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ORIGINAL PAPER

Magnetoelastic biosensor for the detection of *Salmonella typhimurium* in food products

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Abstract In this article, a magnetoelastic sensor immobilized with polyclonal antibody for the detection of *Salmonella typhimurium* in food products is described. The remote query nature of magnetoelastic sensors enables the detection of bacterial species in sealed and opaque containers. Bacterial binding to the antibody on the sensor surfaces changed the resonance parameters, and these changes were quantified by the shift in the sensor's resonance frequency. Response of the sensors to increasing concentrations (5×10^1–5×10^8 cfu/ml) of *S. typhimurium* in three different food products (water, fat-free milk and apple juice) was studied and similar responses were observed. These results were also further ascertained by Scanning Electron Microscopy (SEM) studies. A detection limit of 5×10^3 cfu/ml, with a sensitivity of 139 Hz/decade was obtained for the sensors tested in water samples, as compared to 129 Hz/decade in apple juice and 127 Hz/decade in fat free milk. A $2 \times 0.4 \times 0.015$ mm sensor was employed in all the investigations. The dissociation constant K_d and the binding valencies for *S. typhimurium* spiked in water samples was 435 cfu/ml and 2.33 respectively; as compared to 309 cfu/ml and 2.38 for apple juice; and 1389 cfu/ml and 1.85 for fat free milk samples. Bacterial binding was specific and a divalent binding was observed.

Keywords Magnetoelastic · Biosensor · Antibody · *Salmonella typhimurium* · Langmuir-Blodgett

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Introduction

The ability to determine the occurrence of food contamination due to foodborne pathogens at every stage of food production, processing, and distribution is crucial to improving the safety of our food supply. There are more than 250 known food borne diseases caused by bacterial and viral infections in the United States. Annually, these foodborne diseases result in an estimated 76 million illnesses, 325,000 hospitalizations, 5,000 deaths, and 6 billions dollars [1] in unneeded expenditure. Bacterial contamination accounts for 91% of total foodborne diseases [2, 3]. *Salmonella* sp., *Escherichia coli, Listeria monocytogenes*, *Staphylococcus aureus, Campylobacter jejuni, Campylobacter coli and Bacillus cereus* were found to be main source of bacterial contaminations in our food supply [4]. Reports of illnesses due to bacterial (*Salmonella sp.*) contaminations of different food products such as, pasteurized milk, [5–7] tomatoes, [8] eggs, [9, 10] orange juice, [11] milk, [6, 12–14] chicken, [15–18] ground beef [19] and cheddar cheese [20] are well documented.

Today, a plethora of detection techniques exist to detect foodborne pathogens [21]. Traditional methods such as enzyme linked immunosorbent assay (ELISA) [22] and polymerase chain reaction (PCR) [23] are time consuming, as well as impractical for in-situ real-time measurement and in vivo biological monitoring.

Existing biosensor technologies such as surface acoustic wave devices, [24, 25] optical [26] and thickness-shear mode resonators [27] offer sensitivity, but are limited by their need for direct physical contacts. For example quartz crystal microbalance (QCM) needs direct physical contacts (i.e. electrical wiring) to obtain sensor response which prohibit their usage in the sealed containers. Magnetoelastic sensors don't need direct electrical contacts for the actuation and data acquisition; this makes magnetoelastic sensors suitable for the remote detection of pathogens. The current investigation mainly focuses on the development of a biosensor that addresses these limitations through remote and real-time sensing of pathogens in food products.

Magnetoelastic materials are amorphous ferromagnetic alloys that usually include a combination of iron, nickel, molybdenum and boron. A magnetoelastic material exhibits a physical resonance when it is subjected to a time varying magnetic field. This resonance can be monitored by using a pickup coil without the need for direct physical connections. Because of this unique advantage, numerous magnetoelastic materials applications have been proposed, including the detection of calcium oxalate and brushite; [28] ricin, [29] urea [30] and endotoxin [31].

This paper presents the results of an investigation using a biosensor consisting of a magnetoelastic platform immobilized with a polyclonal antibody (serving as the bio-molecular recognition element) for the detection of *Salmonella typhimurium* in food products such as water, fat-free milk, and apple juice.

