Surface Characterization of a Direct, Label-Free Piezoelectric Immunosensor Platform

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Abstract

The present work provides surface characterization for all steps involved in the fabrication of a direct label free piezoelectric immunoprobe, using techniques such as Ellipsometry, Atomic Force Microscopy, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) and X-Ray Photoelectron Spectroscopy (XPS). Active amino groups were successfully provided on the quartz crystal surface through plasma deposition of allylamine polymer film (ALL) [1] and were further utilised for antibody (Ab) immobilization. Results from this work show the possibility of producing simple, direct piezoelectric immunoprobes through appropriate antibody orientation without the need for labeled compounds. The combination of surface analytical, optical and mass balance techniques is confirming the effectiveness of these immunosensor fabrication strategies.

Keywords: Quartz Crystal Microbalance (QCM), immunosensor, surface characterization, gold nanoparticles

1. Introduction

The choice of antibody immobilization strategy is a key process in immunosensing device development, influencing the sensitivity and reproducibility of the sensors. Orientation controlled methods of antibody immobilization through its Fc region include the use of protein A (PA) [1], a cell wall protein from *Staphylococcus aureus,* that has a natural affinity for the Fc region of IgG molecules [2]. In this work we have made use of these properties combined with the cross linking capacity of glutaraldehyde (GA) [2,3] and the surface increase capacity of gold nanoparticles (GNP) [3, 4]. Both routes were investigated on the plasma deposited allylamine (ALL) on the SiO_2-covered gold surface of the Quartz Crystal Microbalance (QCM) crystal for further development into a piezoelectric immunosensor.

2. Materials and methods

2.1. Allylamine plasma deposition

ALL films were deposited on QCM SiO_2-covered crystals, in a cylindrical, capacitively coupled plasma reactor with a modified active electrode. An Ar discharge was maintained with a RF source (Dressler Integro 133) powered at 20 W during 20 min with the pressure set at 45 mTorr, Ar flow of 6 sccm and an equivalent ALL flow of 12 sccm.

2.2. Antibody immobilization process

Two different antibody immobilization routes were investigated. The first involved the use of GA as a cross linker (2.5% in PBS pH 7.4), followed by PA (250 µg mL^{-1}) for optimal antibody (Ab) orientation and consecutively highly sensitive antibody-antigen interaction. The second route made use of manometer-sized gold (20 nm) particles (GNP) in combination with PA. These two routes are shown schematically in figure 1. The unstable Schiff base produced by the reaction between the active amino groups and the aldehyde function of the GA is stabilized using sodium cyanoborohydrate for reducing the double bond of the imine.

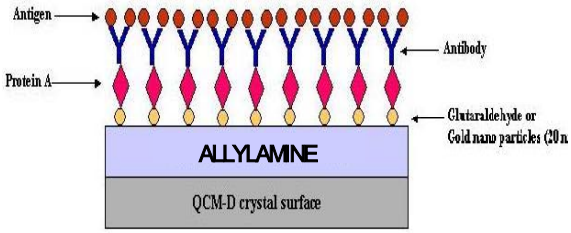


Figure 1. Proposed direct, label-free immunosensor schemes.

2.3. Film Characterization

XPS analyses were performed using an Axis Ultra Spectrometer (KRATOS, Analytical, Manchester, UK) equipped with a monochromatic A1 150 W (hv=1486.6 eV) source, operating at 150 W. The presence of active primary amino groups of the ALL film was investigated by XPS analysis of the derivative formed by exposure to trifluoromethylbenzaldehyde (TFMB) in gas phase at 45°C. An ION-TOF IV (Munster, Germany) was used for all ToF-SIMS analyses. Oscillating frequency changes (ΔF) were

detected and plotted in real time using a Quartz Crystal Microbalance (QCM-D, Q-Sense, Gothenburg, Sweden) at the resonant frequency of 5 MHz and at the 15, 25 and 35 MHz overtones. Topographic imaging of the samples was performed using Atomic Force Microscopy NT-MDT, (Moscow Russia) and imaging ellipsometry from Nanofilm Technology GmbH (Göttingen Germany).

3. Results

3.1. XPS results

ALL deposition resulted in an N/C ratio of 0.23 whereas oxygen content was below 7% with an O/C ratio of 0.08. The C1s fitting showed the presence of C-N and CNO bonds and some COOR functionalities. Figure 2 shows the XPS survey and high-resolution spectra of the derivatized ALL. The C1s spectrum of derivatized sample (with TFBA) shows the presence of the 292.88 CF3 component, indicating the appearence of derivatized primary amino groups to with an activity of about 1.5 %.

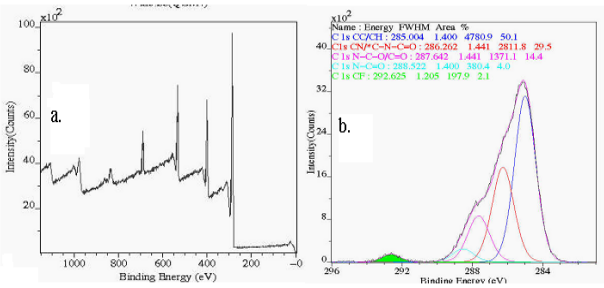


Figure 2. XPS survey and C1S high-resolution spectra for the determination of primary amino groups.

