

Mesoporous TiO₂ Coating for Increased Sensitivity of Love Wave Delay-Lines for Heavy Metal Detection

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Abstract:

This work deals with the design of a highly sensitive acoustic Love waves biosensor for heavy metal detection in liquid medium. Biorecepting *Escherichia coli* bacteria are immobilized on polyelectrolyte multilayers, and are used to detect the presence of cadmium by modification of viscoelastic properties of bacteria. The different setups of sensor elaboration are measured through the real-time resonant frequency shifts of the sensor. The impact of the addition of a titania mesoporous coating is studied, and compared to previous results [1]. Frequency variation for bacteria immobilization is doubled with titania compared with bare silicon dioxide surface. The improvement of the acoustic platform sensitivity is highlighted with cadmium as typical heavy metal. A complementary study was done by Atomic Force Microscopy (AFM) and allowed us to validate the assumptions.

Key words: Love waves, biosensor, Titania mesoporous, AFM, PDMS cell

Introduction

The presence of cadmium in the environment, where it comes from agricultural and industrial activities, is regarded as a toxic environmental pollutant [2]. This metal can lead to heart disease and anemia, and also seems to contribute to autoimmune diseases of the thyroid and classified as a human carcinogen. Therefore, the determination of Cadmium heavy metal traces is of considerable importance. Conventional methods have been used for the Cadmium heavy metal analysis in water and food such as inductively coupled plasma mass spectrometry (ICPMS), atomic absorption (AA), and spectroscopy, inductively coupled plasma (ICP). These methods provide very complete information, but they present significant logistical constraints, in terms of cost and especially time. For these reasons, there is a strong expectation of new analytical methods for fast detection and at low cost, principally a method to ascertain the presence of metal ions *in situ* in the environment, with high sensitivity. The sensor can be seen as a potential answer to this need. In recent years, biosensors have become an integral part of major technological advances, resulting from the fusion of the two

most important technologies of this century: the electronics and biotechnology.

In this context, several sensors have been developed for detection of toxics, including heavy metals such as Cadmium. Among them, Love-wave bacteria-based sensors appeared as very promising devices, combining real-time and highly sensitive surface acoustic wave device with living whole cells which are considered presenting decisive advantages compared to enzymes or other bioreceptors. In particular, bacteria can adapt to an adverse environment, which should increase the sensor lifetime. However, this sensor showed some drawbacks for applications in biosensing, particularly due to the guiding layer of SiO₂ on the surface. The presence of this layer provides a guided wave and this confinement within a few micrometers near the surface, confers sensitivity to the device. But, although it has a number of advantages, silica suffers from imperfect stability when exposed to aggressive chemical treatments (surface functionalization, cleaning, etc.), or in the presence of saline media such as biological buffers conventionally used.

In this paper, we propose to add a titania (TiO_2) layer on the silica surface, and to study its effect on the sensitivity and the stability of a Love-wave bacteria-based biosensor. TiO_2 is favored by its known properties of non-toxicity, stability (biologically and chemically inert enough), it is also inexpensive, easy to coat on a surface. Furthermore, mesoporous TiO_2 could lead to a better sensitivity, as it was already demonstrated for gaseous applications.

Love wave sensor

The Love wave device is a structure composed of a semi-infinite piezoelectric substrate with interdigital transducers (IDTs) oriented to allow a pure shear horizontal (SH) wave propagation perpendicular to X crystallographic axis. A $4\mu\text{m}$ SiO_2 guiding layer, deposited on the substrate through PECVD, confines the acoustic wave energy near the surface to maximize the sensor sensitivity [3]. Once placed in the retroaction loop of a radio-frequency amplifier, the synchronous frequency of the device is close to 117 MHz. Any modification of mass or viscoelastic parameters on the sensor surface can be electronically measured in real-time as a frequency shift.

Titania mesoporous film preparation

The titania mesoporous film is prepared via the sol-gel process, which enables the full control of the film structural properties [4]. The solution was spread by spin-coating at 1000 rpm/s acceleration and 2000 rpm velocity, on the acoustic path of the sensor. Then, the device spent 10 hours in a climatic chamber set at 75% relative humidity and at ambient temperature. A dedicated thermal cure is finally applied to obtain a homogeneous porous film: first, from ambient to $400\text{ }^\circ\text{C}$ with a speed of $5^\circ\text{C}/\text{min}$ for 5 hours; and finally, back to the ambient temperature at the same rate. The film thickness was about 100 nm.

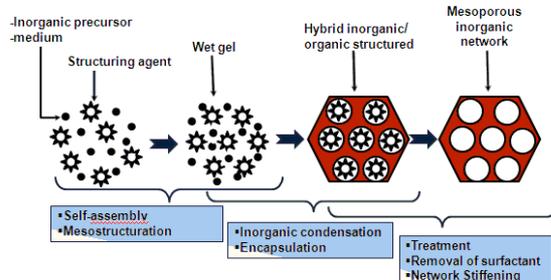


Fig. 1 Scheme of the sol-gel process [7]

PDMS cell

A chip made of Poly (dimethylsiloxane) (PDMS) was used in order to protect the IDTs from the

liquid and define a specific area on the acoustic path for bacteria immobilization via polyelectrolyte multilayer (PEM) and for heavy metals detection (Fig. 2). This polymer, as chamber for the fluid, presents relevant properties such as biocompatibility, ease of cleaning and reusability [5, 6].