Materials and methods

Sensor platform

METGLAS® 2826MB alloy, obtained from Honeywell International (Conway, SC), was used as the sensor platform material. The composition is $Fe_{40}Ni_{38}Mo_4B_{18}$ and its theoretical value of the saturation magnetostriction is 12 ppm [32]. Initially, sections of the as-received, magnetoelastic ribbon material were mechanically polished using standard metallographic polishing techniques to reduce the thickness from 30 to 15 μm, thereby decreasing the initial mass. Following this, the individual sensor platforms were made using an auto-controlled, micro-dicing saw. The diced sensor platforms were then ultrasonically cleaned in methanol to remove any organic film and other debris left by the dicing process. To improve the environmental stability, as well as bioactivity, of the biosensors, thin layers of chromium and gold were coated onto the surfaces of the magnetoelastic particles using a Denton™ (Moorestown, NJ) high vacuum RF sputtering system.

Antibodies and *Salmonella typhimurium* cultures

Rabbit polyclonal antibody (1mg/ml) to *Salmonella.* sp was purchased from Abcam Inc (Cambridge, MA) and immobilized onto magnetoelastic biosensors using the Langmuir–Blodgett (LB) film technique. The functional performance of the biosensors was evaluated with bacterial suspensions. *S. typhimurium* (ATCC 13311) obtained from the American Type Culture Collection (Rockville, MD) was confirmed for identity and propagated in the Department of Biological Sciences at Auburn University. The suspension was serially diluted with water to prepare bacterial suspensions ranging from 5×10^1–5×10^8 cfu/ml. All test solutions were prepared the same day as biosensor testing and maintained at 4°C. Apple juice and fat-free milk were purchased from a local grocery store in Auburn, AL. The apple juice was the Kroger® store brand and the fat-free milk was the Parmalat® brand.

Monolayer deposition

The Langmuir Blodgett (LB) technique was used for antibody immobilization on the magnetoelastic sensors. Antibody monolayers were carried out using a LB film balance KSV 2200 LB, (KSV Chemicals, Finland). This system mainly consists of a Wilhelmy-type surface balance (Sensitivity range 0–100 mN/m), a teflon trough of dimensions 45×15 cm^2, and a teflon barrier (0–200 mm/min) driven by a variable speed motor, all of which are encased in a laminar flow hood. To minimize variations that may arise due to vibrations, the trough is mounted on a marble table and aided by interposing rubber shock absorbers. The subphase temperature (20 ± 0.1°C) control was achieved by circulating water through a quartz tube coil at the bottom of the trough. The magnetoelastic sensors were submerged into the deionized water (DI H_2O) in the LB trough. A monolayer from the antibody suspension was formed by allowing the spreading of 100 μl antibody solution (1 mg/ml) to

run down a wetted glass rod that was partially submerged into the subphase (deionized water). When the antibody solution reaches the air-water interface, it splits into a monolayer due to surface forces [33]. After spreading, the monolayer was allowed to equilibrate and stabilize for 10 min at 20°C.

Monolayers were then compressed using a computer controlled compression barrier at a rate of 30 mm/min, until the pressure reached 22 ± 0.05 mN/m. The pressure was then held constant and vertical antibody film deposition was carried out at a rate of 4.5 mm/min. Multiple monolayers of antibody were obtained by successive dipping of the sensors through the monomolecular film deposited at a water-air interface. Seven monolayers containing antibodies were transferred onto the magnetoelastic sensor surface. Only one surface of the magnetoelastic sensor was coated with the antibody.

