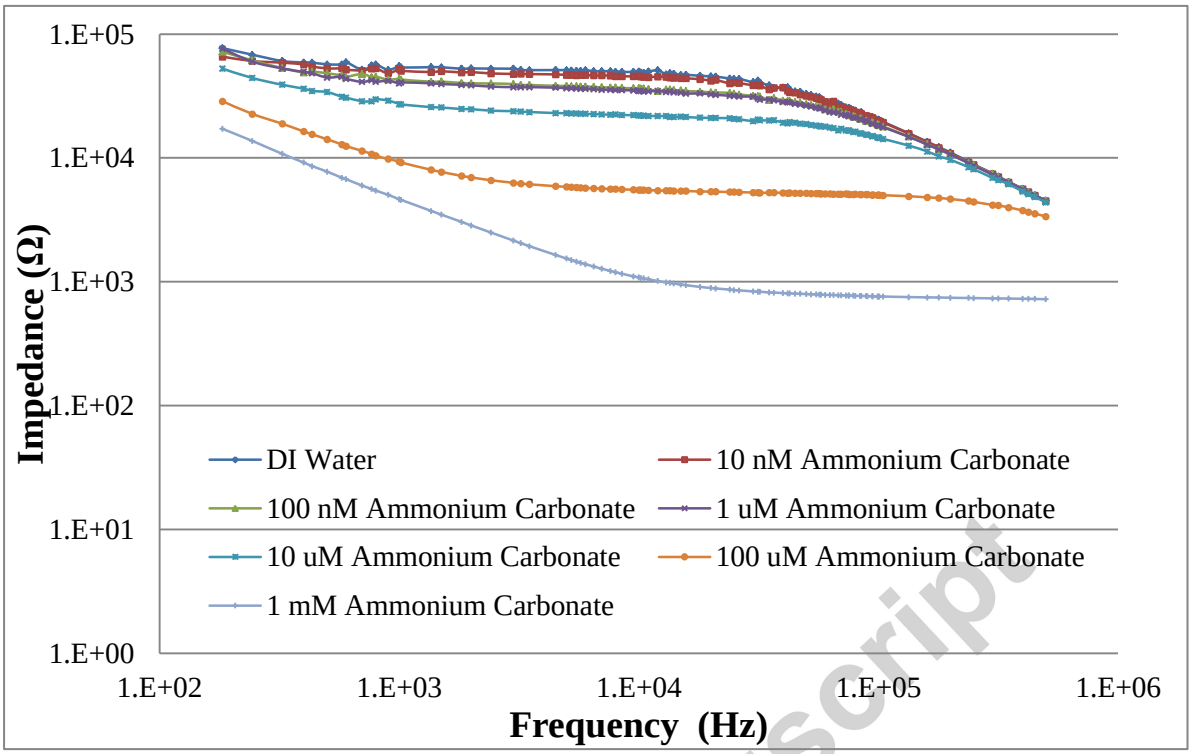


Fig. 1.