N/C and O/C ratios from XPS analysis for both immobilization schemes are shown in figure 3 (a and b). Quantitative analysis of the ALL-GA sample indicated a decrease of the N/C ratio (0.21) and an increase of the O/C ratio up to 0.13. This shows that the GA is successfully immobilized on the ALL surface. In the GNP-PA route the addition of GNP (8% atomic concentration) led to a decrease of the N/C ratio, whereas O/C ratio increased slightly. Concerning the presence of PA on the modified surfaces, quantitative analysis indicated that the N/C ratio decreased to 0.18. PA deposited on Au resulted in an N/C ratio of 0.13 whereas when the PA was immobilized directly on ALL, this ratio was 0.23.

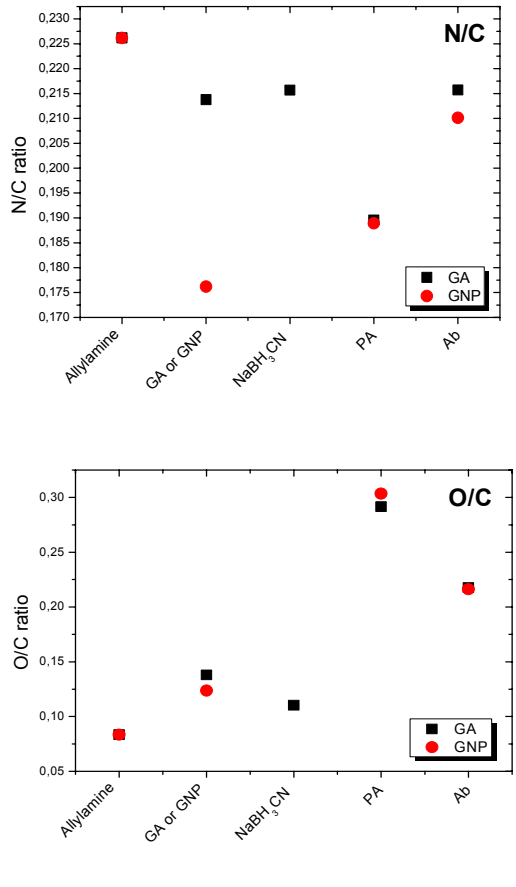


Figure 3. N/C (a.) and O/C ratios (b.) from XPS analyses for all functionalisation steps involved in Ab immobilization.

This, as well as the similarity of the C1s spectra, are indications of the successful immobilization of PA onto the functionalized surfaces. The Ab immobilization on the other hand cannot univocally be determined by XPS without the use of labelled compounds. Even though the N/C ratio increased slightly (0.21) it is still similar to the ALL (0.23).

ToF-SIMS results

ToF-SIMS spectra provided information about each immobilization step. The ion fragments for the characterization of the ALL and ALL-GA-$NaBH_3CN$ layers are shown in figure 4. Decrease in ALL fragments and appearance of C_5H_7O are indicative of GA coverage.

