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Oriented transfer of proteins for biosensor applications

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INTRODUCTION: The orientation of antibodies upon their immobilisation on different surfaces is a crucial factor affecting their recognition properties. This phenomenon is strongly dependent on surface properties such as composition, reactivity, wettability, roughness etc. [1-2]. Some polymeric substrates, such as Zeonor, are used in bio-medical or -technological devices, mainly because of their interesting optical properties, low production time and cost. However, their surface modification/functionalisation is more difficult to perform as compared to other widely studied metallic substrates (such as gold or silicon).

Our objective is to overcome these problems by performing a two-step experiment. The first step uses the advantages of well-known modified metallic/semiconducting substrates (in particular organosilane-modified silicon) for the orientation of antibodies [3-4]. The second step consists of transferring the oriented antibodies onto a second substrate, which may have unfavourable characteristics. This transfer process is based on the template-imprinted nanostructures technique developed by Ratner et al. [5]. In this poster, we focus in particular on the transfer process of proteins from one substrate to the other.

METHODS: The transfer process may be described briefly as follows: a selected protein (fibrinogen, avidin or antibody) is microcontact printed onto a SiO₂ surface, a sugar layer is then spin coated on top of the patterned surface. This is followed by the deposition of a polymer-like layer using a 13.56 MHz RF capacitively coupled plasma in a PECVD reactor. This upper polymer-like layer is then glued to a second substrate. Finally the substrates are separated. The microprinted pattern of proteins is therefore transferred to the second substrate. During these experiments, the wettability, film thickness, film morphology were characterised using contact angle measurements (CA), spectroscopic ellipsometry (SE) and atomic force microscopy (AFM), respectively. Fluorescence microscopy was also used to determine the presence of tagged-proteins on the transferred substrate.

RESULTS. We first demonstrate (using AFM) the transfer of microprinted patterns of non oriented

proteins (fibrinogen – Fig. 1 left). We then show that, when using Cy5-labelled anti-human IgG, such patterns are composed of proteins (as revealed by fluorescence microscopy – Figure 1 right). Finally the function of the transferred proteins is investigated using antibodies, which are recognised by antigens after transfer.

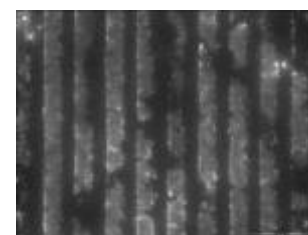
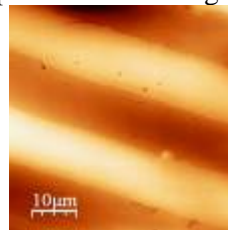


Fig. 1: AFM image (50µm) of fibrinogen microprinted lines after transfer process (left). Fluorescence image of microcontact printed lines of Cy5-labelled anti-human IgG after transfer process (right).

CONCLUSIONS: In this poster, we have demonstrated the transfer of protein from one substrate to another without modifying the protein and its properties. Particularly, the recognition between antigens and antibodies was also proved after transfer of antibodies.

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