ISSN : 0974 - 7435 Volume 10 Issue 24

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(24), 2014 [16430-16433]

Specificity of polyclonal antibody-based magnetostrictive biosensors

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ABSTRACT

Magnetoelastic (ME) biosensors were developed by immobilizing polyclonal antibody using Langmuir-Blodgett (LB) technique onto a magnetostrictive platform, which can offer wireless and remote detecting, a unique advantage over conventional sensor platforms. Due to the magnetoelastic nature of the amorphous magnetostrictive alloy, the sensor exhibits a physical resonance when it undergoes a time-varying magnetic field, a shift in resonance frequency occurs when its mass changes due to bacteria binding, and the bound bacterial cells were visibly confirmed by scanning electron microscopy (SEM) micrographs, then the density of bacteria bound on the biosensor was calculated. The specificity to detect different kinds of bacteria, including *Salmonella* species and non-*Salmonella* species was studied. The results show that the prepared polyclonal antibody-based biosensor shows excellent specificity to detect bacteria of *Salmonella* species, and especially prefers to bind *Salmonella typhimurium*, almost zero affinity for non-*Salmonella* species of *Listeria monocytogenes*.

KEYWORDS

Magnetostrictive; Biosensor; Antibody; *Salmonella*; Specificity.

INTRODUCTION

Magnetostrictive materials are made of amorphous ferromagnetic alloys composed of Iron, Nickel, Molybdenum and Boron. Due to the magnetoelastic nature of the amorphous alloy, the sensor exhibits a physical resonance when exposed to a longitudinal time-varying magnetic field and a DC biasing field. By employing a suitable bioprobe for detecting pathogens, a biosensor can be developed. This characteristic allows the biosensors to be monitored remotely without the use of direct physical connections, such as wires, enabling detection of environmental parameters from within sealed, opaque containers[1-3].

Antibodies are immune system-related proteins called immunoglobulins, which can selectively bind target antigen due to their special structure and different amino acid sequence[2-4]; hence, it was selected as the bioprobe in this research.

As well known it is necessary for biosensors to have good specificity in practical applications. Therefore, an essential test for the characterization of any biosensor is to investigate the specificity of the bioprobe (the bio-molecular recognition element). Hence it is of important research value to investigate the specificity of the developed biosensors.

In this work, Polyclonal Antibody immobilized magnetostrictive biosensors with the size of *5mm* x *1mm* were prepared and the specificity of the magnetostrictive sensors for detecting different kinds of bacteria, including 8 kinds of *Salmonella* species and 1 kind of non-*Salmonella* species was studied.

MATERIALS AND METHODS

Materials

The magnetostrictive material used in this work was purchased commercially from Honeywell International Corporation (2826 MB); its composition is $Fe_{40}Ni_{38}Mo_4B_{18}$ with a thickness of about 30μm. The flat sensor samples were diced from this magnetostrictive material after dry polishing. Polyclonal Antibody used in this work was purchased from AbCam Company. All kinds of bacterial cells were prepared in the department of Nutrition and Food Science at Auburn University.

Sensor platform

The biosensor is comprised of two main parts: the first part is magnetostrictive substrate used as transducer; the second part is Polyclonal Antibody layer used as the bioprobe for binding bacterial cells, which was immobilized onto the sensor surface using the Langmuir—Blodgett (LB) method. The biosensor exhibits a physical resonance when it undergoes a time-varying magnetic field, thus emitting magnetic flux, this can be monitored remotely without the use of direct physical connections, due to the magnetoelastic nature of the amorphous magnetostrictive alloy[5]. Schematic illustration of the wireless nature of the magnetostrictive biosensors was shown in Figure 1.