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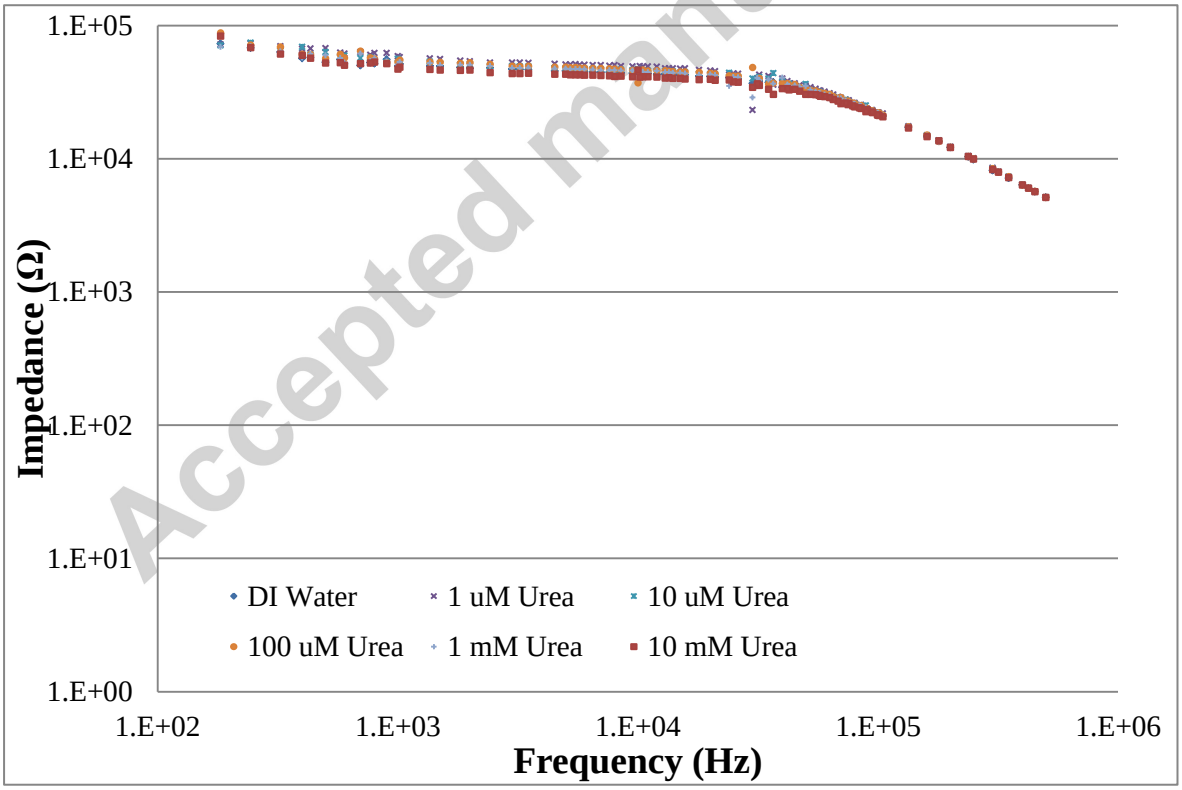

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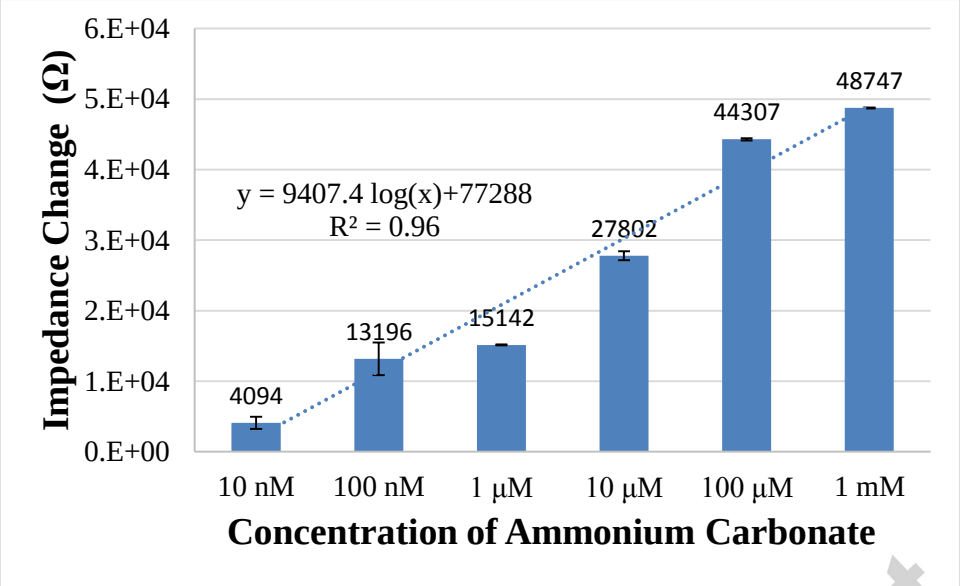

