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Original article

Multipurpose Love acoustic wave immunosensor for bacteria, virus or proteins detection

Immunocapteur polyvalent à ondes acoustiques de Love pour la détection de bactéries, de virus ou de protéines

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Abstract

A multipurpose Love acoustic wave biosensor is described in this article. As mass loading is one of the main effect involved in acoustic wave sensors, a great range of biomolecules could be detected using such sensors. In this way, the antibody/antigen binding property was used to immobilise the target species. We first compared different coupling agents to link the antibodies sensitive layer to the SiO_2 sensor surface. Results showed that GPTS monolayer, allowing covalent attachment of antibodies bioreceptors, is better suited than DTSP and protein G. It permits to obtain a dense, stable and reproducible sensitive layer of antibodies. Then, different biological species with different size and shape like proteins, bacteriophages or bacteria were detected using such sensor. Different models have been chosen to validate the effective detection of a large species range: an anti-mouse antibody has been used to simulate small molecules (< 10 nm) like proteins or toxins, bacteriophage M13 for species lower than 1 μm like virus, and *Escherichia coli* for bacteria which are typically longer than 1 μm. Each kind of species were successfully quickly detected from few seconds for small proteins to one hour for bacteria, with detection threshold down to 4 ng/mm^2 for protein and 10^6 cfu per milliliter for bacteria.

Résumé

Un biocapteur à ondes acoustique de Love est étudié dans cet article. L'effet de masse est un des principaux effets impliqués dans ce type de capteur et il est donc possible de détecter une grande variété de biomolécules différentes. De plus, les propriétés d'affinité et de sélectivité du couple anticorps/antigène sont utilisées pour l'immobilisation des espèces cibles. Une comparaison de différents agents de couplage a été menée afin d'accrocher de manière optimale la couche sensible d'anticorps sur le SiO_2 en surface du capteur. Les résultats ont montré qu'une monocouche de GPTS, qui permet le greffage covalent de la couche bioréceptrice d'anticorps, est plus adaptée que le DTSP ou la protéine G, permettant ainsi d'obtenir une couche stable, dense et reproductible. Cette plate-forme générique de détection a été utilisée avec des espèces de tailles et de formes différentes comme des protéines, des bactériophages et des bactéries. Pour cela nous avons utilisé comme modèle un anticorps de souris pour les espèces inférieures à 10 nm, le bactériophage M13 pour les espèces inférieures à 1 μm et une bactérie *Escherichia coli* pour les bactéries qui sont typiquement supérieures à 1 μm. Chacune de ces espèces a pu être détectée rapidement en des temps allant de quelques secondes pour les anticorps à une heure pour les bactéries, avec un seuil de détection de 4 ng/mm^2 pour les protéines et 10^6 cfu par millilitre pour les bactéries.

Keywords: Immunosensors; Love wave; Covalent grafting; Bacteria; Silanization

Mots clés : Immunocapteurs ; Onde de Love ; Greffage covalent ; Bactéries ; Silanisation

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1. Introduction

Infections by pathogenic bacteria cause a serious threat to public health with periodic outbreaks arising from food and water contaminations. Access to safe drinking water is a major problem for a large part of the world's population. At least 1.8 million people die every year from diarrhoeal diseases, with 88% of these deaths being attributed to unsafe water supply, inadequate sanitation and hygiene [1]. *Escherichia coli* (*E. coli*), a Gram negative bacteria, normally inhabits the intestinal track of humans and warm-blooded animals. Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens and may cause diseases under certain conditions, e.g., from a contaminated environment exposure or in the case of an immune system response dysfunction. This organism may cause several infections like peritonitis, septicaemia, infantile gastroenteritis, tourist diarrhoea and hemorrhagic diarrhoea. For these reasons, *E. coli* have been used (with *Streptococcus*) as a biological indicator of faecal contamination in drinking water since the 1890. Rapid detection of micro-organisms in food and water to prevent infection, illness and economic loss has always been a challenge [2].

