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Wireless, Remote-Query, and High Sensitivity *Escherichia coli* O157:H7 Biosensor Based on the Recognition Action of Concanavalin A

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***Escherichia coli* O157:H7 is detected using a remote-query (wireless, passive) magnetoelastic sensor platform to which a 1 μm thick layer of Bayhydrol 110 and then a layer of functionalized mannose is applied. The multivalent binding of lectin concanavalin A (Con A) to the *E. coli* surface O-antigen and mannose favors the strong adhesion of *E. coli* to the mannose-modified magnetoelastic sensor; *E. coli* is rigidly and strongly attached on the mannose-modified sensor through Con A, which works as a bridge to bind *E. coli* to the mannose-modified sensor surface. As *E. coli* is bound to the sensor, its resonance frequency shifts, enabling quantification of *E. coli* concentration with a limit of detection of 60 cells/mL and a linear logarithmic response range of 6.0×10^1 to 6.1×10^9 cells/mL. The analysis can be directly conducted without incubation and completed in 3 h or less.**

Escherichia coli (*E. coli*) O157:H7 is a pathogenic bacterium capable of contaminating foodstuffs causing food poisoning, urinary tract infections, inflammation, and peritonitis.[1] Rapid, accurate methods for bacterial detection are essential with respect to food poisoning as well as water contamination and, therefore, for treatment options. Detection methods developed over the past several decades include DNA and RNA probes,[2,3] piezoelectric quartz crystal techniques,[4] surface plasmon resonance (SPR),[5,6] polymerase chain reaction (PCR)-based analysis,[7,8] flow cytometry methods,[9] immunomagnetic separation (IMS) analysis,[10,11] bulk acoustic wave impedance biosensor,[12] microelectromechanical analysis,[13−15] bioluminescent assay,[16] fluorescent in situ hybridization (FISH),[17] optical tweezers,[18] and nucleic acid sequence-based amplification (NASBA).[19] Most conventional methods are time-consuming, often requiring 1−2 days to obtain results. Although much faster detection methods such as immunosensors or DNA chips are becoming available, they have failed to gain wide acceptance due to the relatively high degree of user expertise required and the high cost of labeling reagents. As a result, a rapid, quantitative, sensitive, and specific method for one-step bacterial detection is highly sought after.

The development and application of wireless, passive magnetoelastic sensors have been reported over the past 10 years.[20−37] Typically, the sensor is a free-standing, ribbon-like magnetoelastic thick film coupled with a chemical or biochemical sensing layer such as an enzyme. In response to an externally applied time-varying magnetic field, the magnetoelastic sensor mechanically vibrates at a characteristic resonance frequency, launching a return magnetic field that can be remotely detected using a pickup coil.[21,22] No physical contact between the sensor and the detection system are required for signal telemetry. Since the sensor is powered by the interrogation signal, no internal power supplies, i.e., a battery, are needed. Various magnetoelastic sensors have been developed including sensors for remote-query monitoring of gastric pH,[22] glucose,[23] organophosphorus pesticides,[33] and microorganisms.[25−27,35−37] A magnetoelastic immunosensor for the detection of *E. coli* O157:H7 was reported[25] through im-

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mobilizing alkaline phosphatase-labeled anti-*E. coli* O157:H7 antibody on the sensor surface, with the mass change associated with the antibody−antigen binding reaction amplified by biocatalytic precipitation of 5-bromo-4-chloro-3-indolyl phosphate. Other magnetoelastic biosensors based on antigen−antibody reaction include one for *Salmonella typhimurium*,[26] and the use of a biosensor array for the simultaneous quantification of multiple bioagents including *E. coli* O157:H7, staphylococcal enterotoxin B, and ricin.[27] Magnetoelastic sensors for the detection of *Pseudomonas aeruginosa* (*P. aeruginosa*)[34] was reported on the basis of the growth curves of bacteria. The reported wireless magnetoelastic microorganism biosensors were based on either the antibody−antigen binding reaction in combination with enzyme amplification[25−27] or the growth curve of the microoganism during the incubation.[34] The antibody−antigen binding reaction cannot be used for the detection of unknown species, and the incubation method is not suitable for rapid detection.