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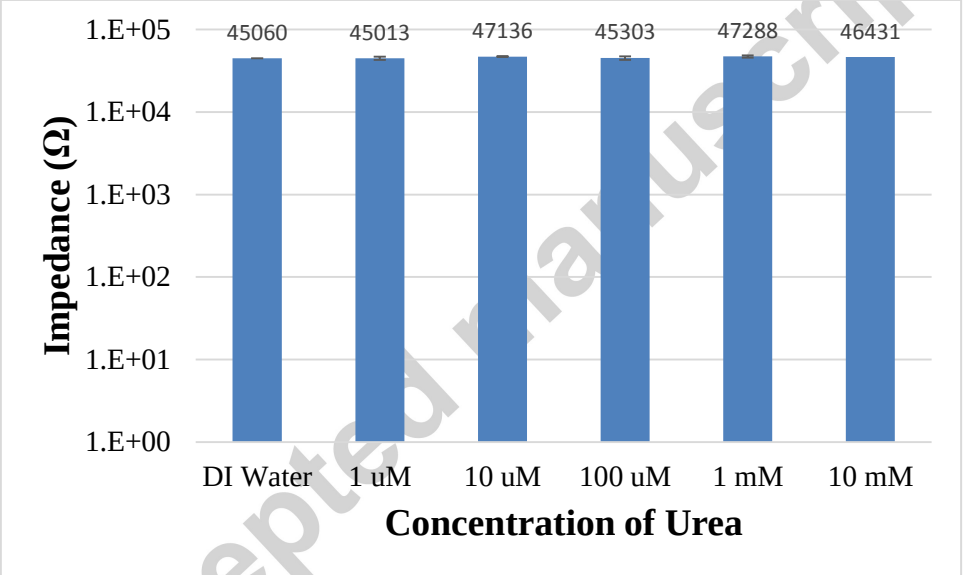

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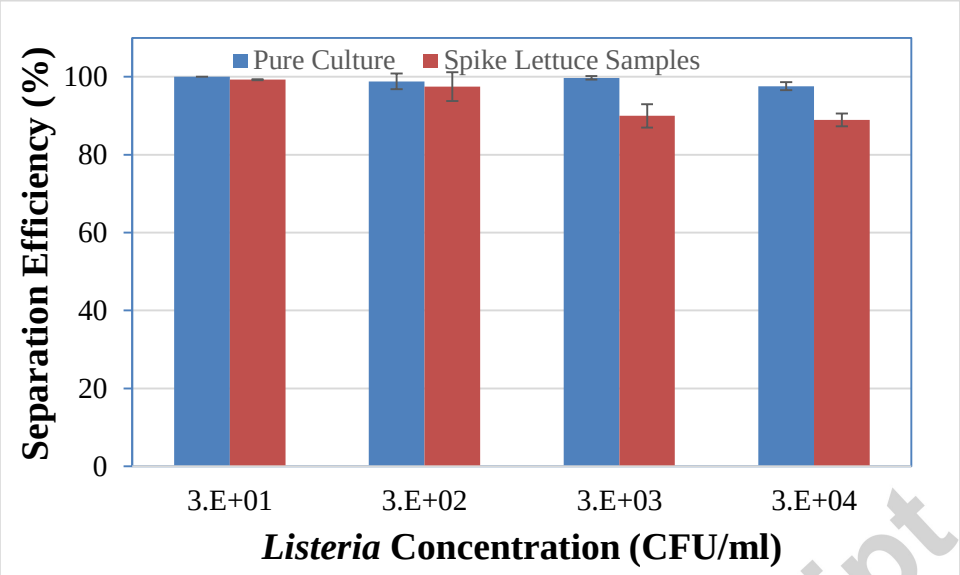