Principle of operation

A schematic drawing illustrating the wireless nature of the magnetoelastic resonance biosensor and basic principle for detecting bacterial cells is shown in Fig. 1. The frequency spectrum of the sensor was obtained by sweeping an AC magnetic interrogation field over a pre-determined frequency range while monitoring the response of the sensor using a pickup coil. At the resonance frequency of the sensor, the conversion of the magnetic energy into elastic energy reaches a maximum and the sensor undergoes a magnetoelastic resonance. For a thin, ribbon-shaped sensor of length L vibrating in its basal plane the fundamental resonant frequency of the longitudinal vibrations is given by [34].

$$f = \sqrt{\frac{E}{\rho(1-\sigma^2)}}\frac{1}{2L} \tag{1}$$

where E denotes modulus of elasticity, σ is the Poisson's ratio, ρ is the density of the sensor material, and L is the longitudinal dimension of the sensor. Due to the shape of the ribbon-like sensor the magnetic permeability is greatest along its length; hence an incident magnetic field generates longitudinal vibrations in the sensor from almost any orientation except normal to the basal plane of the sensor.

When the test temperature and humidity are constant, the resonant frequency change of the magnetoelastic sensor depends only on the mass change (Δm) on its surface. If the mass increase is small compared to the mass of the sensor the shift in the resonant frequency is given by: [35]

$$\Delta f = -\frac{f}{2}\cdot\frac{\Delta m}{M} \tag{2}$$

where f is the initial resonance frequency, M is the initial mass, Δm is the mass change, and Δf is the shift in the resonant frequency of the sensor.

Equation (2) shows that the resonance frequency shifts linearly, decreasing with increasing mass on the sensor surface. Hence binding of the target organism onto the biosensor surface causes a mass increase with a corresponding decrease in fundamental resonance frequency.

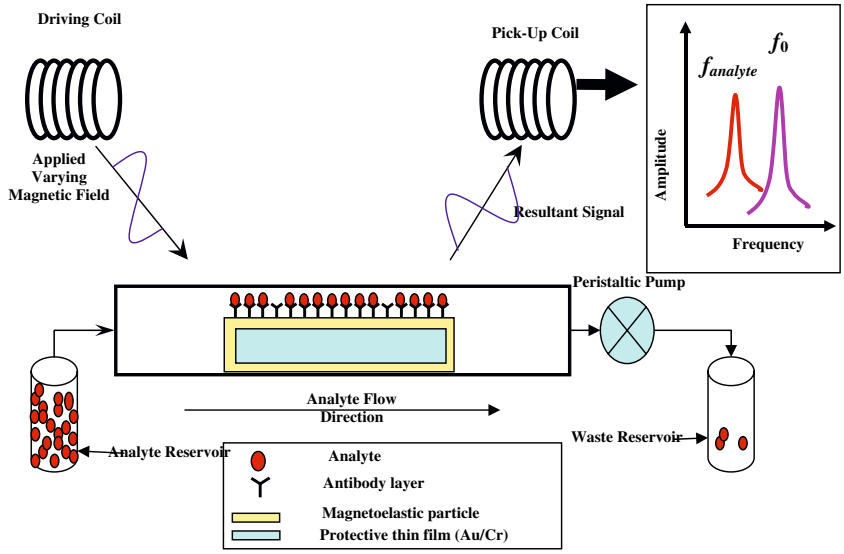

Fig. 1 Schematic drawing illustrating the wireless nature of the magnetoelastic biosensors and the basic principle for detecting bacterial cells. The fundamental resonant frequency of the biosensor is f_0 without antigen binding, which shifts (decreases) to $f_{analyte}$ due to the increased mass of antigen binding to antibody immobilized on the sensor surface

Bacterial binding measurements

After the preparation of biosensors, they were exposed to increasing concentrations (5×10^1 cfu/ml–5×10^8 cfu/ml) of *S. typhimurium* spiked in different media (distilled water, fat-free milk and apple juice) at a flow rate of 100 µl/min. A peristaltic pump (Ismatec Reglo Digital peristaltic pump) was used to control the flow rate of the media containing the pathogens. The resonance frequency of the sensors was measured using an HP network analyzer 8751A with S-parameter test set both before and after the binding of bacterial cells to the immobilized antibody on the sensor. About of 801 points were recorded over the frequency range with an 11.31 s sweep time. A standard open circuit calibration was used to minimize experimental errors in the test set up. A personal computer was used to acquire data at 2 min intervals. Each data point (Fig. 3) represents the mean values obtained from five independent sensors, subjected to study under identical conditions.

