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Effect of surface roughness on performance of magnetoelastic biosensors for the detection of *Escherichia coli*

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ABSTRACT

Escherichia coli are bacteria that must be controlled in the food industry and the hospital sector. Magnetoelastic biosensors offer the promise of rapid identification of these and other harmful antigens. In this work, strips of amorphous Metglas 2826MB3 were cut to size (5 mm × 1 mm) with a microdicing saw and were then coated with thin layers of Cr and Au, as verified by Rutherford backscattering spectroscopy (RBS). Several sensor surfaces were studied: 1) as-cast strip, wheel side; 2) as-cast strip, free surface; and 3) thinned and polished surface. A layer of cystamine was applied to the Au-covered magnetoelastic substrate, forming a self-assembled monolayer (SAM), followed by antibodies, using a modified Hermanson protocol. The cystamine layer growth was verified by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The biosensors were exposed to solutions of bacteria and the resonant frequency of the sensors was measured with an impedance analyzer for times up to 100 min. Reductions in the resonant frequency, corresponding to bacteria capture, were measured after optimizing the signal amplitude. For times up to 40 min, high capture rates were observed and thereafter saturation occurred. Saturation values of the frequency shifts were compared with the number of bacteria observed on the sensor using fluorescence microscopy. Parameters associated with capture kinetics were studied for different sensor surfaces. The rough surfaces were found to show a faster response, while the thinned and polished sensors showed the largest frequency shift.

1. Introduction

The Center for Disease Control and Prevention (CDC) estimates that 48 million Americans suffer from some kind of food-borne illness annually [1]. This problem is universal. Legislation regulating the Brazilian Health Surveillance Agency (ANVISA) was recently revised to modernize the agency and make it more agile by encouraging it to incorporate recent technological advances. One of the obligations of ANVISA is to supervise the food industry through Good Manufacturing Practices Certificates. The food industries are constantly being asked to control various pathogens, which may lead to food-borne diseases. One of the controlled pathogens is a group of bacteria, the pathogenic *Escherichia coli* strains. *E. coli* bacteria normally live in the intestines of people and animals and are harmless. However, several types of *E. coli* may cause diarrhea or other illnesses. There are six pathogenic strains of *E. coli*, which are associated with diarrhea, and these are referred to as the diarrheagenic *E. coli* [2]. Since food-borne illnesses affect millions of persons every year, it is necessary to have an efficient way to rapidly detect the presence of pathogenic bacteria.

Standard methods such as colony counting or polymerase chain reaction (PCR) are both sensitive and specific to the target pathogens. However, the preparation methods are time consuming and are difficult to apply in real-time in-situ measurements. A number of biosensor technologies have been developed based upon surface plasmon resonances [3,4], piezoelectric effects [5,6], and electrochemical methods [7]. These techniques demand electrical connections to power the sensors and collect the signals associated with the presence of the bacteria. Even more recent electrochemical methods (such as iontophoresis-based blood glucose monitoring [8] or the use of mesoporous silica nanoparticles for the dual detection of pH and temperature [9]) as well as the recent electroporation techniques for cell transfection [10,11] all require electrodes for their operation. The wiring necessary to connect these devices necessarily limits their application to very controlled environments.

The limited accessibility of the above-mentioned sensors due to their electrical connections is not an issue for certain magnetic sensors, which can be queried remotely. Magnetoelastic sensors employing strips of an amorphous alloy have been in use for some time now as antitheft devices [12]. Magnetoelastic biosensors for the detection and quantification of pathogens have been studied more recently [13,14]. In this case, an oscillating magnetic field causes a strip of amorphous alloy having a biological recognition layer on its surface to oscillate at

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its natural resonant frequency. As the pathogens are deposited onto the amorphous strip, the oscillation frequency is reduced, allowing the detection of the pathogens. This is in many ways similar to the functioning of the quartz crystal microbalance (QCM) [15]. This technology has been developed to the point where a recent patent [16] proposes a practical system wherein a large number of small sensors could be sprinkled onto a fresh fruit or vegetable which is suspected of being contaminated. The small sensors would be coated with antibody or phage bio-recognition elements. As the pathogens are deposited on the sensor, the frequency shifts of the sensor would be detected by a planar coil detector [17,18], thus turning the bacteria detection process more efficient and convenient.

Sensors based on the immobilization of antibodies on a substrate have been widely used in different biosensing or bioanalytical techniques for the detection of pathogens [19]. A large number of chemical routes are available for immobilizing antibodies on biosensor surfaces. Strategies employing thiols, which can be immobilized on gold surfaces with high-affinity and without any prior surface modification, provide strong site-directed orientation. After depositing thin layers of Cr (for increased adherence) and Au, several types of layer could be applied. In the present case, the initial layer consisted of cystamine, a thiol which forms self-assembled monolayers. The chemisorption of thiols on the Au surface occurs spontaneously and is due to the relative inertia of the Au, the strong interaction with S and the formation of densely packed layers. For the bacteria capture, antibodies have been used [20] with magnetoelastic biosensors as well as phages [21]. A recent paper has discussed the relative stability of antibodies and phages for pathogen detection with magnetoelastic biosensors [22]. In the present work, antibodies were employed for biological recognition as will be described below.