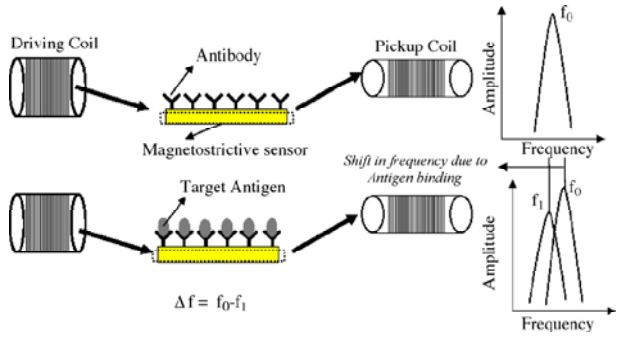

Figure 1 : Schematic illustration of the wireless nature of the magnetostrictive biosensors and principle for bacteria detection

Experimental

After biosensors were prepared, they were immersed in 1 ml solution of different kinds of bacterial cultures with a concentration of 1×10^9 cfu/mL for 30 minutes for binding bacterial cells. The resonance frequency of the sensors was measured using a HP network analyzer 8751A with S-parameter test set at 87511A before and after bacterial cells binding. Then the sensors were removed and exposed to Osmium tetra Oxide (OsO_4) vapor for one hour to fix the bacterial cell's wall. Finally, SEM images were taken using JEOL 7000F, operating at 5kV, which was used for investigating the physical distribution and density of the bacterial cells attached to the sensor surface.

RESULTS AND DISCUSSIONS

Scanning electron microscopy (SEM)

Polyclonal antibody immobilized sensors with the size of 5mm x 1mm were exposed to the following eight kinds of *Salmonella* species, *S. typhimurium, S. Heldelberg, S. typhi, S.Eteritidis, S.Mission, S.Thompson* and *S.Montevideo, S.Panama,* and other kind of non-*Salmonella* species of *Listeria monocytogenes*, all the bacteria solutions were adjusted to

have the same concentration of 10^9 CFU/ml. SEM images were taken for all the sensors to clearly show the binding of bacterial cells. Figure 2 shows typical SEM images of the polyclonal antibody immobilized biosensors after exposed to different kinds of bacteria with the same concentration of 10^9 CFU/ml, which showing different density on the sensor surfaces, with highest binding density for *S. typhimurium*, and almost no binding for *Listeria monocytogenes.*