Hill plot and determination of dissociation of binding

The association and dissociation of an antibody and antigen in solution can be expressed by:

$$n\mathrm{St} + \mathrm{Ab} \underset{K_d}{\overset{K_a}{\rightleftharpoons}} \mathrm{St}_n\mathrm{Ab} \tag{3}$$

where, St represents *S. typhimurium* in solution that comes in contact with the sensor, Ab represents the immobilized antibody on the sensor in solution and St_n Ab is the bacteria-antibody complex, and k_a and k_d are the association and dissociation rate constants, respectively. The equilibrium constant or, the affinity (K), is given by:

$$K_\mathrm{d} = \frac{1}{K_\mathrm{a}} = \frac{[\mathrm{St}]^n[\mathrm{Ab}]}{[\mathrm{St}_n\mathrm{Ab}]} \tag{4}$$

The dissociation of the reaction is chiefly governed by the strength of the bonding between the bacterial cells in solution and the immobilized antibody on the sensor. This is further dependent on the type of immobilization technique used. Stronger antibody-bacterial binding is indicated by lower K_d values, which in turn implies higher sensitivity of the biosensor [36].

The dissociation constant (K_d) and Hill coefficient (n) were calculated using the Hill plot. The degree of binding can be estimated using the Hill coefficient (n) which is the slope of the Hill plot [37]. Association constant (K_a) can be estimated from the ordinate intercept and its reciprocal gives the dissociation constant (K_d). The Hill plot can be constructed by plotting log θ versus log (C) where C is the concentration of *S. typhimurium* and θ is given by the equation:

$$\theta = \frac{Y}{1-Y} \tag{5}$$

where $Y = \Delta f/\Delta f_{\max}$ and Δf denotes the shift in frequency obtained after bacterial binding to the antibody immobilized sensor and $\Delta f_{\max}$ was considered as the maximum frequency shift response that can be measured before the sensor reaches saturation (i.e. saturation point) [38]. This was obtained from the sigmoid curve fitting to the sensor response data.

Microscopic analysis of sensors

Confirmation of antibody–bacteria binding at the sensor surface was conducted by Scanning Electron Microscopy (SEM). Previously assayed biosensors were exposed to osmium tetraoxide (OsO_4) vapor for one hour to fix the bacterial cell wall in order to facilitate SEM observations. Then, sensors were mounted onto aluminum stages with carbon adhesive tape followed by gold (Au) sputtering (60 nm thickness) at 0.08 mbar Argon (Ar) gas pressure to obtain a conductive surface for SEM imaging. The sensors were examined using a JEOL-7000F SEM, operating at 5 kV. The physical distribution and density of the bacterial cells attached to the sensor surface were examined using these SEM procedures as described [39].

Results and discussion

Response curves

Figure 2 depicts the dynamic response of the magnetoelastic biosensor, when exposed to increasing concentrations (5×10^1 cfu/ml to 5×10^8 cfu/ml) of *S. typhimurium* suspensions in water. A peristaltic pump was used to flow the bacterial suspension at a constant flow rate of 100 µl/min for 10 min allowing 1 ml of each concentration to pass over the sensor. To simulate real world conditions, the bacterial suspensions were not recycled. It was found out from the experiments that sensors have shown a steady state response after 10 min. So the response at 10 min was taken as the resultant frequency shift for these experiments.

