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Magneto-elastic biosensors: Influence of different thiols on pathogen capture efficiency

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ABSTRACT

Magneto-elastic biosensors have mass sensitivity to biological species, offering reliability and reproducibility in the detection of pathogens such as *Escherichia coli*. In this work, amorphous ribbons of Metglas 2826MB3 were coated with layers of Cr and Au by DC magnetron sputtering and cut to 5 mm × 1 mm. The influence of different thiols on captured pathogens was studied. The compounds cystamine (CYS), cysteamine (CYSTE) and mercaptopropionic acid (MPA) were deposited on Au-covered surfaces, followed by antibodies. The roughness parameters Ra and Rq were determined using atomic force microscopy (AFM) and micrographs from scanning electron microscopy with a field emission gun (FESEM) were also utilized. Biosensors formed with MPA showed an increased efficiency for attracting *E. coli* compared to biosensors with CYS and CYSTE, but large standard deviations were observed, making reproducibility and reliability difficult for that biosensor. Sensors tested with CYSTE showed greater efficiency and a lower detection limit than sensors with CYS. The results indicated that the size of the carbon chain and the terminal grouping influence the effectiveness of immobilization on magneto-elastic biosensors.

1. Introduction

The Center for Disease Control and Prevention (CDC) estimates that up to 48 million persons become ill every year in the United States due to food-borne illnesses, with up to 3000 deaths [1]. In May of this year, General Mills, producer of wheat flours Gold Medal, Wondra and Signature Kitchen, recalled certain lots of their products from the supermarkets because of a relation with the appearance of the *Escherichia coli* bacteria in up to 20 American states [2]. According to the CDC, the average time to diagnose these bacteria is from 1 to 3 days, after obtaining the sample. The detection and rapid quantification are essential for environments which require the control of this pathogen. Magneto-elastic biosensors may be an alternative method for this function since they can be queried remotely in a reliable and secure manner [3].

E. coli is a pathogenic agent, which can cause urinary tract infections or infections of the bloodstream. Transmission may occur through the consumption of contaminated food, mainly raw or undercooked meat and milk, as well as through the consumption of raw vegetables. The control of this pathogen is critical in the food and hospital sectors [4].

The detection of pathogens on the functionalized surfaces of magneto elastic biosensors may occur through the formation of self-assembled monolayers (SAM) followed by a specific antibody. The binding of antibodies on a surface modified with a SAM is mediated by crosslinking agents such as EDC and Sulfo-NHS, creating reactive intermediaries and providing the connection with the functional groups of the surface.

Organic compounds with a thiol group at one of its extremities and the other having a carboxyl (R-COOH) or amine ($R\text{-}NH_2$) group may be used for the formation of the SAM. We decided to produce biosensors using the organic compounds cystamine (CYS), cysteamine (CYSTE) as well as mercaptopropionic acid (MPA) to form the SAM [5].

The formation of the SAM on a gold surface is strongly affected by the nature and roughness of the substrate, by the solvent employed, by the time and temperature of exposure, by the concentration of the compound, as well as by the length of the thiol molecule utilized. A recent paper [6] has studied the surface functionalization of gold nanoparticles with thiol-based SAMs. Thiol-functionalized gold nanoparticles have proven to be very useful as biosensors [7]. The length of the thiol chain affects the stability of the applied SAM as well as the electron transfer during the formation, thereby influencing the detection sensitivity of the biosensor [8–11].

The cystamine ($C_4H_{12}N_2S_2$) molecule is a disulphide with two atoms of sulphur (S) in the middle as well as amine groups at each of its extremities [12]. When the bond between the two sulphur atoms is broken, the formation of the SAM may occur. In the case of CYS, two thiols are formed when the S—S bond is broken, leading to a closer coupling of the thiols forming the SAM [13–15].

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Cysteamine (C_2H_7NS) is the simplest stable aminothiol, having a structure similar to that of cystamine, however with one sulphur atom and a single amine group. The formation of the SAM occurs by breaking the bond with the sulphur and the formation of a bond with the gold substrate.

The molecule of mercaptopropionic acid ($C_2H_6O_2S$) is a short chain thiol with a sulphur and a carboxylic group (COOH) at one of its extremities [14,16]. To form the SAM, the S—H bond is broken, allowing the bonding of the sulphur with the gold surface and promoting an organized and oriented layer [14].

The use of immobilized antibodies on magneto-elastic biosensors, their advantages and disadvantages, has been discussed at some length [17]. Hoping to improve the detection sensitivity of the magneto-elastic biosensor [17,18], this work proposes to analyze the effect of SAM formation (with CYS, CYSTE and MPA) on gold-coated ribbons of Metglas 2826MB3. For this purpose, the functionalized ribbons were exposed to solutions of *E. coli* bacteria and the variations of the resonant frequency were measured as a function of time with an impedance analyzer. Since the organic compounds employed differ in their structure, the length of their carbon chains and in the terminal group responsible for connecting to antibodies, we might hope to obtain insights into the importance of these factors in determining the sensitivity of the biosensors. The biosensors were also characterized by atomic force microscopy (AFM) in the tapping mode and field emission scanning electron microscopy (FESEM).