In this work, strips of amorphous Metglas 2826MB3 ribbon were cut to size and were then coated with thin layers of Cr and Au, as verified by Rutherford backscattering spectroscopy. Several surfaces with differing degrees of roughness were studied: surfaces from as-cast ribbons as well as polished surfaces. Recent work [23,24] has emphasized the importance of surface roughness in determining biosensor kinetics. In their book [23], Sadana and Sadana have presented various models based on fractal analysis in an attempt to describe both diffusion effects and surface roughness (inhomogeneity) and their effects upon biosensor kinetics. The importance of inhomogeneities indicates that controlling surface roughness would be a convenient way to help regulate the binding kinetics of the sensors. For our sensors, cystamine was applied to the substrate, forming a self-assembled monolayer [25], followed by antibodies, using a modified Hermanson [26] protocol. Several sensor surfaces with roughness in the range $R_a = 0.3–0.52$ μm were studied and the bacteria capture was described and discussed.

2. Material and methods

The amorphous alloy Metglas 2826MB3 was supplied by the Metglas Corp. of Conway, SC. The approximate composition in wt.% is $Fe_{45}Ni_{45}Mo_7B_3$. The material has a saturation magnetostriction $\lambda_s =$ 12 ppm, a Curie temperature $T_c = 353$ °C, a density $\rho = 7.9$ g/cm^3, a Young modulus E = 100–110 GPa and a crystallization temperature $T_x = 410$ °C. The Poisson ratio σ is about 0.33 [27].

We received the alloy in the form of 2-inch wide ribbons. The average thickness t is about 30 μm, however we have measured values ranging from about 32 μm near the edges of the ribbon to about 27 μm near the center. A microdicing saw was used to cut the sensors to dimensions 5 mm × 1 mm and thus guarantee them to have the same resonant frequency. Since the mass sensitivity $S_m = df/dm$ of our sensors depends upon their thickness (see equation in Section 3), an effort was made to choose sensors from the same relative position on the ribbon. Some ribbons were cut as-cast and then coated by DC magnetron sputtering (AJA model ATC 2000) with a thin layer of Cr, for adherence, and then Au to form the sensor substrate. These ribbons were maintained at a thickness of about 30 μm, but the Cr/Au films were deposited on either the wheel side (rough surface of Fig. 1b) of the ribbon or the free side (smooth surface of Fig. 1a). Other ribbons were first mechanically polished on both sides using a Struers Tegramin 20 polishing system with 0.05 μm alumina and demineralized water. After 1.5 h of polishing, we reduced their thickness to about 15 μm, then we deposited the Cr and Au layers and we cut the ribbons (polished surface of Fig. 1c). We note that the sensors that we cut after depositing the Au layers had cleaner borders. There is a substantial difference in the roughness of the surfaces shown in Fig. 1a, b, and c. Using a Mitutoyo Surface Roughness Tester, model SJ-301, values of

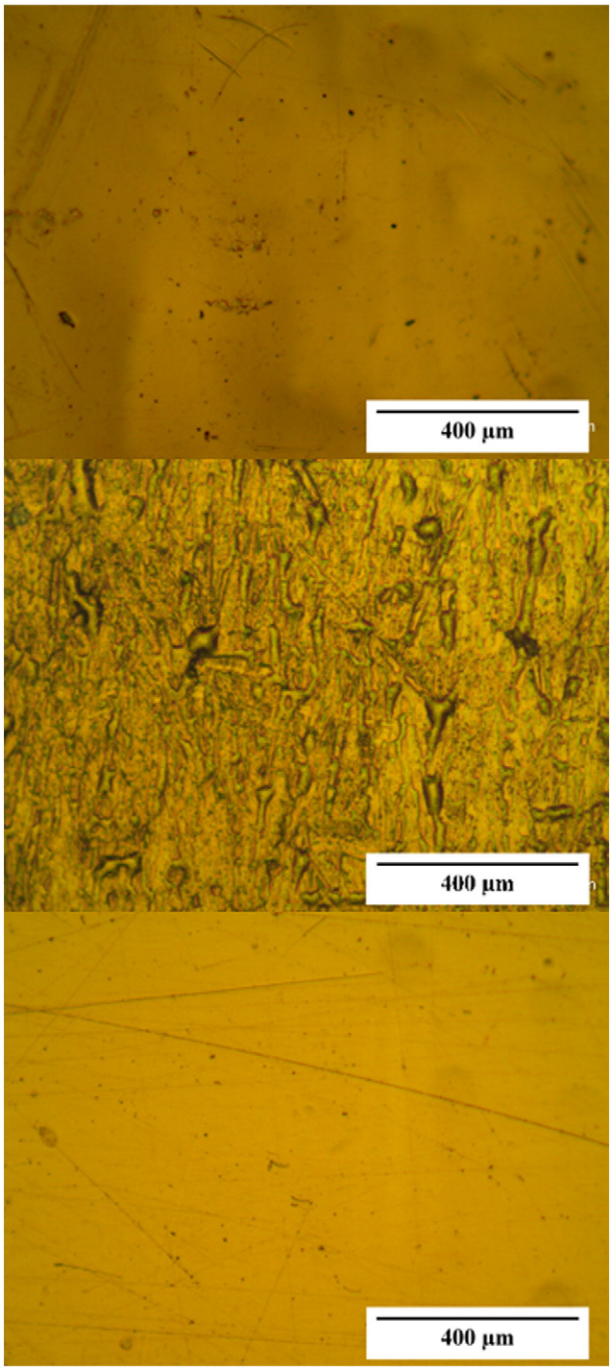

Fig. 1. Micrographs of surfaces used for biosensors. (a) Top image shows free side of as-cast amorphous ribbon. (b) Middle image shows wheel side of as-cast amorphous ribbon used for biosensors. (c) Bottom image shows surface of polished amorphous ribbon with thickness of about 15 μm.