In this Article, a wireless magnetoelastic biosensor for direct and rapid detection of Gram-negative bacteria, *E. coli* O157:H7, without incubation is reported. As the direct adsorption of bacteria on the magnetoelastic sensor results in little response with low sensitivity, sensor sensitivity is enhanced by using concanavalin A (Con A) which increased the binding sites of *E. coli* on the sensor surface.[38] The multivalent binding of Con A to the *E. coli* surface O-antigen sustains the strong adhesion of *E. coli* to the mannose-modified biosensor surface by forming bridges between mannose and the *E. coli* surface O-antigen.[38] The specific binding property of Con A to O-antigen and mannose promotes the specificity of the sensor in response to Gram-negative bacteria. The wireless operation characteristics of the magnetoelastic sensor are of great utility in an aseptic environment for microbial analysis.

EXPERIMENTAL SECTION

Materials and Reagents. Mannose and Con A were purchased from Jie Hui biology Co. (P.R. China). Phosphate, acetone, calcium chloride, and manganese acetate were purchased from commercial sources and used as received. A 400 nM Con A solution in saline was prepared by dissolving the required amount of Con A in a pH 7.2 phosphate buffered saline (PBS) solution. Dimethyl aminopropyl-3-ethylcarbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich and used as received. All chemicals were of analytical grade.

Bayhydrol 110, an anionic dispersion of aliphatic polyester urethane resin in water/*N*-methyl-2-pyrrolidone solution (50%, w/v) was purchased from Bayer Corp. (Pittsburgh, PA). *E. coli* O157:H7 was generously provided by the Department of Life Science of Hunan Normal University (China). The culture medium (CM) is a nutrient broth (NB) prepared by adding 0.5 g of beef extract, 1 g of peptone, and 0.5 g of sodium chloride in 100 mL of distilled water. After the pH was adjusted to 7.2 using sodium hydroxide, the mixture was sterilized in an autoclave at 121 °C for 20 min. A 100 mL aliquot of the NB was seeded with *E. coli* and incubated for 18 h at 37 °C; the broth culture was then stored at 4 °C as a stock solution. The concentration of *E. coli* in the stock solution was determined by plate counts (PC). Distilled and deionized water was used throughout the experiment.

Freestanding magnetoelastic thick films composed of Metglas alloy 2826 ($Fe_{40}Ni_{38}Mo_4B_{18}$) purchased from Honeywell Corp. (Minneapolis, MN) were used throughout this work as the sensor substrate. Each sensor was 22 mm × 6 mm × 28 μm in size. The resonant frequency of an uncoated sensor in air is approximately 102.5 kHz.

Sensor Fabrication. Figure 1 represents the route of the sensor fabrication. The magnetoelastic sensor was ultrasonically cleaned in a Micro Cleaning solution, followed by a water and acetone rinse, and then dried in air. About 2 μL of 40% Bayhydrol 110 (density of 1 g/cm^3) was applied upon the sensor, resulting in a 1 μm thick protective film. The polyurethane-coated sensor was dried in the air and then heated at 150 °C for 2 h to form a robust protective membrane, which offered a NH group for linking the modified mannose and protected the iron-rich magnetoelastic substrate from corrosion.

Mannose was carboxyl functionalized[39] as shown in Figure 1a. Briefly, 0.5 g of mannose, 7.4 mL of 1.35 M sodium monochloracetate, 1 mL of 10 M sodium hydroxide, and 1.6 mL of water were added to a 25 mL flask. After stirring for 7 h at room temperature, 2 mL of 1 M monopotassium phosphate was added into the flask to stop the reaction. The resulting solution was adjusted to pH 5.2 with hydrochloric acid.

A 50 μL aliquot of the carboxyl funcionalized mannose solution (16.67 g/L) containing 0.46 g/L EDC and 0.38 g/L NHS was applied on the polyurethane-coated sensor and dried in air. The mole ratio of NHS and EDC to mannose is 3.2 and 2.9%, respectively. Activated by EDC and NHS, mannose was bonded on the sensor surface through an amide bond between polyurethane and mannose.

