PROOF-OF-CONCEPT EXPERIMENT REPORT

CHARACTERISATION OF PROTEIN COATING AND ELECTRODE SURFACE OF BIOSENSORS

Author(s): Laura Andolfi

Istituto Officina dei Materiali - CNR-IOM

Q2 Building - Area Science Park Strada Statale 14 km 163,5 - 34149 Basovizza – Trieste

ISTITUTO OFFICINA DEI MATERIALI

Proof-of-Concept experiment details

Main aim of the proposal

The development of biosensors able to identify and quantify biomarkers in physiological samples for the rapid diagnosis of myocardial infarction, as well as for other pathologies, requires the precise assembling of linker that immobilize antibody in oriented and functional way. For this purpose the company used two strategies: polymetric scaffolds based on carbon nanostructures and self-assembled monolayer on gold electrode.

The problem experienced was that electrodes with same functionalization and coating procedure show different performances, which compromises the reproducibility of the sensor response. To understand the reason of this behaviour we were asked for the identification of a technique suitable to visualize the protein assembled on the electrode to evaluate the coating quality. Moreover tha same technique should also be able to visualize the self-assembling on the gold electrode to evaluate quality of functionalization of the surface.

Approach and results

We proposed epifluorescence microscopy to characterize the protein coating on polymeric and gold electrodes. Once the protein is bound to the first antibody immobilised on the electrode, a second antibody labelled with a fluorescent probe recognizes and binds to the protein and it is used for fluorescence microscopy. In Fig. 1 the preliminary results obtained with the antibody assembled on the polymeric scaffold based on carbon nanostructures (CN).

At a first analysis it was clear that the fluorescence signal from the antibody, can highlight the homogeneity of distribution on the electrode, independently from antibody concentration. Likewise, also the antibody concentration can be assesed by the same technique.

To better evaluate the proposed approach, we performed a comparison of CN electrodes coating with gold electrodes where the antibody immobilisation was achieved by using a thiolated self-assembled monolayer. The results are shown in Fig. 2. In general we observed that for the gold electrodes, the fluorescence signal is higher and more spread on the electrode, while for the CN electrodes the signal is again low and poorly spread on the surface. These results are in agreement with the performance of the electrodes as tested by the company, which indicates that the proposed technique can be adopted for the required characterization.

