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Tailoring odorant-binding protein coatings characteristics for surface acoustic wave biosensor development

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ABSTRACT

In this study, wild type bovine odorant-binding proteins (wtbOBPs) were deposited by matrix-assisted pulsed laser evaporation (MAPLE) and utilized as active material on surface acoustic wave (SAW) biosensors. Fourier transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM) were used to determine the chemical, morphological characteristics of the protein thin films. The FTIR data demonstrates that the functional groups of wtbOBPs do not suffer significant changes in the MAPLE-deposited films when compared to the reference one. The topographical studies show that the homogeneity, density and the roughness of the coatings are related mainly to the laser parameters (fluence and number of pulses). SAW biosensor responses to different concentrations of *R*-(−)-1-octen-3-ol (octenol) and *R*-(−)-carvone (carvone) were evaluated. The obtained sensitivities, achieved through the optimization of deposition parameters, demonstrated that MAPLE is a promising deposition technique for SAW biosensor implementation.

1. Introduction

During the last years, an increase in the number of sensors applications in various areas such as the environmental control, process industry, security, and biodefense markets, has resulted in increasing of research efforts to develop newer approaches that can provide high sensitivity, accuracy, selectivity and, in general, better performances for sensors [1]. Within this context, the use of functional protein thin films as active material represents an emerging and rapidly growing solution in the field of biosensors. In particular, piezoelectric sensors based on quartz crystal microbalance (QCM) and SAW devices, and using biomolecules as sensing material, have attracted the attention of the scientific community for vapour phase applications [2]. Specifically, SAW biosensors can overcome the intrinsic low-selectivity of polymer coated SAW chemical sensors, and, at the same time, ensure the high sensitivity and fast response time typical of these sensors [2,3]. However, the established idea that biomolecules can maintain their three-dimensional structure and, hence, their prescribed functionality, only in an aqueous environment, has delayed the development of SAW biosensors for in-air applications and, to date, only a limited number of works has been proposed [4–6]. In particular, the sensing capabilities of OBPs and their property to preserve their full functionality when exposed to air environment have been recently demonstrated to detect odorant molecules [7].

Regardless of the application and the operating principle of the biosensor, great attention is devoted to the method of applying biomolecules in order to preserve the chemical composition and natural conformation of deposited protein molecules, so that the biological activity is not altered. Specifically, an important issue with SAW sensors is the uniform application of the sensing layer along the wave propagation path, in order to prevent high attenuation [8,9]. As an alternative to conventional deposition techniques, such as drop casting, MAPLE has the potential to create thin films of controlled thickness on surfaces of various substrates [10–14] and in particular on SAW devices [15,16]. Nevertheless, as compared with other techniques used for depositing sensitive biological compounds onto SAW devices, MAPLE technique presents the advantage of improved and intimate adhesion of the sensing material onto the active area of the sensor, controlled morphology, homogeneity and uniformity of the surfaces, as well as confinement of the material within the active area by the use of masks. Another advantage is the use of very small amount of materials, which is important when it involves the use of expensive biological or other type of compounds.

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Therefore, this technique is of great interest in the area of protein thin film processing for various applications, from multifunctional coatings for biological application to biosensors. In MAPLE, a material, for example a biomolecule, is dissolved in a solvent in concentrations of 0.1–5%, and the mixture is, then, frozen, resulting in a solid target. When the laser light irradiates the target, the solvent is evaporated and the dissolved material (the organic material) is collected on a substrate. Earlier work with MAPLE has demonstrated that with an appropriate choice of experimental parameters, such as laser wavelength, fluence and pulse duration, type of solvent, target and substrate temperature, and background gas pressure, MAPLE is capable of providing conditions for "soft" ejection and deposition of biological molecules without significant modifications of the chemical structure and functionality.

In this work, MAPLE is used for the deposition of wtbOBPs as active material on SAW devices and FTIR and AFM analysis were used to determine the chemical, morphological and surface wettability of the protein thin films. The homogeneity, the density and the roughness of the obtained coatings were correlated to the laser parameters. The capabilities of MAPLE were demonstrated by the responses of the SAW biosensors to concentrations of octenol and carvone, and, in particular, by the different sensitivities obtained changing the laser parameters.

2. Material and methods

2.1. MAPLE deposition of wtbOBPs on SAW devices

The purification of wtbOBPs was performed as described in [7]. The target for MAPLE system was prepared by dissolving a protein solution (Tris/HCl, 10 mM, pH = 8) in distilled water (0.2% in weight). The MAPLE set-up was described elsewhere [11–14]. Shortly, a "Surelite II" pulsed Nd:YAG laser system (Continuum Company) (266 nm, 6 ns pulse duration, 10 Hz repetition rate and a laser spot size of 0.02 cm^2) was used to irradiate the frozen targets. The laser fluence used in this study was set at 200 or 320 mJ/cm^2, while the pulses were varied between 12k and 46k pulses.