Detection of *E. coli*. The as-prepared sensor was vertically and freely placed in a 2.5 mL vial which was stood in a coil

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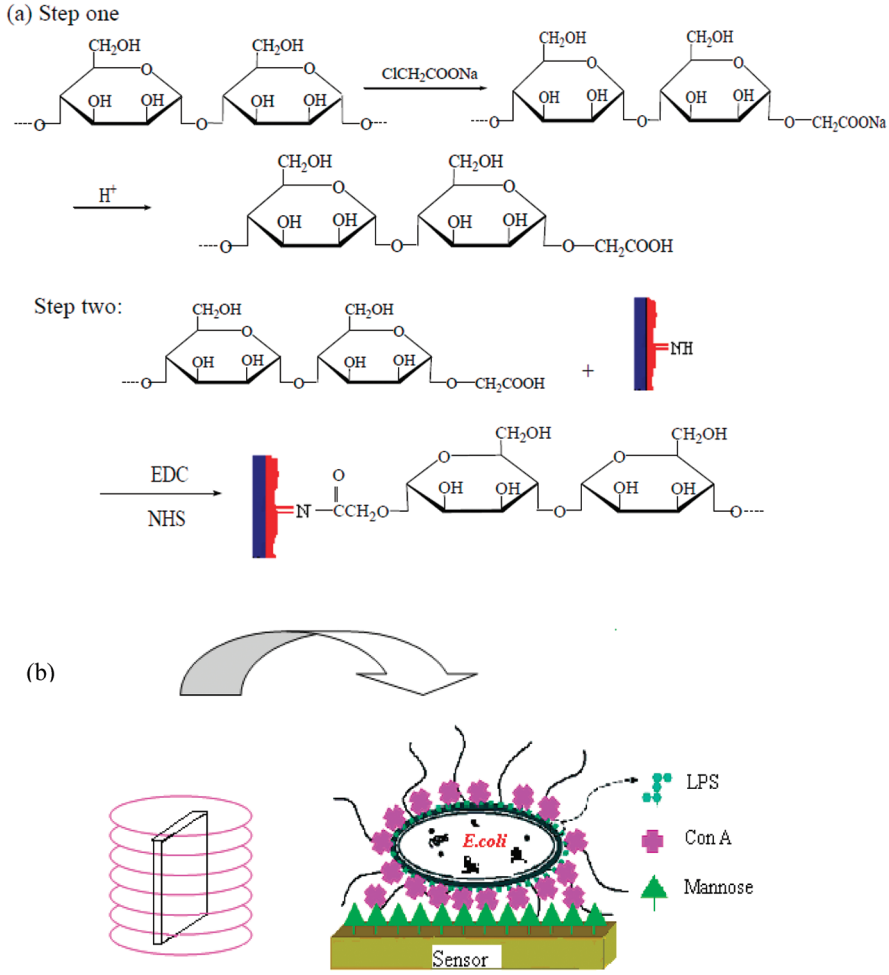

Figure 1. (a) Fabrication sequence of sensor. (b) Illustration depicting the structure of the mannose-magnetoelastic sensor. The sensor is placed within a vial containing the test solution, which is then placed inside a signal telemetry coil. The electronic sensor-reader electronics are interfaced to a computer via an RS232 port for data display and storage.

connected to a magnetoelastic sensor reader. The resonance frequency was monitored by the magnetoelastic sensor reader, using a frequency counting technique of the pulse-wise excited sensor, the details of which can be found in the literature.[22,23] The electronics are interfaced to a computer via an RS232 port to facilitate measurement control and data processing.

Different quantities of *E. coli* were added in the 2.5 mL sterilized vial in which 1.5 mL of CM or water was added. With addition of Con A, the resonance frequency of the sensor was recorded every 1 min. The temperature was kept at 37 ± 1 °C using a thermostatic water jacket.

RESULTS AND DISCUSSION

Sensor Response. Otto et al.[40,41] show that the direct adhesion of *E. coli* onto the mannose immobilized sensor surface is quiet flexible. Structural studies indicate that the major bacterial cell wall component in Gram-negative bacteria such as *E. coli* consists of a class of glycoconjugates called lipopolysaccharides (LPS).[42] LPS are capped by a single O-antigen subunit consisting of glucose, *N*-acetylglucosamine, galactose, and rhamnose.[38] The distinct surface LPS can be recognized by specific lectins. Con A, a multivalent binding protein isolated from Jack bean (*Canavalian ensiformis*), is the most widely used and well-characterized mannose- and glucose-binding lectin. Con A can aggregate on specific terminal carbohydrates of bacterial surface LPS with different binding ability.[38] The multivalent binding of Con A to the *E. coli* surface O-antigen glucose receptor favors the strong adhesion of *E. coli* to mannose immobilized on the sensor as schematically shown in Figure 1.

