

A PROTEIN-RESISTANT POLYMERIC INTERFACE FOR BIOAFFINITY SENSING

J. Vörös¹, N.P. Huang¹, S.DePaul¹, M. Textor¹, J. A. Hubbell², N. D. Spencer¹

¹ *Laboratory for Surface Science and Technology and the* ² *Institute of Biomedical Engineering, ETH Zürich, Switzerland*

INTRODUCTION: A novel platform technology for tailored interfaces in sensor applications have been developed based on PEG derivatised cationic polymers. These molecules spontaneously assemble on anionic oxides and provide highly protein resistant surfaces. Furthermore, they can be functionalised to improve selectivity and sensitivity in bioaffinity sensing applications. In the model biotin-streptavidin system, a specific sensor response to streptavidin was achieved by the functionalising the metal oxide surface with biotinylated PEG-containing polymer.

Non-specific protein adsorption is a problem that plagues a wide array of biomedical applications such as serum contacting sensors. Protein adsorption often leads to a sensor response that is not analyte specific. Since metal oxides are commonly present in the biosensor applications, a method for measuring only specific target analytes while eliminating non-specific binding on oxides would be an important development in biosensor technology.

A class of materials based on poly(ethylene glycol) (PEG), a hydrophilic polymer with many properties similar to water, has been found to be remarkably resistant to protein adsorption, and many strategies for the immobilisation of PEG onto surfaces have been developed. [1] The polymer, poly (l-lysine)-g-poly(ethylene glycol), consists of a poly(l-lysine) (PLL) backbone that has been grafted with PEG side chains, some of which were biotinylated. The assembly of the polymer film onto the surface is based on the electrostatic interaction of the positively charged polymer backbone and the negatively charged metal oxide surfaces. These properties make this interface design a useful platform for many sensing applications. Among which, the Optical Waveguide Lightmode Spectroscopy (OWLS) technique was chosen because it is an online, direct, label-less and highly sensitive technique with high throughput capabilities.

METHODS: The OWLS technique involves the incoupling of the evanescent field of a He-Ne laser into a planar waveguide which allows for the direct online monitoring of macromolecule

adsorption.[2] It is highly sensitive (i.e. $\sim 1\text{ng}/\text{cm}^2$) up to a distance of 100 nm above the surface of the waveguide. Furthermore, a measurement time resolution of 3 seconds allows for the study of adsorption kinetics

10mM HEPES (pH 7.4) buffer solution and SiO₂-TiO₂ waveguides (Microvacuum Ltd, H) were used for all of the experiments. The modified waveguides were prepared in situ by exposing the waveguides to 1 mg/ml polymer solution for twenty minutes in a flow cell apparatus, followed by rinsing with buffer. Then the PLL-g-PEG-biotin modified waveguides were exposed to Control Serum N (human) (Roche, CH) for thirty minutes and then washed in buffer. At the end of the measurement selectivity was tested with 50 $\mu\text{g}/\text{ml}$ streptavidin (SIGMA, USA) for 20 minutes followed by a rinse with buffer.

Mass data were calculated from the thickness and refractive index values derived from the mode equations. [2] All OWLS experiments were conducted in a BIOS-I OWLS instrument (ASI AG, CH).

RESULTS AND DISCUSSION: The first part of the experiment, shown in Figure 1, demonstrates the protein resistant behaviour of the treated oxide surface.

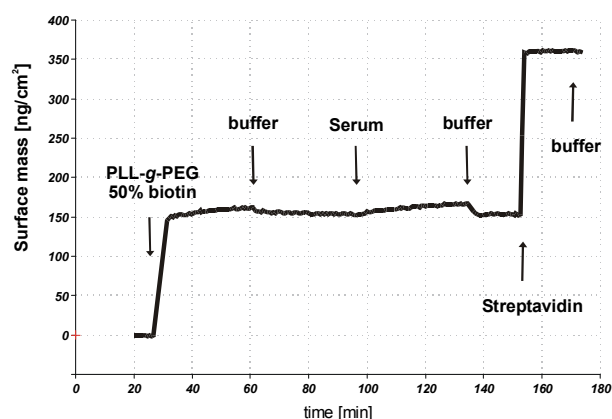


Fig. 1: Selective sensor response of the biotin derivatised PLL-g-PEG on streptavidin exposure.

Waveguides coated with the PLL-g-PEG-biotin exhibit protein adsorption of less than 2 ng/cm² after exposure to human blood serum. This value is two-hundred-fold lower than the adsorption seen on untreated waveguides. The second part of the experiment demonstrates the specificity of the sensor. A monolayer of streptavidin quickly and irreversibly adsorbs onto the same PLL-g-PEG-biotin functionalised surface after exposure to a 50 µg/ml streptavidin solution.

The adsorbed amount of the PLL-g-PEG polymer onto a SiO₂/TiO₂ surface depends on the pH and ionic strength of the buffer solution in which it is dissolved. For example, at pH values higher than the pK of the polymer (pK~10) and lower than the isoelectric point of the surface (IEP~4), the mass of adsorbed polymer decreases. Subsequent human serum albumin adsorption experiments at pH 7.4 indicate that the protein resistant property of the PLL-g-PEG layer depends on the mass of polymer adsorbed [2].

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