

IMMOBILISATION OF STREPTAVIDIN ON POLYSTYRENE

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INTRODUCTION: The measurement of certain analytes in blood is an important aspect of clinical diagnosis. There is a need for tests (immuno assays) with high sensitivity to identify and detect enzymes, therapeutic drugs or other specific proteins. In order to achieve this we use a biosensor based on fluorescence measurement [1]. To improve analytical performance, allyldextran monolayers were coated on gamma-irradiated polystyrene wells (γ -PS). This study reports the coating of polystyrene wells with allyldextran and chemical properties of the surface analyzed by X-ray Photoelectron Spectroscopy (XPS), contact angle and Total Internal Reflectance Fluorescence Spectroscopy (TIRF) measurements based on a diploma work of one of the authors [2].

METHODS: Surface modification procedures are based on allyldextran layer absorbed on the bottom surface of the polystyrene wells. Allyldextran grade (MW 150'000, Pharmacia 253758-01) provided by Pharmacia is used for all experiments. It is a form of dextran substituted with allyl groups.

Allyldextran solutions with different concentrations 0 – 10'000 $\mu\text{g/ml}$ of allyldextran are prepared in water (18.2 M Ωcm at 25°C) which has been degassed with nitrogen for about 1h. The wells are filled with the allyldextran solution and stored in a closed box for 16 ± 3 hours. After washing again with water the γ -PS chips are used for further treated with 30mM sodium (meta)periodate NaIO_4 (Fluka 71859). After washing with PBS and coupling in a bicarbonate solution (100mM NaHCO_3 , pH 9.3) with 40 $\mu\text{g/ml}$ of Streptavidin (Pierce cat. No. 21122) or Neutravidin (Pierce cat. No. 31000)

The avidin-biotin system has many applications in both research and technology. Due to the very high affinity ($K_a=10^{-15}\text{M}^{-1}$) of the complex.

XPS scans were performed to determine the surface composition in order to analyze changes due to the

transformations induced by surface treatment and to detect otherwise contamination. XPS of the samples was performed on a Kratos AXIS Ultra System, utilizing an aluminum K α monochromatized X-ray source ($E=1486.6\text{ eV}$) and a hemispherical energy analyzer. For this study, XPS spectra were recorded at take-off angles of 0°, 45°, 70° and 80° with respect to the surface. Spectral binding energies were referenced to the C-H peak assigned to 285.0 eV. Evaluation of the XPS data was done by CasaXPS (v. 2.11) and Tougaard-Quasas software packages.

The surface modification and its effects on wettability were analyzed using contact angle measurements. They were performed with a contact angle measuring system G10 by Krüss GmbH (Hamburg, Germany) at the DGR of EPFL (Prof. H. Harms). The contact angle measurements were performed on air-dried sample by placing a water drop on the polystyrene surface. The measured contact angle was the advancing angle between the surface and the water drop.

Total Internal Reflectance Fluorescence measurements were performed at DiaMed by an immunosensor device [3].

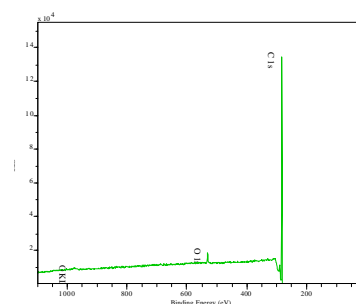


Fig. 1 XPS survey of γ -PS radiated Polystyrene

RESULTS:

A typical XPS survey for a polystyrene surface is shown in Figure 1. Carbon C1s peak and the oxygen O 1s are observed. This survey is the standard survey for the gamma irradiated

polystyrene wells prior to any surface treatment. The oxygen amount of the pure gamma irradiated samples ($\tilde{\alpha}$ -PS) is less than approx. 2 atom%.

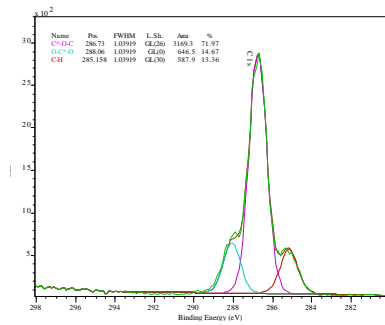


Fig. 2 C1s high resolution XPS of pure allyldextran powder

Fig.2 shows AC1s high resolution scan of pure allyldextran powder is shown in Figure 2, which is compared to allyldextran coating as shown in figure 3.

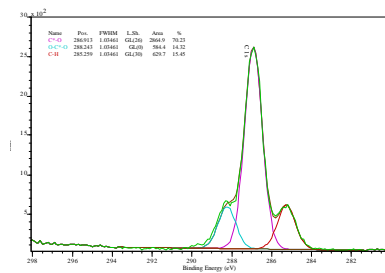


Fig. 3 C1s high resolution XPS of $\tilde{\alpha}$ -radiated Polystyrene coated with an allyldextran layer; $\theta = 0^\circ$

SUMMARY: The allyldextran coating provides a stable, hydrophilic surface on γ -radiated Polystyrene wells with repetitive XPS results. Surface treatments were optimised using XPS and contact angle measurements. Functional biotin binding assay using fluorescence measurements of surfaces treated with allyldextran activated with iodate and coupled to Neutravidin seem to indicate that the Neutravidin is immobilised. Surfaces treated with allyldextran followed by iodate activation and Neutravidin are highly hydrophilic and express very good non-specific adsorption characteristics.

REFERENCES: ; ¹ Pierce catalogue 2001-2002 ; ² C. Houhouet-Boigny, EPFL diploma thesis submitted Feb. 2002 to the Mat. Institute; ³ Patent Application WO 01/14859 A1

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