PDMS was mixed in 10:1 weight ratio (silicone rubber: curing agent). An aluminium mold, previously developed [7], has been used to fabricate the PDMS chip including two separated tanks for simultaneous measurements on both delay lines. PDMS is then poured on the mold and cured for 2 h at 65°C to achieve a reticulation. This chip ensured a liquid chamber open for micropipette analyte delivery.

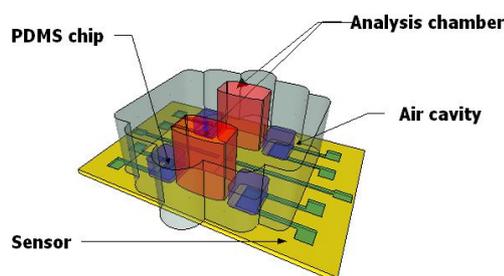


Fig. 2 PDMS cell with open cavities fixed on the sensor

AFM devices

For AFM experiments, we used a Bioscope II AFM (Veeco-Brucker, Santa Barbara, CA) installed in the NSI platform. This AFM is equipped with a G scanner (maximum XY scan range of $150\mu\text{m} \times 150\mu\text{m}$ with vertical range Z of $12\mu\text{m}$). In this study, AFM data (images, Force curves) were obtained in air with the Tapping mode. The study by AFM can provide additional data to those obtained by Love wave (surface state, roughness, bacteria morphology, elasticity, etc.). The tip was in Si_3N_4 with stiffnesses of the order of 50 N.M^{-1} .

The scan rate was at 0.5Hz for all the data presented in this paper.

For each AFM operation are registered 4 images (height trace and height retrace, deflection image, signal error and friction image). We limit to present in this work the height trace image for more clarity, and images are flattened.

AFM and Acoustic results for oleyelectrolytes multilayers (PEM) deposition

The PEM consists in self-assembled molecular bilayers of Poly (allylamine hydrochloride) (PAH cationic layer) and poly (styrene sulfonate)

(PSS anionic layer), used for bacteria immobilization on the sensor surface. The sequential depositions of oppositely charged solutions via a layer-by-layer self-assembly (LBL) method presents many advantages, including simplicity, low-cost and low temperature of deposition.

The real-time characterization of PEM coating is based on the modification of the acoustic wave phase velocity, due to the surface perturbation induced by material deposit and is presented in Figure 3. An increase of the steady-state frequency shift can be observed reproducibly for the first PAH-PSS bilayer (PAH1, PSS1) when using the mesoporous layer compared to the classical Love wave structure: 9.7kHz instead of 3.4kHz with and without TiO₂ mesoporous layer respectively, whereas the cumulative frequency shift after the complete PEM is quite similar. This could be attributed to the electrical charge of TiO₂, opposite to that of PAH, as well as to the presence of pores of a few nanometers in diameter, both improving the first layer attachment. Additional studies by AFM (tribologic: friction and phase) are in progress to verify these proposals.

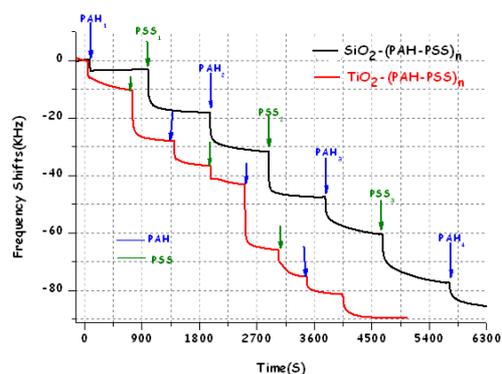


Fig.3 Typical real-time frequency shift for PEM deposition on SiO₂ (4 μm) and SiO₂ + TiO₂.

In order to investigate the mechanisms of structural evolution of TiO₂/PEM, the surface morphologies of the nanostructured thin films on silica and mesoporous titania were analyzed with AFM in tapping mode (Fig 4).

One can observe a clear evolution of the PEM surface when compared to the TiO₂ substrate, suggesting that the surface is recovered differently.

These results show the appearance of small granules as circles on the first bilayer PEL1-PAH, in agreement with a formation from a first layer (PAH1) inside the pores of TiO₂(Fig4-f).