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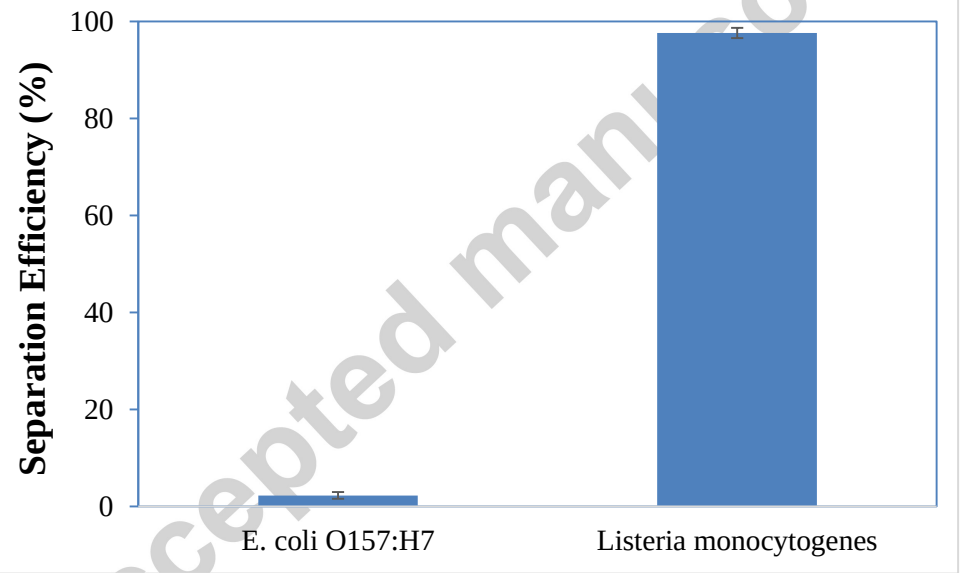

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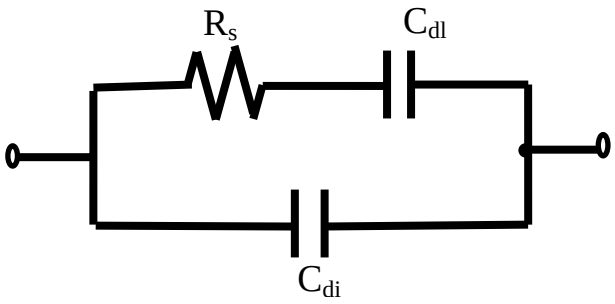
Fig. 3.



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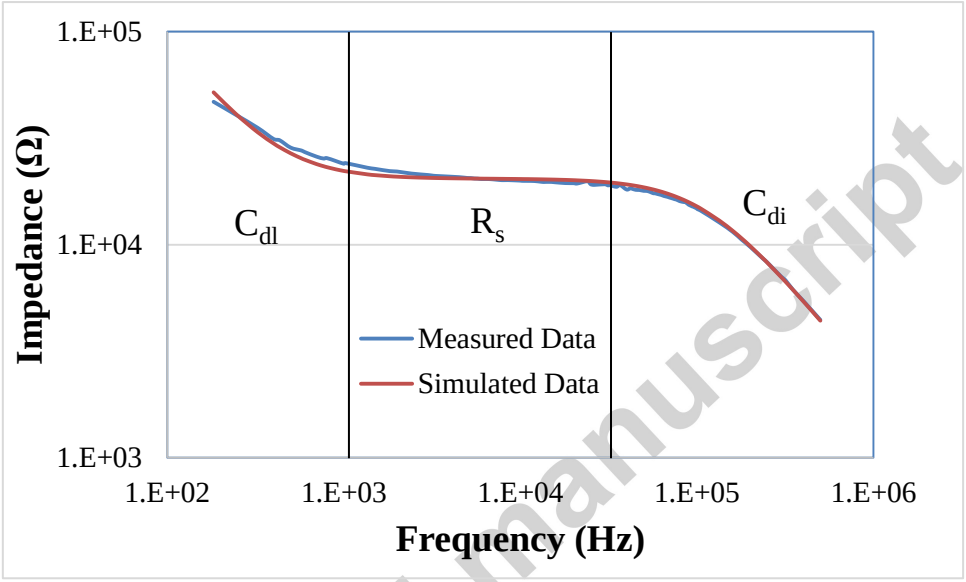