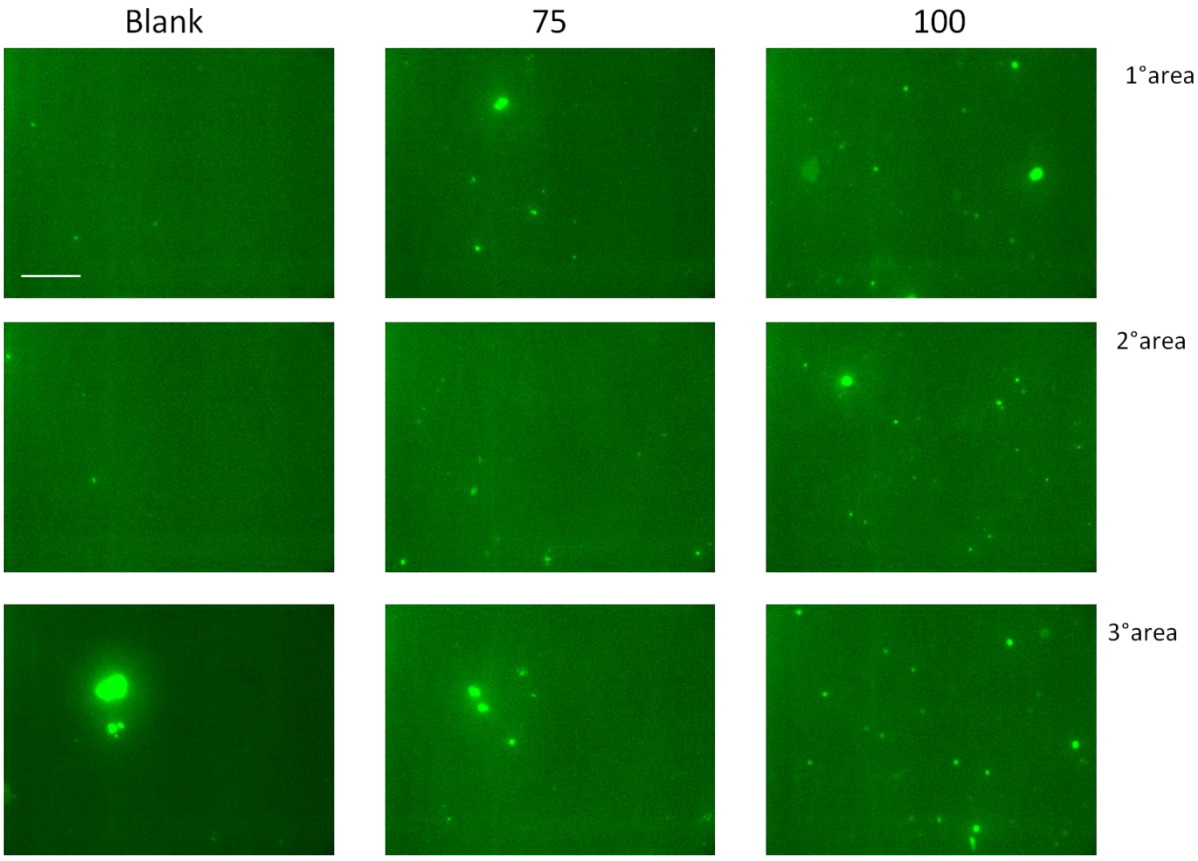

Fig.1: Fluorescence images of specific antibody bound on CNTs electrode acquired with a 40x objective; FITC filter. Blank is bare CNTs in absence of antibody, 75 and 100 are different concentration of antibody. Images were acquired on 3 different areas for each samples (Bar 50 µm)

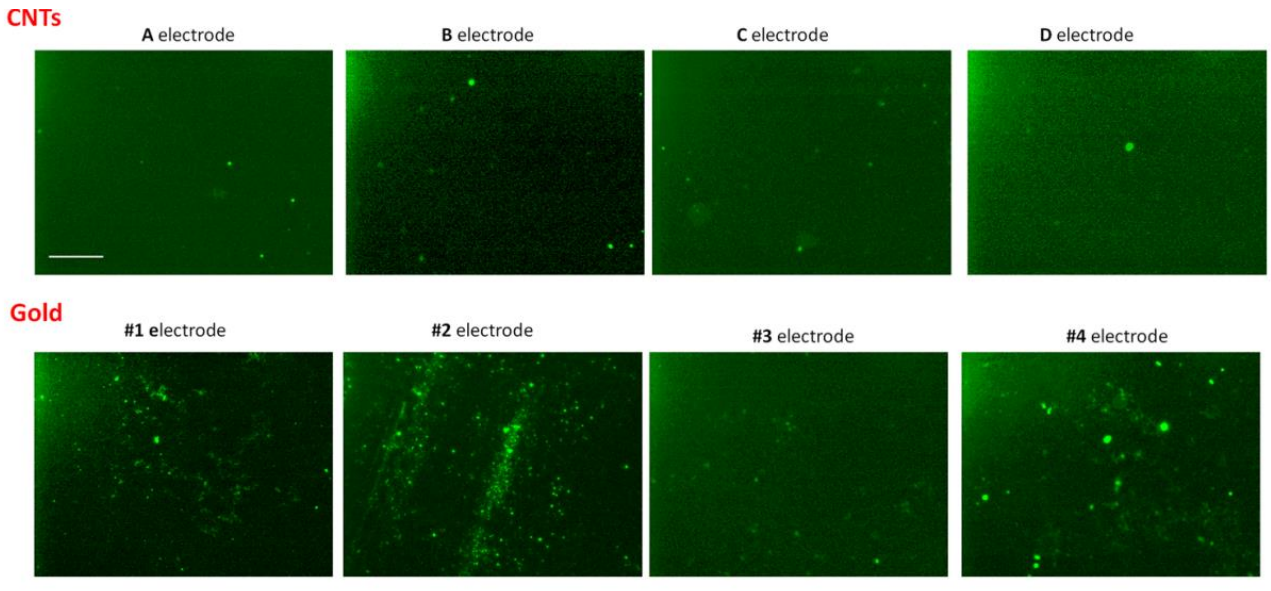

Fig.2: Fluorescence images of antibody bound on CNTs electrode and gold electrode acquired with a 40x objective; FITC filter. Bar 50 µm.

To evaluate the reproducibility of antibody coating on functionalized gold electrodes we acquired fluorescence images of several electrodes (N=14) (Fig.3). Same electrodes were also tested for antigen recognition (Fig.3).

