Fabrication of optical gratings coated with Si_3N_4 for efficient immunosensing monitored by Optical Waveguide Spectroscopy

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Biosensors are nowadays a powerful tool to enable the detection of biological interactions. The novel Electrochemical Optical Waveguide Spectroscopy technique (see figure 1) incorporates an electrochemical cell to a grating coupler optical biosensor [1]. In this way, this system allows obtaining electrochemical measurements such as cyclic voltammetry or impedance spectroscopy while monitoring the immobilization kinetics through optical measurements with similar accuracy than Surface Plasmon Resonance based techniques. Moreover, by numerical methods, it is possible to simulate both the refractive index change and the thickness of the adsorbed film and its mass, exhibiting a very high sensitivity of 1 ng/cm^2. The system keeps this sensitivity measuring changes at the sensor surface closer than 200 nm [2].

In the field of optical label-free biosensing, the most used transducer is a sensor chip with gold surface which presents an easy functionalization and provides information about the success of the biomolecular adsorption on the surface. Depending on the application, the possibility of choosing a surface would remain a challenge. In the case of the grating couplers, the substrate must be a transparent material to allow the light coupling in the waveguide. There are only a few materials commercially available covering the sensor chip: insulators such as SiO_2, TiO_2, Ta_2O_2, or transparent conductive materials like the Indium Tin Oxide (ITO), which allow electrical measurements to be coupled with the optical sensing.

Silicon nitride is one of the most important materials for electronic-based biosensors [3]. Several widely available methods for the immobilization of biological elements on this substrate rely on silanization procedures. But for immunosensing applications, a fast-to-functionalize and efficient procedure for the immobilization of antibodies onto the surface by covalent binding is required. In figure 2, a new approach for the direct immobilization of CHO groups onto silicon nitride substrates has been described previously [4]

To test the functionality of such an approach, commercial optical grating chips were covered with a Si_3N_4 layer by Low Pressure Chemical Vapor Deposition. Silicon nitride offers a number of advantages compared to other coating materials, such as the absence of undesirable impurities and the good control of the film composition and thickness. This is especially important for ultrathin layers used in optical spectroscopy measurements. The silicon nitride surfaces were chemically oxidized and subsequently silanized with an aldehyde organosilane self-assembled monolayer by using the vapour phase method leading to the direct and covalent binding of anti-human serum albumin (anti-HSA) antibodies and used for the specific detection of different concentrations of HSA proteins with a sensitivity of 1 ng/cm^2 and detection limit of 10^{-8}M (Figure 3).

References:

[1] L. Diéguez et al., Soft Matter, **5** (2009) 2415-2421.
[2] J. Vörös et al., Biomaterials **23** (2002) 3699-3710.
[3] A. Tliti et al., **25** (2005) 490-495.
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Figures:

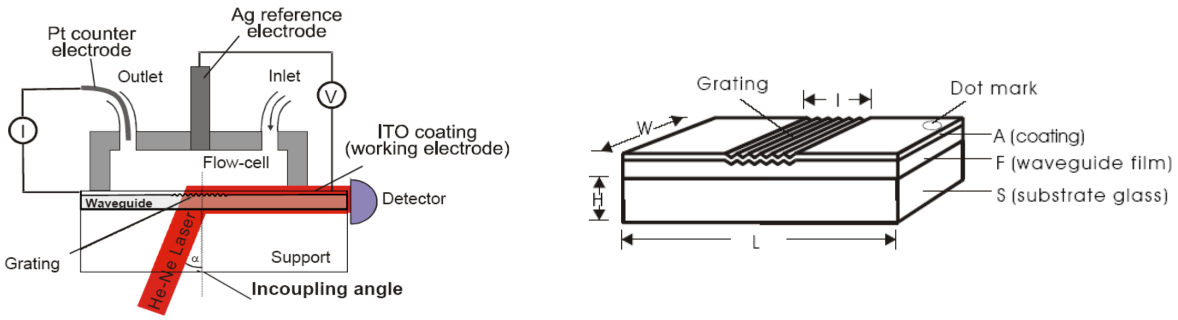

Figure 1: (left) Electrochemical Optical Waveguide Spectroscopy set-up.
(right) Optical grating coated chip.

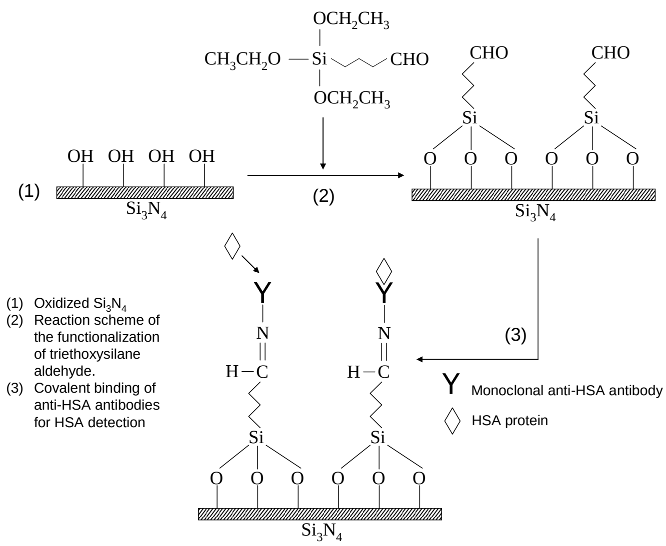

Figure 2: Direct immobilization of CHO groups onto silicon nitride f or the binding of anti-HSA antibodies.

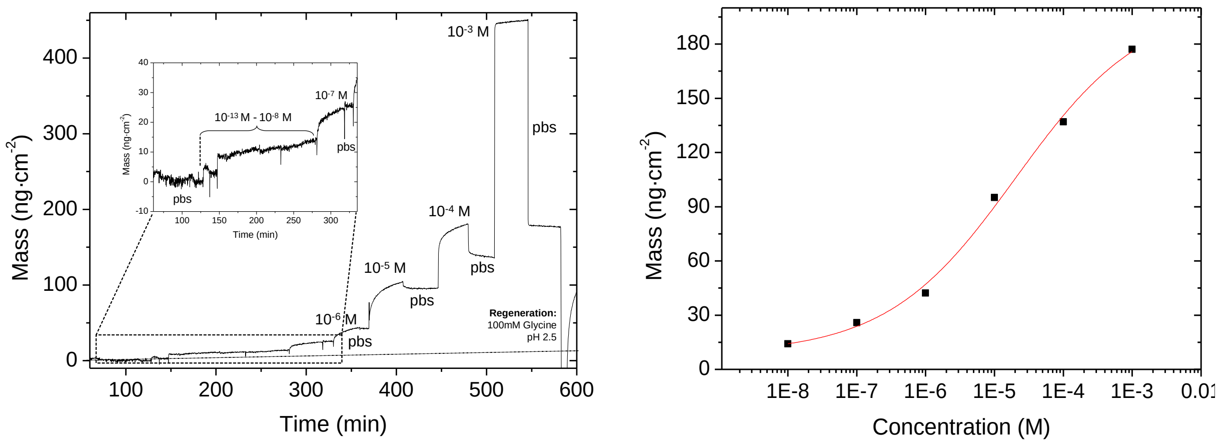

Figure 3: (left) HSA immunosensing reaction monitored by Optical Waveguide Spectroscopy.
(right) Relationship between the adsorbed mass and the concentration.