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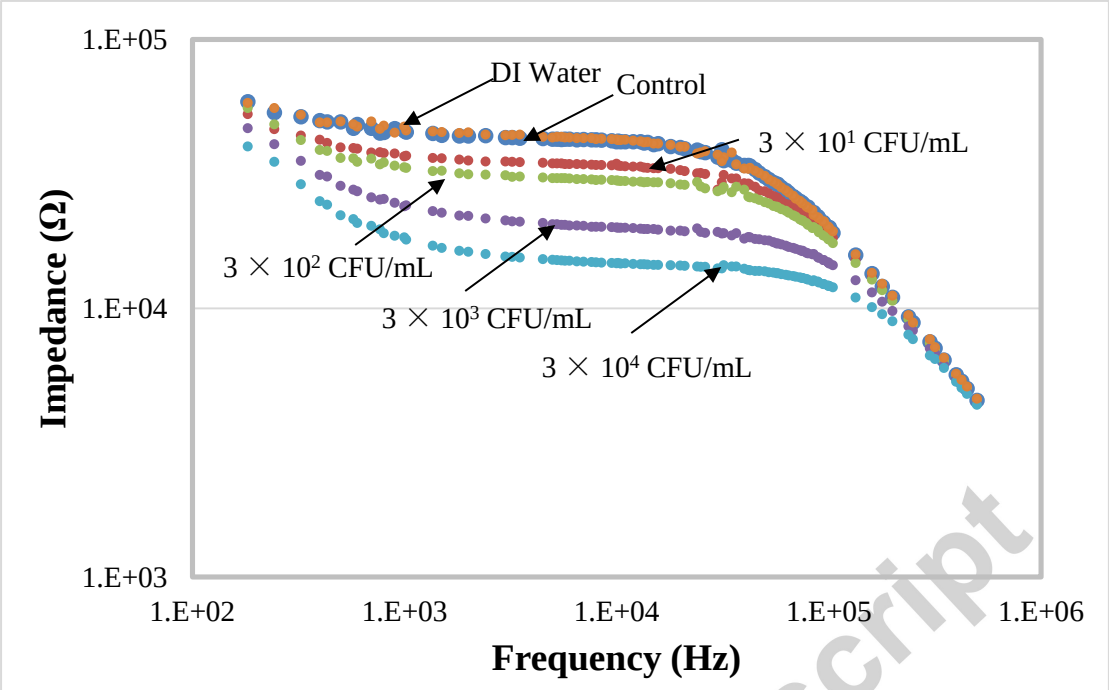
Fig. 4.



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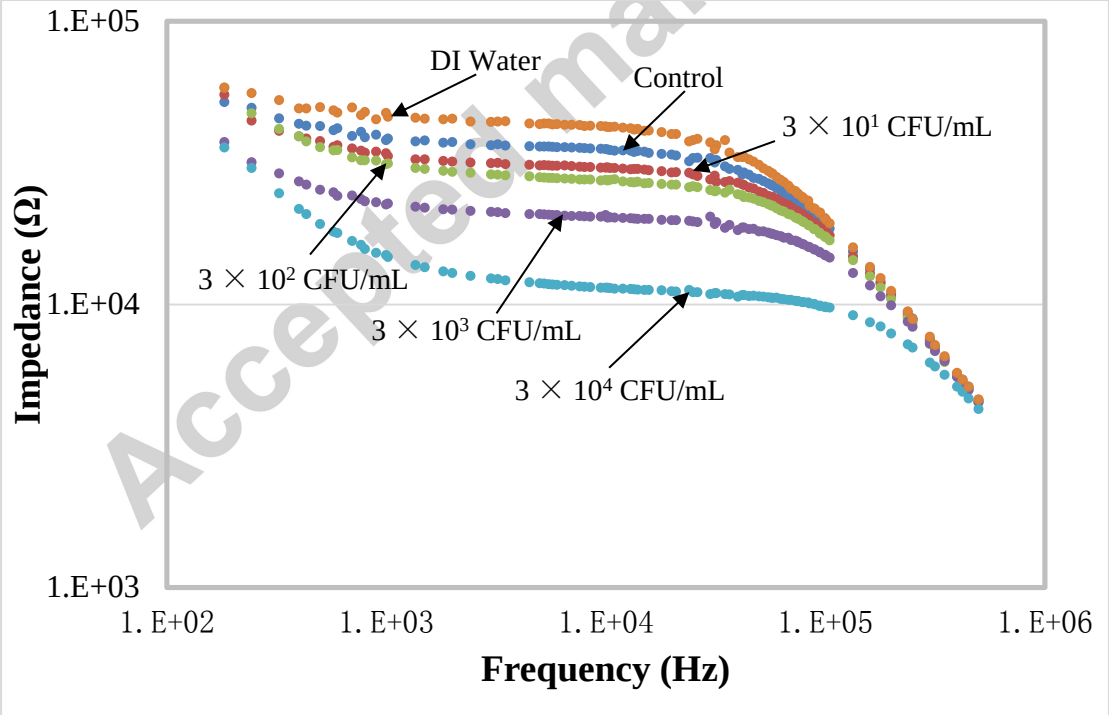