The substrates were 2-port SAW resonators fabricated on α-quartz substrates (ST-cut, *x* propagation) with metallic electrodes made of a thin Au film (2 nm thick) [7]. Each substrate was cut in slide ($25 \times 6.5\ mm^2$) containing 6 resonators and was kept at room temperature during the deposition.

2.2. Thin film analysis and testing

2.2.1. Morphological and structural studies

The morphological characterizations of wtbOBP coating deposited on the surface of SAW resonators were performed by AFM with a "XE 100 AFM Setup" from Park. The measurements in non-contact mode were performed to analyse the films surface roughness and morphology on several different areas.

The structure composition of the deposited proteins was investigated by FTIR spectroscopy with a Jasco FT/IR-6300 type A spectrometer in the range 400–7800 cm^{-1}. All spectra were obtained by transmission measurements, 16 scans and with CO_2/H_2O correction. Only the 500–4000 cm^{-1} interval of the spectra was chosen for comparison; the signal intensity is not relevant because the thicknesses of the measured samples were different.

2.2.2. Ultrasonic characterizations

The SAW biosensors were tested before and after wtbOBP depositions by microwave probes using a Network Analyzer. The resonance frequency shifts of SAW devices point out the change in mass on the sensor surface due to the protein adhesion. The surface densities of the wtbOBP coatings obtained with different laser parameters were calculated considering the mass sensitivity of SAW devices as reported elsewhere [17].

2.3. SAW biosensor system and measurement setup

SAW devices are used as frequency control elements in the feedback path of RF oscillators. This configuration provides a simple, effective and accurate method for monitoring small variations in SAW velocity typical of the SAW biological- and chemical-based sensors. Details on the conditioning electronics are reported in [7]. The SAW biosensor responses, given by frequency shifts of the oscillators, were measured with a frequency counter (HP 53131A) and a multiplexer module (Agilent 34980A and 34941A). All data were acquired using a custom LabVIEW™ routine. The frequency of a uncoated device was also measured to evaluated the sensing capability of the wtbOBP coating layers. The SAW biosensors were tested in N_2 atmosphere upon exposure to concentrations of octenol and carvone vapours. To obtain different concentrations of odorants, sensors were exposed to a total flux of 100 sccm controlled by two flow meters: the main for the gas carrier and the second for the odorant. Different concentrations of vapour were obtained fluxing N_2 in a bubbler containing pure and liquid odorant at room temperature. Before measurements, the frequency baseline was obtained exposing the SAW biosensor to a flux of pure N_2. Then, the odorant concentration was added to the system until saturation of the frequency responses was reached. For each odorant, measurements at different concentrations were performed and the frequency shifts at saturation were recorded to evaluate the sensitivity. Finally, the detection limits were calculated considering a maximum noise level of 10 Hz.

3. Results and discussion

3.1. Morphological and structural results

The morphology and homogeneity of the deposited active layers onto the SAW device are directly correlated to its response towards the tested compounds, which imply the necessity of morphological surface analysis and correlation of its characteristics with the deposition parameters.

Together with number of pulses, laser fluence is one of the main determinant parameter for the deposition characteristics, not only structural, but morphological as well. In this study, the influence of both laser fluence and number of pulses on the morphology of the deposited protein is shown by the 3D AFM images of the deposited (Fig. 1a–f) and drop cast protein thin films surfaces (Fig. 1g).

For the two laser fluence used, although the surface of the deposited wtbOBPs protein thin films was characterized by the presence of granular structures, the films were uniform and homogenous. The typical heights of the grain like structure were in the range of 50–150 nm and the average surface roughness (measured over $40 \times 40\ \mu m^2$) calculated for several areas and samples had maximum values of 26 ± 0.6 nm. There was only a 10% decrease in the average surface roughness calculated for the surfaces obtained with low fluence.

However, the grains seem to agglomerate and form island like structure, with porous aspect, when the number of pulses increased from 12k pulses to 46k pulses for both fluences used (Fig. 1b, c, e and f). It can be seen that cracks may appear when larger number of pulses are used (Fig. 1c and f).

These features can be explained by the size and speed of the matrix/proteins clusters generated during the ablation of the MAPLE target. In our case the process can be compared to laser ablation of a solution with spatially heterogeneous absorption, as the solvent is almost transparent to laser radiation, so the laser energy