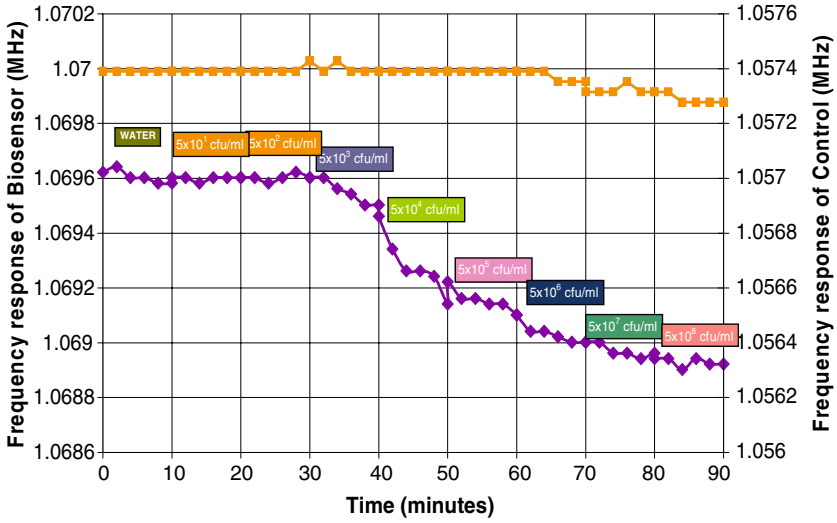

Fig. 2 Magnetoelastic biosensor (♦ – left hand side scale on the *y*-axis) and Control sensor (■ – right hand side scale on the *y*-axis) dynamic response when exposed to different concentrations (5×10^1 through 5×10^8 cfu/ml) of *S. typhimurium* suspensions in water. Water with no bacteria was used as the reference. Data was recorded at two minute intervals. Each concentration of bacterial suspension was run for 10 min at a flow rate of 100 µl/min

Dose response

Figure 3 shows the comparative responses of $2 \times 0.4 \times 0.015$ mm sensors, when exposed to increasing concentrations of bacteria suspended in water, fat-free milk, and apple juice. As can be seen, the shift in the resonance frequency of all the sensors shows a similar trend in the different foods. The first detectable response due to the presence of bacteria occurs at a concentration of 5×10^3 cfu/ml. Dose response is linear over five decades of bacterial concentrations. The sensor sensitivity was measured as a slope of the linear portion of the dose response, is 139 Hz/decade ($R > 0.99$, $P < 0.001$) for the samples tested in water, 127 Hz/decade ($R > 0.99$, $P < 0.0001$) and 129 Hz/decade ($R > 0.99$, $P < 0.0001$) in fat free milk and apple juice respectively. When the control sensor exposed to increasing concentration of *S. typhimurium*, a change in the resonance frequency (~50 Hz) was seen at very high concentration of 5×10^6 cfu/ml. This small change in the frequency can be associated to the very little non specific binding of bacterial cells to the sensor surface.

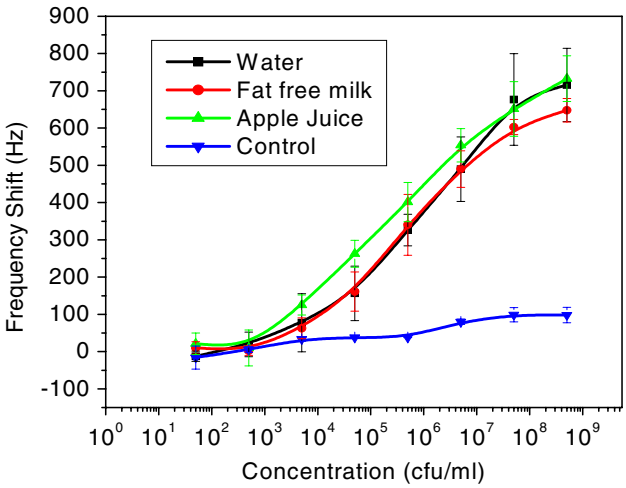

Fig. 3 Magnetoelastic biosensor's responses, when exposed to increasing concentrations (5×10^1–5×10^8 cfu/ml) of *S. typhimurium* suspensions in water (■ – $\chi^2 = 0.048$, $R^2 = 0.99$), fat free milk (• – $\chi^2 = 0.32$, $R^2 = 0.99$), and apple juice (△ – $\chi^2 = 0.26$, $R^2 = 0.99$). Control (▼ – χ^2=5.22, $R^2 = 0.72$), represents the uncoated (devoid of antibody) sensor's response. The curves represent the sigmoid fit of signals obtained. Experimental values were obtained by averaging values obtained from five individual experiments carried out under identical conditions