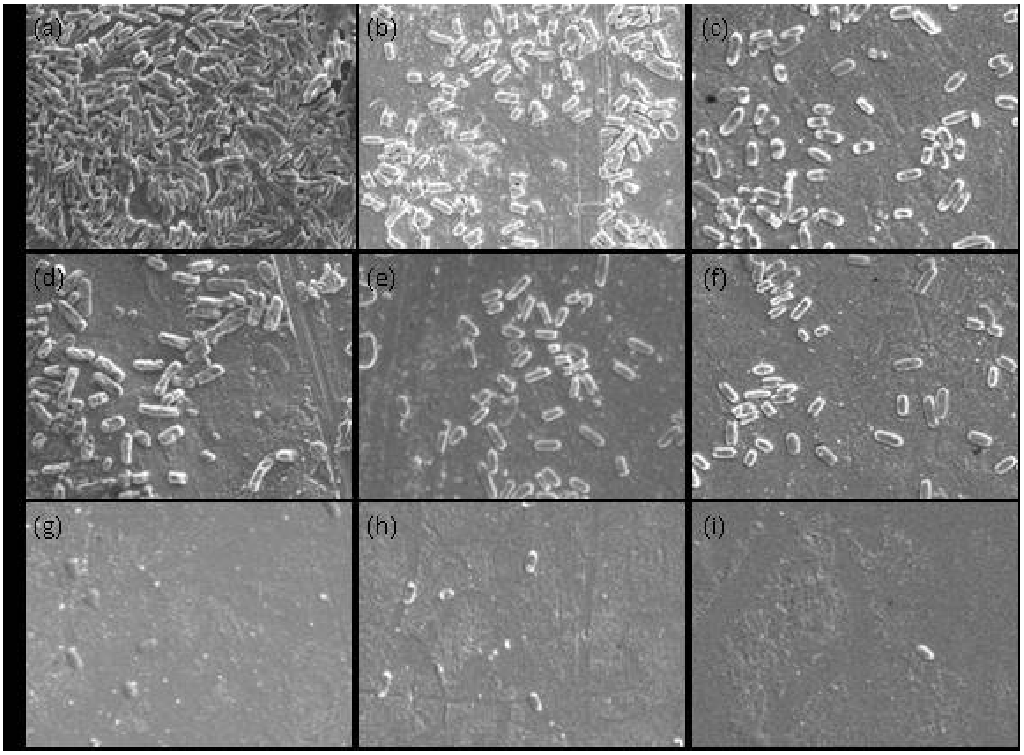

Figure 2 : Typical SEM images of the biosensors after exposed to different kinds of bacteria with the same concentration of 10^9 CFU/ml (5mm x 1mm); (a)*S. typhimurium,* (b)*S. Heldelberg,* (c)*S. typhi,* (d)*S.Eteritidis,* (e)*S.Mission,* (f)*S.Thompson* and (g)*S.Montevideo,* (h)*S.Panama,* and (i) *Listeria monocytogenes,* (a) ~ (h) represents bacteria of *Salmonella* species, (i) represents non-*Salmonella* species

Statistic density of the bacterial cells

In order to investigate the statistical density of the different kinds of bacterial cells, the number of cells bound on each sensor surface was counted from SEM images, and the actual density of bacterial cells were obtained by dividing the total number of cells by the area of the surface. Figure 3 shows the statistic density of the different kinds of bacterial cells attached to the polyclonal antibody immobilized sensor surface.

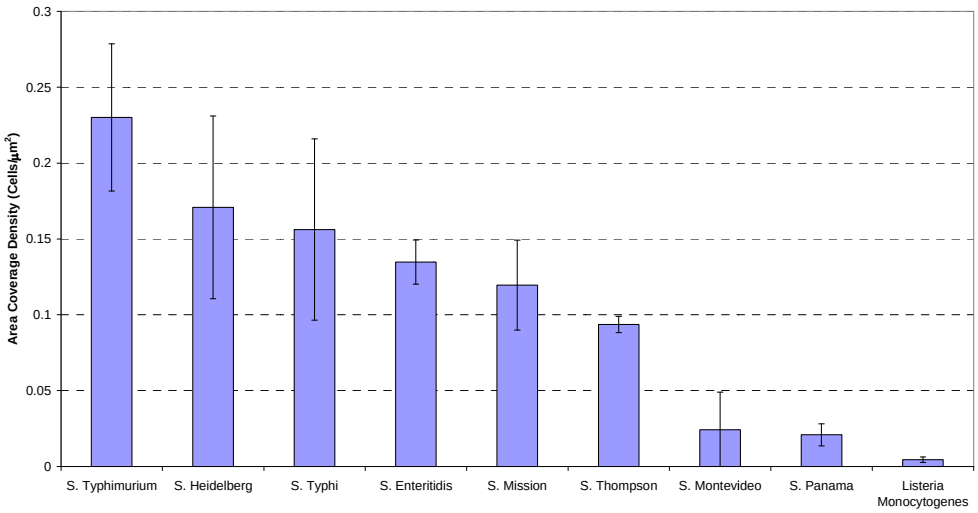

Figure 3 The statistic density of the bacterial cells attached to the polyclonal antibody immobilized sensor surface after exposed to different kinds of bacteria with the same concentration of 10^9 CFU/ml.

Figure 3 shows that the polyclonal antibody preferred to bind *Salmonella typhimurium*, though existed some affinity to other *Salmonella* species, but almost zero affinity was seen with *Listeria monocytogenes*. Thus, the conclusion could be drawn that the polyclonal antibody exhibited excellent specificity to *Salmonella* species bacteria.

CONCLUSIONS

Polyclonal antibody immobilized magnetostrictive biosensors were prepared with a unique advantage over conventionally used platforms, which needs no physical contact in order to obtain a response. The specificity to detect different kinds of bacteria, including *Salmonella* species and non-*Salmonella* species was studied. It was found that polyclonal antibody showed excellent specificity to detect bacteria of *Salmonella* species, and especially prefered to bind *Salmonella typhimurium*, almost zero affinity for non-*Salmonella* species of *Listeria monocytogenes*.

ACKNOWLEDGEMENTS

This project was supported by National Natural Science Foundation of China (51271039), PAPD of Jiangsu Higher Education Institutions and USDA Grant 2011-51181-30642A.

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