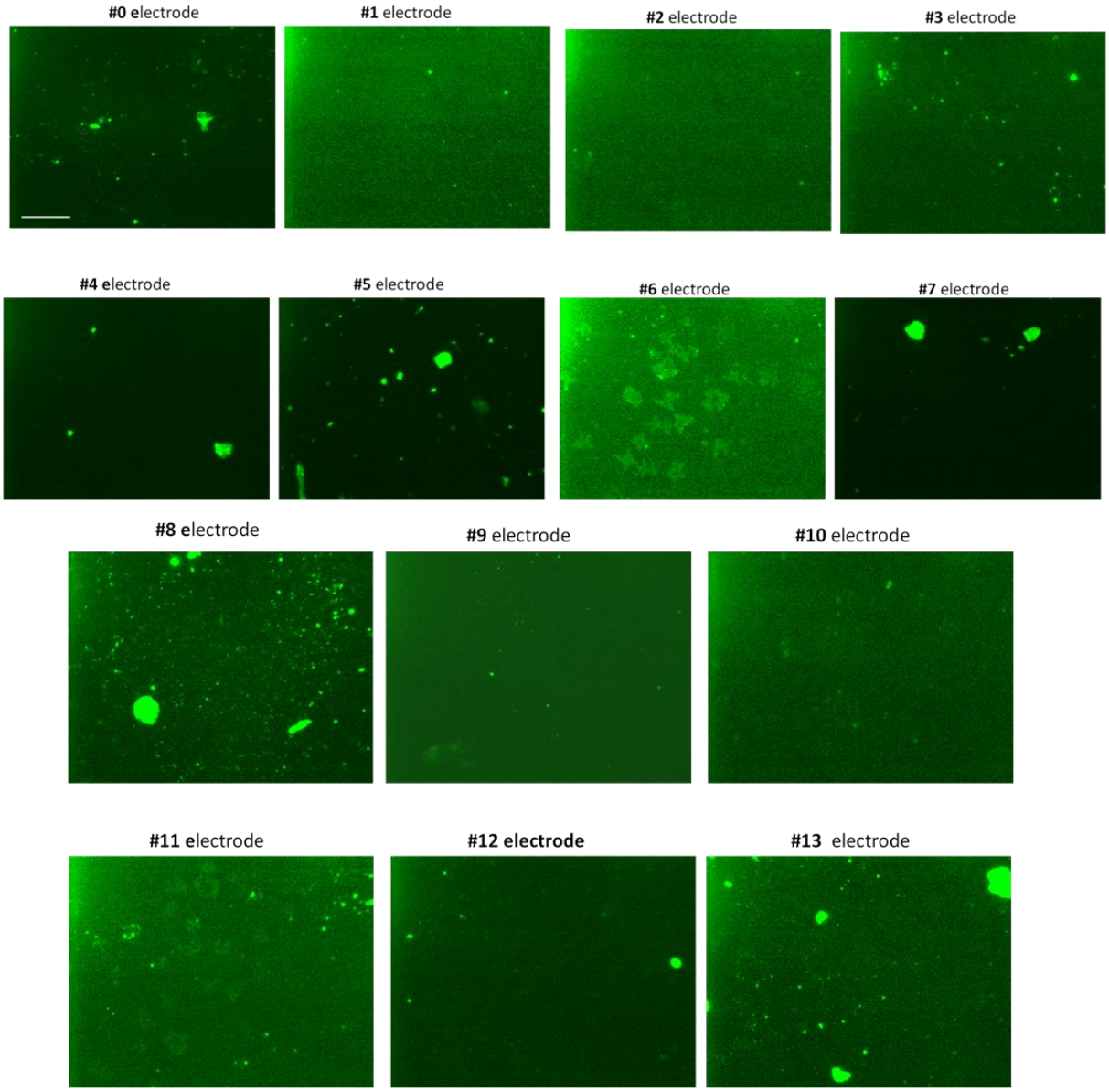

Fig.3: Fluorescence images of specific antibody bound to SAM functionalized gold electrodes acquired for several electrodes, 40x objective and FITC filter (Bar 50 µm).

The epifluorescence microscopy was able to highlight a serious issue of coating heterogeneity that varies among the samples: the fluorescence signal was higher and more homogeneous on some electrodes, while for others the signal was not homogenous indicating a poor protein coating and, as result, different performances of the electrodes.

These images were in agreement with the electrode performances observed by the company. Hence these results indicated that, even with the gold electrodes, the difficulty to obtain a reproducible and high performance of the electrode is likely associated with the difficulty to obtain a reproducible coating and with a high antibody density, and a dedicated characterization of the coating homogeneity is required to standardize the sensor production.

To visualize the surface of the gold electrodes we also proposed the use of Atomic Force Microscopy (AFM). Images in contact mode were acquired in air by using a NanoWizard II Atomic Force Microscope (JPK Instruments, Berlin, Germany). Some of the test images acquired are shown in Fig. 4. The AFM was used also to quantify the roughness of the bare gold which was very high and did not allow us to observe a real difference with the gold+SAM electrode.