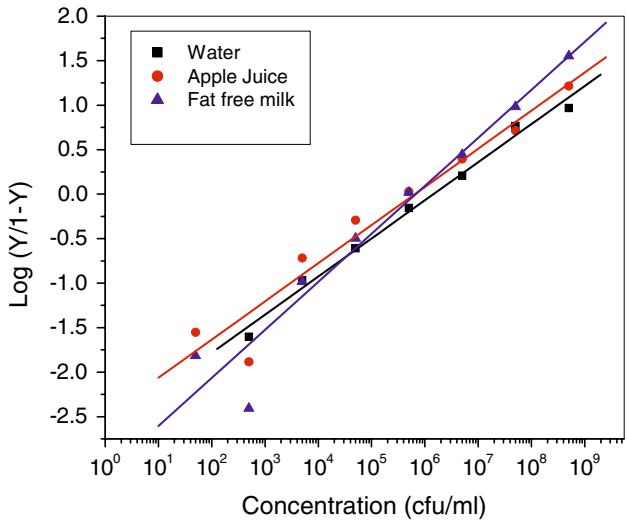

Fig. 4 Hill plots of binding isotherms showing the ratio of occupied and free antibody sites as a function of bacterial concentrations spiked in different food samples. The straight line is the linear least squares fit to the data. (Water: slope = 0.43 ± 0.02, $R = 0.99$; Apple juice: slope = 0.42 ± 0.04, $R = 0.97$; Fat-free milk: slope = 0.54 ± 0.05, $R = 0.97$)

Table 1 The dissociation constants and binding valencies of magnetoelastic sensor in different liquid media

	Hill coefficient (n)	Binding valency ($1/n$)	K_d (cfu/ml)
Water	0.43	2.33	435
Apple Juice	0.42	2.38	309
Fat free milk	0.54	1.85	1389

From the dose responses obtained, the Hill plot (shown in Fig. 4) was derived using the principles explained in the experimental procedure section. The dissociation constant, K_d and the binding valencies were calculated. Table 1 summarizes the Hill plot results obtained for the sensors when exposed to water, fat-free milk, and apple juice. All the tests conducted with different solutions showed a multi-valent binding, indicating that the bacteria in solution are binding to the immobilized antibody at more than one site. As compared to biosensors tested in water and apple juice samples, biosensors tested in fat free milk had a higher K_d value and smaller binding valency, which is also in agreement with the frequency shift data from the dose response curves. The lesser amount of binding in the fat free milk can be due to lesser number of antibody sites available for the bacteria binding caused by proteins in the fat free milk blocking few of the antibody sites.

SEM observations

In order to confirm the frequency shifts caused by binding of *S. typhimurium* to the sensor with immobilized antibody, SEM micrographs were taken for all the samples after binding of *S. typhimurium*. Figure 5 shows typical SEM micrographs of the biosensor surface after exposure to *S. typhimurium* suspensions in various media. The control sensor showed only minimal capture when exposed to higher concentrations of bacteria (5×10^8 cfu/ml). Results from SEM study confirm that the observed frequency shifts are the result of capture of *S. typhimurium* cells by the immobilized antibody. The change in resonance frequency generated by the sensors before and after being exposed to bacterial suspensions can be attributed to the increase in mass of the bound analyte (*S. typhimurium*) on the sensor surface. Ten different regions of each sensor surface were examined and photographed using SEM. Sensor surface bacterial densities of 0.105, 0.075 and 0.105 cells/μm^2 were observed on the