Figure 2 shows the responses of the sensor modified with or without mannose to the addition of the mixture of *E. coli* and Con A. The frequency shifts of the unmodified sensor in the response to *E. coli* indicate the inherent attaching property of microorganisms on the sensor surface. The modification of the sensor with mannose results in a greater frequency shift as compared with the unmodified sensor, indicating the enhanced adhesion ability of *E. coli* to the mannose immobilized sensor due to the multivalent binding of Con A to the *E. coli* surface O-antigen and mannose. In an 8.3×10^7 cells/mL *E. coli* solution, the Δf of the mannose applied sensor was about 240 Hz, while that of the unmodified sensor was 80 Hz. To further investigate the role of Con A, Con A and *E. coli* were introduced into the test

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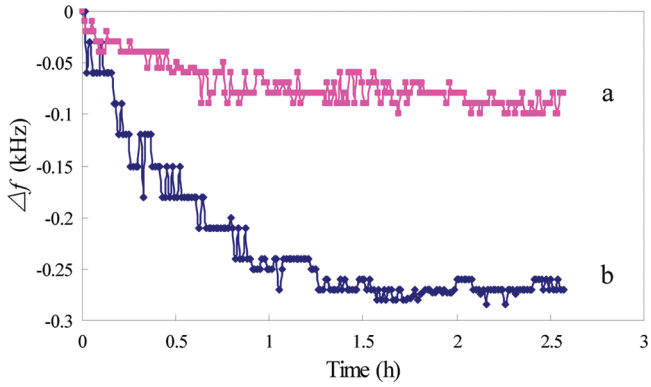

Figure 2. Influence of mannose: (a) mannose was not applied to the sensor and (b) mannose was applied to the sensor. The Con A concentration was 100 nM, with 8.3×10^7 cells/mL *E. coli.*

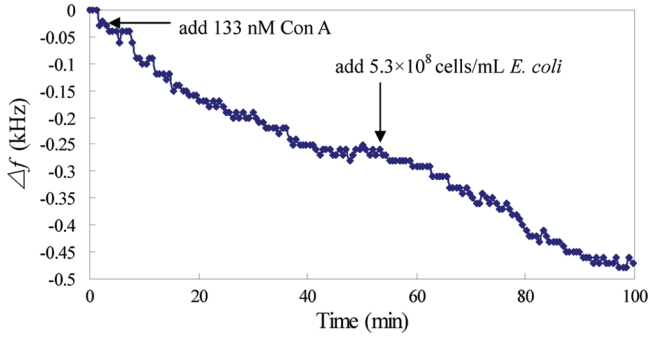

Figure 3. Frequency change vs time when Con A solution and *E. coli* were sequentially added to the mannose sensor. The Con A concentration was 133 nM.

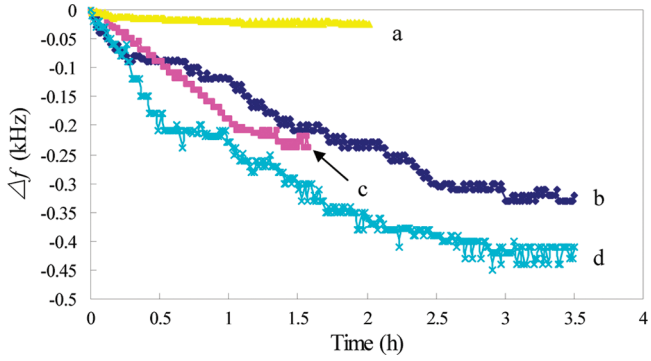

Figure 4. Frequency change vs time with the sensors exposed to different solutions: (a) blank; (b) 9.4×10^9 cells/mL *E. coli*; (c) 100 nM Con A; (d) 9.4×10^9 cells/mL *E. coli* and 100 nM Con A.

solution serially, and the response is shown in Figure 3. The addition of 133 nM Con A results in a frequency shift of 260 Hz within 40 min, and following the addition of 5.31×10^8 cell/mL *E. coli*, the frequency shifts 210 Hz within another 40 min. These results indicate that Con A can bind with both mannose and *E. coli.*

To further confirm the multivalent binding property of Con A, the mannose-modified sensor was exposed to solutions containing Con A or *E. coli*, with the results shown in Figure 4. The sensor responds to either Con A (Figure 4, curve c) or *E. coli* (Figure 4, curve b), with a higher response to *E. coli*, indicating that either Con A or *E. coli* can adsorb on the mannose-modified sensor. While both Con A and *E. coli* are present, the sensor shows the highest response as shown in Figure 4, curve d, confirming the multivalent binding property of Con A, which results in an enhancement of the sensor response.