Also, rapid detection of bacteria like *E. coli* and other pathogens is an important concern for safety and public health in various domains such as environment, food industry or even sewage. Using conventional methods like standard *E. coli* culture on a specific solid medium followed by colonies counting, Enzyme Linked Immuno Sorbent Assay (ELISA) [3,4] and Polymerase Chain Reaction (PCR) [5–7], several hours to several days are needed to detect bacteria; moreover, the tests must be carried out in a specific laboratory. The development of real-time microsensors during the last few years has provided an alternative to these tedious and time-consuming methods. Recently, rapid biosensors based upon various technical achievements such as Surface Plasmon Resonance (SPR), Surface Acoustic Wave (SAW) or impedance measurements have emerged in the literature [8–15]. SPR and acoustic wave bacteria microsensors previously reported present an important detection threshold, such as 5×10^7 colony forming unit (cfu) per milliliter in [16] using a SPR biosensor, whereas others with lower detection thresholds (10^3 cfu per milliliter) suffer from poor specificity [17] with acoustic wave devices. Interestingly, a detection threshold of 10 cfu per milliliter has recently been reported using Electrochemical Impedance Spectroscopy (EIS) in a conventional cell [18–20].

To avoid contamination, a rapid determination of the presence of a virus for instance is also of great importance like the Influenza A virus subtype H5N1, an epizootic known as bird flu. SPR biosensors have already been used to detect such virus, with Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) with a detection threshold down to 10^7 virus per milliliter [21].

Biological detection of virus, bacteria or toxin has to be rapid, sensitive and easily realised. SAW sensors technology has proved its efficiency in liquid media as biosensors [22], especially Love wave sensors, which are designed not only to operate in liquid media but also to present a high surface sensitivity [23]. This handheld technology has shown promising results for the detection of low concentrations of bacteria in quasi real time with good reproducibility. To our knowledge, a sensor able to detect such different species with a same protocol has not been reported yet. In this paper, a versatile Love acoustic wave sensor to detect different biological species from small proteins or toxins (< 10 nm), viruses or bacteriophages (< 1 μm) to bacteria (> 1 μm) in short time using a similar protocol is described.

2. Love wave sensor presentation

The Love wave sensor (Fig. 1) is an electromechanical sensor based on a SAW delay line with metallized interdigital transducers (IDT) to generate and receive an acoustic wave on a piezoelectric substrate. To avoid any coupling of the wave with the adjacent liquid, a shear horizontally polarized wave is generated using an AT-cut quartz substrate oriented so that wave propagation direction is perpendicular to X cristallographic axis. The pure shear horizontal waves, generated with interdigitated electrodes, are then confined within a thin guiding layer at the surface of the device in order to increase the sensor surface sensitivity. Any physicochemical perturbation onto the sensor surface will modify the wave velocity, which can be measured with high accuracy via frequency in an oscillator configuration, confering great measurement resolution to the system.

A sensitive layer is deposited onto the device to trap specifically the target species and, thus, modify the wave propagation. In the case of an immunosensor, this sensitive film is typically composed of a film of antibodies.

We used 0.5 mm thick AT-cut quartz substrate (The Roditi International Corporation Ltd., London, England), Euler angles (0°, 121.5°, 90°) with IDTs consisting of 44 finger pairs of 20 nm titanium (Ti) and 70 nm gold (Au) with a wavelength λ equal to 40 μm, deposited with a lift-off process. The IDTs aperture was 39λ and the propagation path between the IDTs centre was 210λ. The SiO_2 guiding layer thickness, deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) technique, is about 4.4 μm, which results in a synchronous frequency of about 118 MHz. The thickness of this guiding layer has been chosen to approximately maximize the mass effect sensitivity of the device [24]. This device is inserted into a test brass cell providing electrical contacts and sealed with a Viton® seal. The top of

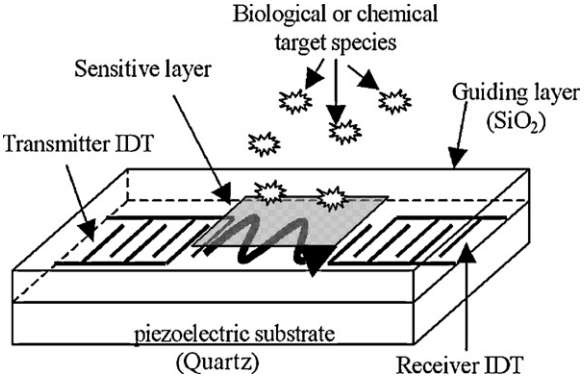

Fig. 1. Schematic representation of a Love wave sensor.