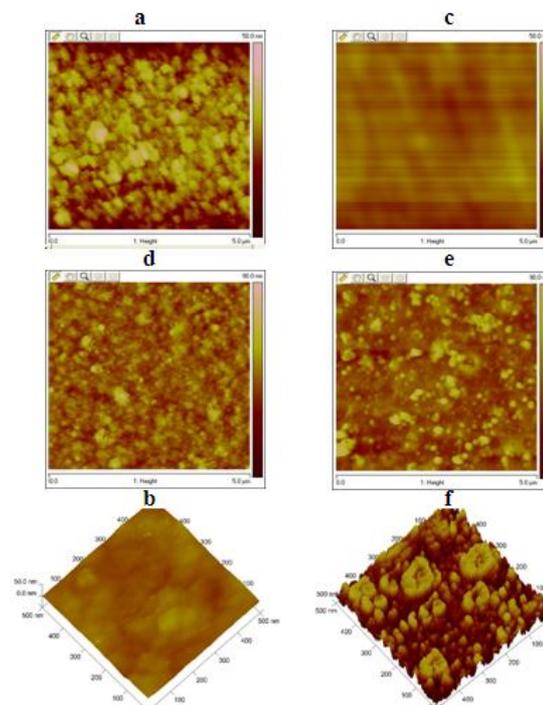


Fig4 SiO₂ and TiO₂ comparison of Tapping-mode AFM images of (a) SiO₂ surface topography (5μm), (b) SiO₂-((PAH-PSS)-PAH) surface topography (5μm), (c) SiO₂-((PAH-PSS)-PAH) 3D-surface (5μm), (d) mesoporous TiO₂ surface topography (5μm), (e) TiO₂-((PAH-PSS)-PAH) surface topography (5μm), (f) TiO₂-((PAH-PSS)-PAH) 3D-surface (5μm)

Acoustic response during Bacteria immobilization and Cadmium detection

Typical real-time frequency during deposition of Escherichia coli bacteria and during exposition to increasing Cadmium (Cd²⁺) heavy metal ion concentrations, in the range from 10⁻¹² M to 10⁻³ M, is represented on Figure 5a (sensor with titania layer). The steady-state frequency variations with both surfaces can be compared on Figure 5b. It can be noted a frequency decrease due to E.coli bacteria immobilization, approximately equal to 20 kHz and 8±3kHz, with and without titania layer, respectively. Then, a better sensitivity to cadmium is also observed, particularly at weak concentrations, when using the mesoporous layer. Indeed, after a few minutes to the introduction onto bacterial cell-based biosensors, cadmium has an increasing frequency effect, previously attributed to changes in viscoelastic properties of the sensitive film, related to modifications in bacteria metabolism [1]. This frequency variation (mean value) is multiplied by 3 times at 10⁻¹² M when using the mesoporous TiO₂ layer. This is due to be the increase of the number of bacteria immobilized on a surface which increases the response to the detection.

Furthermore, reproducibility is consistently enhanced by the titania layer (Figure 5b),

probably partly due to the chemical stability of this material [7].

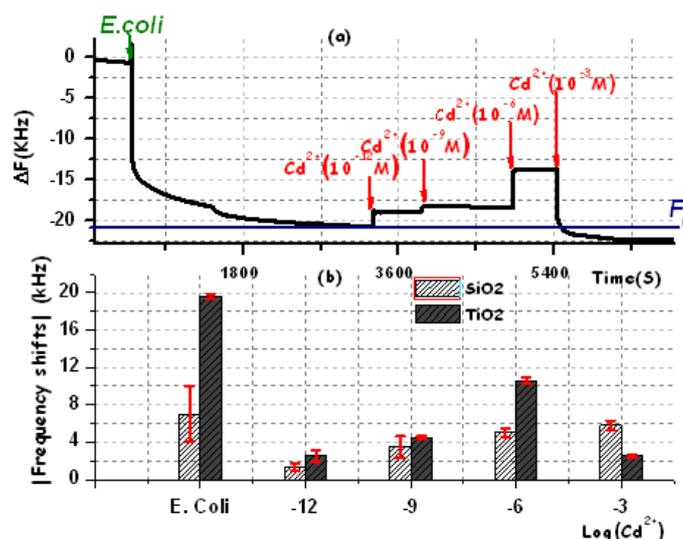


Fig.5 Frequency shifts due to Bacteria (*E. coli*) deposition and to Cadmium injection a) Typical real-time frequency of the sensor with SiO_2 ($4\mu\text{m}$) + TiO_2 (100nm) surface b) Steady state frequency shifts with both sensors (SiO_2 , $\text{SiO}_2+\text{TiO}_2$); mean values and error bars have been calculated from 3 experiments with different delay-lines.

Conclusion

The effect of the addition of a titania mesoporous coating on a Love wave delay-line for a bacterial-based biosensor was studied. An enhanced sensitivity was noted for the first layer of polyelectrolyte PAH₁; this was explained by enhanced electrostatic attraction due to electrical charge of titania and by penetration inside mesopores. Then, immobilization of bacteria and detection of low concentration of cadmium induced frequency responses two to three times those obtained without titania. A better reproducibility was also obtained, even with sensors regenerated a limited number of times. All these reasons demonstrate the interest of using such a titania layer. More studies (Love waves and AFM) are ongoing in order to verify the improvement of aging and lifetime of the so-modified sensor, which shown a complementary characterization between these two methods.

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