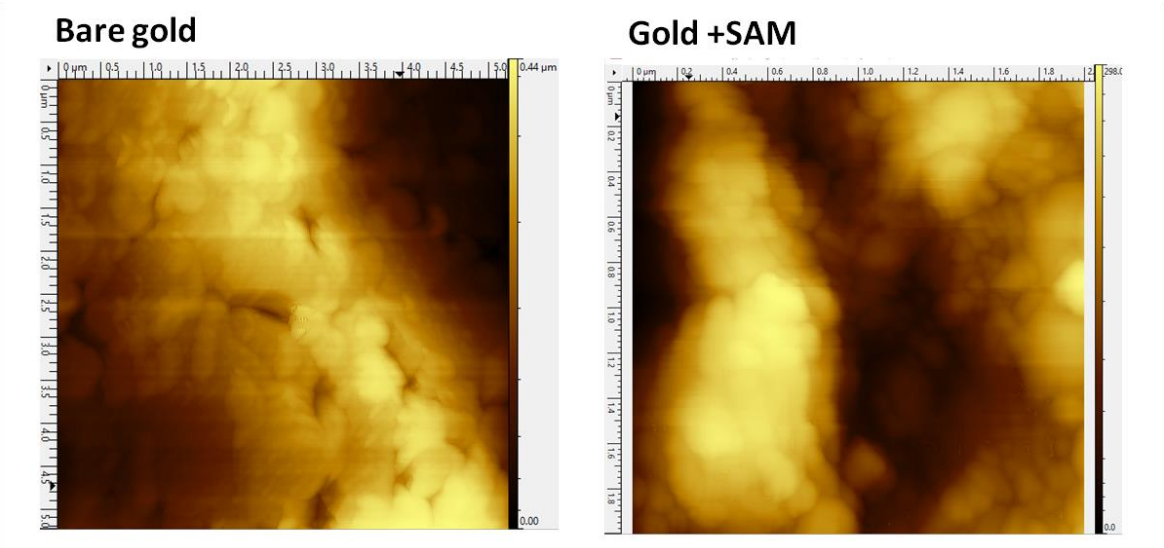

Fig.4: Contact mode AFM images of bare gold electrode and self-assembled monolayer of on gold electrode (cantilever Bruker MLCT K=0.02N/m).

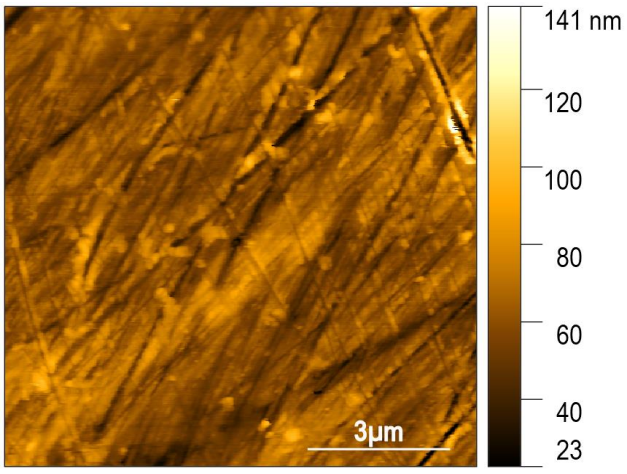

Fig.5: Contact mode AFM images of pure flat gold electrode (cantilever Nanosensors ATEC –FM-20 K=0.7-9 N/m)).

A second set of electrodes made with gold having higher purity and differently fabricated to achieve a more flat surface was images (Fig. 5). In this case we observed a more uniform surface with a notable reduction of surface roughness, which decreases from 117 ± 50 nm of the previous electrodes to 11 ± 2 nm for these new ones (roughness evaluated on 10 µm x 10µm). These results indicated that AFM is a suitable technique to investigate the morphology of these gold electrodes.

Summary and final remarks about the effectiveness of epifluorescence and AFM to evaluate the protein coating quality

To summarize the epifluorescence microscopy can provide a good and reliable analysis of the protein coating on the different electrodes. AFM images demonstrated that the gold electrodes were rough and this might be one of the possible source of variability of SAM formation and as result antibody organization and orientation. The roughness of the electrode surface could play an important role in the assembling of organic linker and then protein assembly, in terms of organization and antibody orientation. Since probably a reduced surface roughness can help to obtain a more controlled assembling of antibody on the electrode, which is fundamental issue for the fabrication of a reliable high performance biosensor, we recommend an extensive characterization of the electrodes with this technique.

This report has been written by Laura Andolfi (Trieste, 13/12/2021)