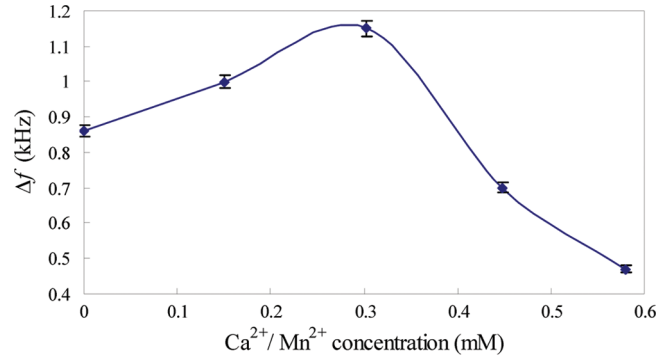

Figure 5. Effect of Ca^{2+} and Mn^{2+}, with 9.7×10^{10} cells/mL *E. coli* and a Con A concentration of 133 nM.

Considering that the in situ growth of *E. coli* may increase the sensor response, the diluted culture medium NB was used as the test solution. The sensor showed a response of 360 Hz in NB solution but 660 Hz in water. The possible reason would be that the organic macromolecules of NB partly blocked the sensor surface, resulting in a decrease in the mass loading of *E. coli*. In addition, the bacterium consumes nutrients, resulting in a decrease in the viscosity, which in turn leads to an increase in the resonance frequency partly offsetting the frequency decrease caused by the bacterium loading.[20] NB was, therefore, not added in the test solution.

Effect of Mn^{2+}/Ca^{2+}. Con A has identical subunits of 237 amino acid residues (MW 26 000). At neutral pH, Con A is predominantly tetrameric with optimal activity.[38] Metal ions Mn^{2+}/Ca^{2+} can bind to Con A; both must be present for carbohydrate binding.[38] As such reactions may affect the binding of Con A to mannose and *E. coli*, the effect of these metal ions on the sensor performances was investigated. Figure 5 shows the sensor responses in the presence of Mn^{2+}/Ca^{2+} at different concentrations with a molar ratio of 1:1. The frequency shifts are increased by 140 and 290 Hz with the addition of 0.15 and 0.303 mM Mn^{2+}/Ca^{2+} (1:1 molar ratio), respectively. This result confirms that Mn^{2+}/Ca^{2+} can promote the binding of Con A to mannose and *E. coli*. However, further increasing the Mn^{2+}/Ca^{2+} concentration to above 0.45 mM results in decreases in the frequency shifts. High Mn^{2+}/Ca^{2+} concentrations are unfavorable to the sensor responses. The possible reason would be that high concentrations of Mn^{2+}/Ca^{2+} could restrain the growth of bacteria and then reduce the sensitivity of the sensor.[43] The optimal concentration of Mn^{2+}/Ca^{2+} is around 0.3 mM.

Detection of *E. coli*. Figure 6 shows the change in resonance frequency versus *E. coli* O157:H7 concentration from 6×10^1 to 6.1×10^9 cells/mL. The resonance frequency decreases with the loading of Con A-bond *E. coli*, in most cases reaching a plateau within 2 h except in the case of 6.1×10^7 cells/mL *E. coli* where 3 h is required for signal saturation. As indicated in Figure 3, the time to reach the plateau is longer than that when only Con A or *E. coli* exists (40 min). It seems that the binding of Con A and *E. coli* onto the sensor surface proceeds in a step by