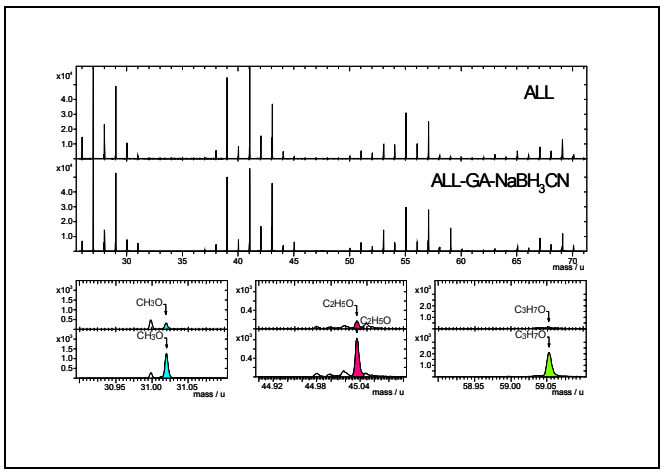


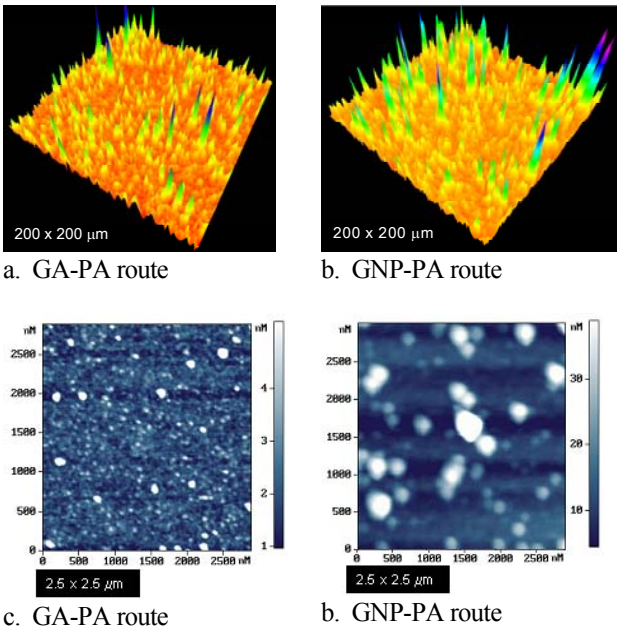
Figure 4. Ion fragments from Tof-SIMS analysis attributed to ALL and ALL-GA-$NaBH_3CN$ layers

3.2. Ellipsometry and AFM measurements

Ellipsometry allows the large scale imaging of surfaces and provides information about the thickness of each layer. The thicknesses layer by layer are shown in table 1 and delta maps of surface for each functionalization step are shown in figure 5 (a and b), for each final immunoprobe. It can be seen that even though for the GA-PA route a thicker layer of PA is immobilized than in the in the GNP route, the subsequent Ab layer thickness is less important (d=34 nm vs 87 nm). The GA-PA method results into a smoother PA surface, whereas the rougher PA surface in the GNP method clearly favors Ab loading. This is confirmed by the AFM pictures shown (figure 5 c and d), where at equal scales a noticeable difference is shown by the two images, with the GNP route resulting in bigger Ab clusters.

Layer thickness (d)	GA-PA	GNP-PA
ALL	**319**	**319**
GNP	**-**	**7**
GA+$NaBH_3CN$	**18**	**-**
PA	**84**	**21**
Ab	**34**	**87**

Table 1. Thickness of added layers determined by ellipsometry



a. GA-PA route b. GNP-PA route

c. GA-PA route b. GNP-PA route

Figure 5. Ellipsomery delta maps (a and b) and AFM pictures (c and d) indicating Ab adsorption for each of the two routes studied.

3.3. QCM results

All main intermediate steps in the antibody immobilization process as well as the Ab-Ag rection were monitored in 'real time' using the QCM. Figure 6 shows typical QCM ΔF curves, for PA, Ab adsorption and Ab-Ag interaction for the anti BSA-BSA couple.

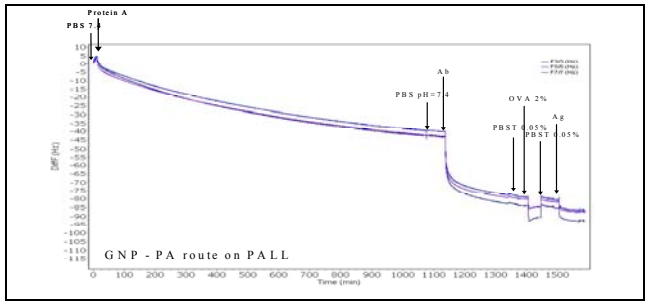


Figure 6. F curves obtained using the QCM for monitoring PA, Ab adsorption and Ab-Ag interaction on ALL-GNP modified crystal surface.

Mass contributions from each antibody immobilization route for every step were determined by the QCM measurements (figure 7).

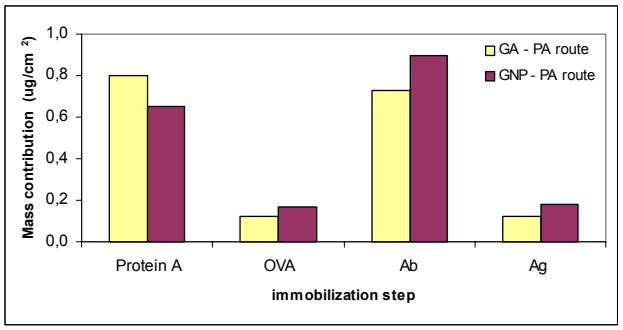


Figure 7. Mass contributions from every immobilization step for each of the Ab immobilization routes studied.

It can be seen that the protein loading for the GA-PA route was slightly higher for the GA-PA route, whereas the Ab loading was more important for the GNP-PA route. This is compatible with the findings from the ellipsometry and AFM studies and could be attributed to the increased roughness provided by the GNP. As a consequence Ag loading was higher for the GNP-PA route.

4. Conclusion

In this paper we propose the fabrication of simple direct label-free piezoelectric immunoprobes, utilizing PA as antibody orienter. The use of GNP seems to be a more efficient way to immobilize Abs, thanks to their capacity to amplify the available active area. Through the combination of surface characterization techniques, it was possible to evidence the presence of each active layer. The use of QCM provides a simple acoustic transducer able to monitor in real time the adlayers for surface functionalization towards immunosensor development as proposed here, and to monitor the Ab-Ag reactions functioning this way as a simple imunosensing device.

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