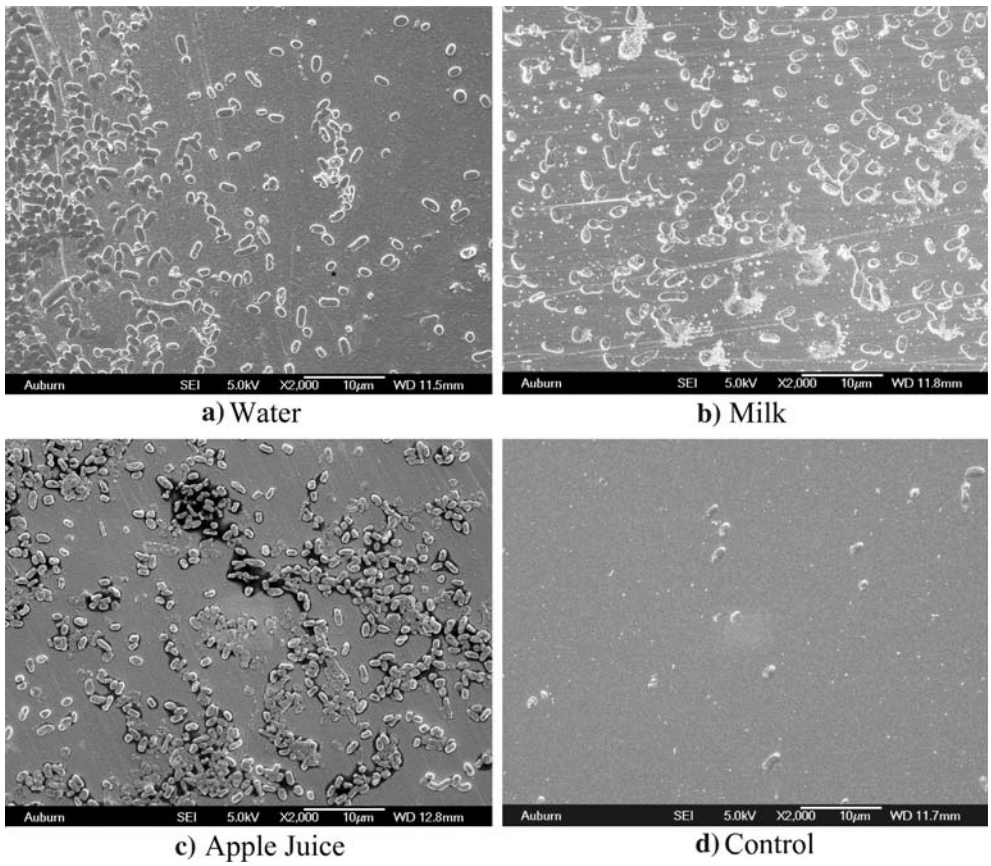

Fig. 5 Typical SEM images of *S. typhimurium* bound to an antibody immobilized magnetoelastic sensor surface. *S. typhimurium* suspensions (5×10^8 cfu/ml) in (**a**) water, (**b**) fat-free milk (**c**) apple juice and (**d**) control (biosensor devoid of antibody and treated with 5×10^8 cfu/ml of bacterial sample)

Table 2 Comparison of bacterial cells counted from SEM images and theoretically expected number of cells calculated from equation (2) according to the measured frequency shifts

	Number of cells from frequency shifts	Number of cells from SEM	% difference in cell counts
Water	64954	84424	29.97
Fat free milk	57681	60293	4.53
Apple juice	66816	84098	25.87

samples which were exposed to *S. typhimurium* suspensions in water, fat-free milk, and apple juice respectively at the highest bacterial concentrations. Table 2 summarizes a comparison between the number of bacterial cells counted from the SEM images and that calculated from the measured frequency shifts. From Table 2 and SEM pictures it is evident that lesser number of bacterial cells were bound to the biosensor in fat free milk samples as compared other media (water and apple juice). This decrease in bacterial binding is proportional to the decrease in the frequency shift response of the sensor. Generally there is good agreement between the two methods of determining the number of cells bound to the sensor surface. The number of cells as determined from SEM measurements is an extrapolation of an average of 10 different regions on the exposed sensors. Variations in uniformity of binding from region to region and the small area viewed in making the analysis lead to an overestimation of up to 29% in the number of SEM counted cells.

Conclusions

The application of polyclonal antibody immobilized magnetoelastic biosensors for the detection of *S. typhimurium* in food products (water, fat-free milk and apple juice) was successfully established. A detection limit of 5×10^3 cfu/ml was obtained for a $2 \times 0.4 \times 0.015$ mm size sensor in all the three different food samples employed in this study. With a response time of approximately 2 min, these sensors show faster response times as compared to acoustic wave devices [40–42]. SEM studies confirmed that the measured frequency shifts were due to the attachment of bacteria to the sensor surface. Similar bacterial capture densities on antibody immobilized sensors for the different test samples show that the performance of the sensor is not affected by the different media, and hence can be readily used for the detection of *S. typhimurium* in various food products.

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