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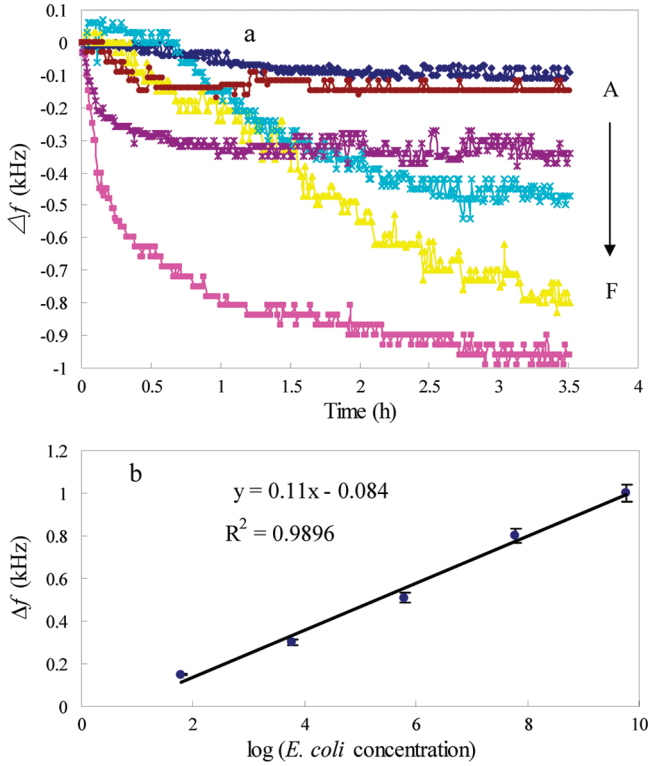

Figure 6. Shift of f_r in response to various concentrations of *E. coli* (a): A, vacant sample; B−F, *E. coli* concentrations of 6.0×10^1, 6.1×10^3, 6.1×10^5, 6.1×10^7, and 6.1×10^9 cells/mL. (b) Calibration curve: 3 h frequency shifts vs log of *E. coli* concentrations. Con A (133 nM) and Mn^{2+}/Ca^{2+} (0.303 mM) were added to the detection solution.

step manner, with *E. coli* following Con A, and as a result more time is required to reach an absorption equilibrium in the presence of both Con A and *E. coli*. The 3 h frequency shift is, therefore, used to quantify *E. coli*, with the calibration curve shown in Figure 6b; the 3 h frequency shift is linearly dependent on the logarithm of cell concentrations ranging from 6.0×10^1 to 6.1×10^9 cells/mL. At 60 cells/mL *E. coli*, the sensor response is distinguishable from the blank response, and the detection limit (LOD) of the mannose-magnetoelastic sensor is, therefore, defined as 60 cells/mL. As for rapid detection of *E. coli*, the LOD of 60 cells/mL is achieved within 3.5 h, comparing favorably with values reported in the literature,[13,35,38,44,45] such as the amperometric detection of *E. coli* based on DNA hybridization and enzyme amplification using microelectromechanical systems with a LOD of 1000 *E. coli* cells,[13] nonlabeled quartz crystal microbalance biosensing detection using carbohydrate and lectin recognitions with a LOD of 7.5×10^2 cells/mL,[38] nanoparticle amplification based quartz crystal microbalance DNA sensing detection with a LOD of 2.67×10^2 cells/mL,[44] and magnetoelastic sensing detection of *E. coli* based on changes in the properties of culture medium during the bacterium growth with a LOD of 200 cells/mL.[35] Using polymerase chain reaction, a LOD of 2 to 3 cells was achieved via a capillary electrophoretic (PCR-CE) technique.[46] The direct detection (without incubation) can be completed within a couple of hours offering rapid detection.

Specificity is another key parameter, in addition to sensitivity and detection time. In biosensing analyses, high specificity is generally achieved by using either antibodies or nucleic acid probes as the detection element, with a large array of antibodies required for identification of unknown agents. Taking advantage of the fact that Gram-negative bacteria such as *E. coli* have distinct surface LPS that can be recognized by specific lectins, such as Con A, the described method can be applied for the detection of Gram-negative bacteria with high specificity and sensitivity.

CONCLUSIONS

A novel wireless biosensor is described, constructed by coating a mass-sensitive magnetoelastic sensor with a mannose polymer upon it for the direct detection of Gram-negative bacteria, *E. coli*. Con A, a multivalent specific binding lectin, was used to increase the binding of *E. coli* on the mannose-coated sensor, and a sensitive response was, therefore, achieved. The multivalent specific binding property of Con A promotes sensor response to any Gram-negative bacteria. As Mn^{2+}/Ca^{2+} can promote the binding of Con A to mannose and *E. coli*, Mn^{2+}/Ca^{2+} were added in the test solution to enhance the sensor response. The highest response was achieved at 0.3 mM Mn^{2+}/Ca^{2+}, with a LOD of 60 cells/mL and an analysis time of 3 h. The proposed sensor shows a linear response in the range from 6.0×10^1 to 6.1×10^9 cells/mL. However, concentrations higher than 0.3 mM of Mn^{2+}/Ca^{2+} resulted in a decrease in the sensor response.

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