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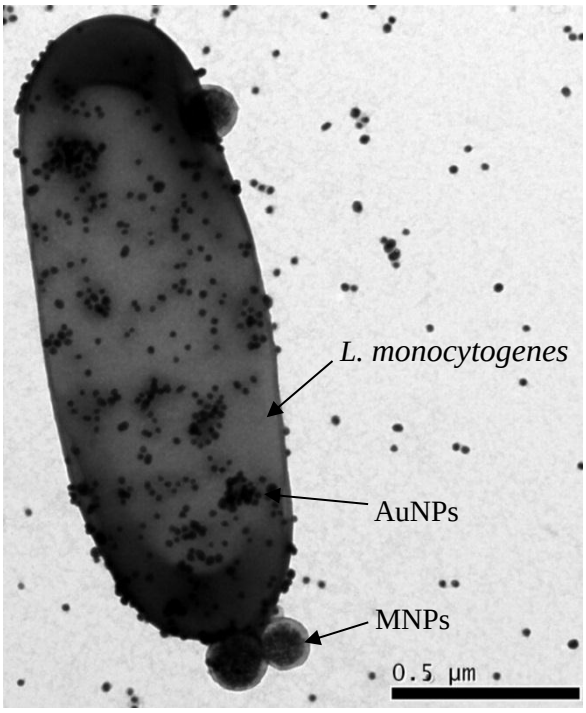

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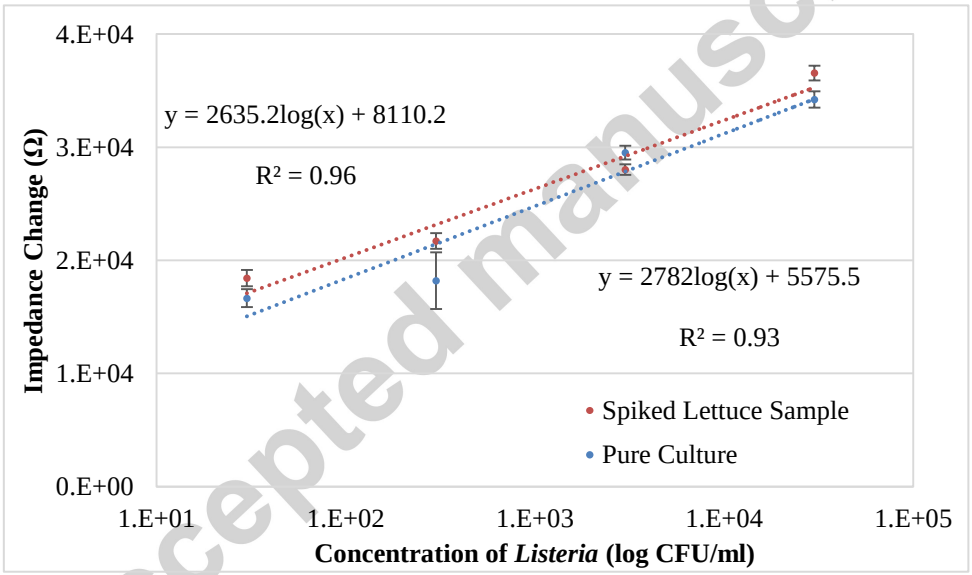

(d)

Fig. 5.

Highlights:

The detection limit of this biosensor was 30 CFU/ml for Listeria monocytogenes.

The microelectrode was free of immobilization and could be reused for > 50 times.

Urease catalysis was successfully used to amplify the detection signal.

The separation efficiency of Listeria was ~90% for pure culture and spiked samples.