2. Experiment

The amorphous alloy Metglas 2826MB3, with approximate composition $Fe_{45}Ni_{45}Mo_7B_3$ in weight percent, was furnished by the Metglas Corp. in the form of two inch wide ribbons with a thickness of about 30 μm [19]. The alloy has a saturation magnetostriction (λ_s) of = ppm, a density of $\rho = 7900\ kg\ m^{-3}$, a Young modulus E of approximately 105 GPa and a Poisson ratio of $\sigma = 0.33$ [20,21]. The ribbons were first mechanically polished on both sides using a Struers Tegramin 20 polishing system with 0.05 μm alumina and demineralized water. After reducing the thickness to about 15 μm, layers of Cr (~128 nm) and Au (~117 nm) were deposited by DC magnetron sputtering [22]. The Cr layer was added to improve the adherence of the gold layer. Previously [22] an elemental analysis of the films using Rutherford backscattering spectroscopy was reported. A micro-dicing saw was then used to cut sensor substrates with dimensions 5 mm × 1 mm × 0.015 mm.

Atomic force microscopy (AFM) was performed using the Shimadzu Scanning Probe Microscope SPM 9700 in the tapping mode with NCHR-10 silicon tips. All parameters were adjusted to permit scans over an area of 1 μm^2. Average roughness values were calculated using the system software and the averaging was done over three areas of 1 μm^2 and the results were averaged. Field emission scanning electron microscopy (FESEM) was performed using a TESCAN model MIRA 3.

Cystamine dihydrochloride (≥98%), cysteamine (≥98%) and 3-mercaptopropionic acid (≥99%) were purchased from Sigma Aldrich. The reagents *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC) and *N*-Hydroxysulfosuccinimide (Sulfo-NHS) used for the dilution and activation of the antibodies were also acquired from Sigma Aldrich. The preparation of each compound studied (CYS, CYSTE, or MPA) for the formation of the SAM proceeded by dissolving the material in ethanol at a concentration of 20 mM, followed by agitating at 1700 rpm for about 24 h at room temperature. After the deposition of the material, the sensor was washed with the solvent in order to remove molecules that are not adhering well and transferred to a micro reaction tube (Eppendorf).

The primary antibody anti-*E. coli* ab137967, acquired from ABCAM, was diluted to a concentration of 0.02 mg mL^{-1} with PBS (pH 7.4). For the CYS and CYSTE sensors we added EDC 20 mM in the proportion 1:100 with the antibody and incubated the result for 2 h at a temperature of 37 °C. For the MPA sensors, we added EDC 15 mM and Sulfo-NHS 35 mM for 2 h at room temperature and later the diluted antibodies for 2 h at 37 °C. After the application of the antibody, the biosensor was washed with PBS (pH 7.4) in order to remove non-specific bonds. For analysis using the fluorescence microscope, the secondary antibody goat anti-rabbit IgG H&L Alexa Fluor 488, also acquired from ABCAM, was applied after being diluted to a concentration of 0.008 mg mL^{-1} with PBS (pH 7.4) [24–26].

The *E. coli* bacteria were transferred to a Petri dish with semisolid Luria Bertani (LB) medium from the original sample [27]. After the growth period had passed (24 h at 37 °C), a single colony forming unit (CFU) was used for inoculation and growth in 100 mL of liquid LB medium. A series of dilutions were carried out using PBS (pH 7.4), starting from the concentrated bacteria. Initially 0.9 mL of the PBS solution and 0.1 mL of the bacteria-containing solution were mixed and this process was repeated until a dilution of 10^{-6} was attained, permitting a direct counting of the CFU in the semi-solid medium. For each analysis, new suspensions of bacteria were produced. The contamination of the biosensors was carried out with an *E. coli* concentration of about 10^9 CFU mL^{-1}.

The shifts in the resonant frequencies were measured as a function of time for 60 min using an Agilent E5061B impedance analyzer. Measurements were taken automatically at intervals of 2 min. Further

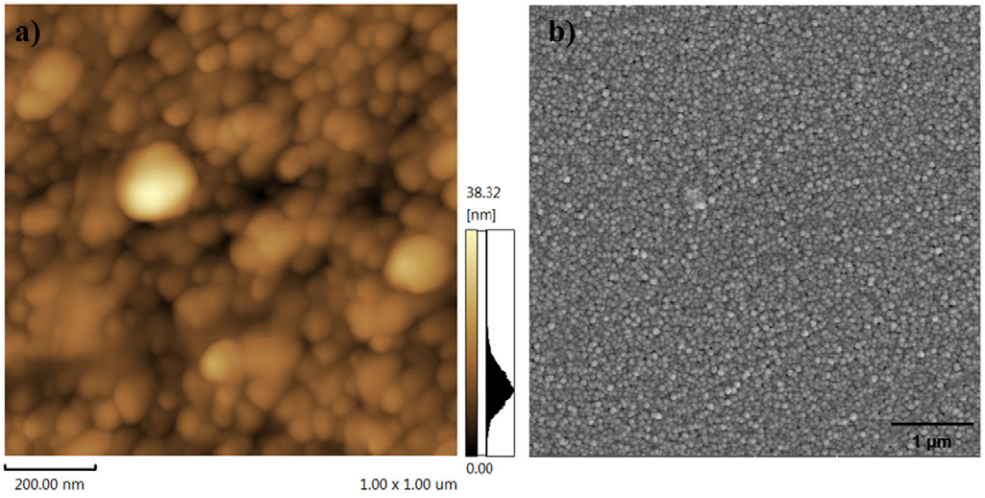

Fig. 1. a) Atomic force microscope image b) FESEM micrograph of magneto elastic sensor substrate covered with Cr/Au by sputtering.