

PLASMA TREATMENT OF SOLID SURFACES FOR BIOMEDICAL APPLICATIONS

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INTRODUCTION: Functional surfaces are very important for biomaterial technology. Among the various methods used for surface modification of biomedical devices plasma process shows significant advantages [1, 2]. In particular, some reactive surfaces obtained by glow discharge down-stream plasma polymerisation of functional vinyl monomers show tailorable functionalities [3-6]. The aim of this study was to prepare specifically functionalised surfaces by means of plasma polymerisation of 4,4-dimethyl-2-vinyl-oxazolinone or isocyanatoethyl methacrylate and to demonstrate superior reactivity of these surfaces.

METHODS: Substrates included silicone-hydrogel contact lenses (Lotrafalcon A, CIBA Vision) [7], silicone wafers, polished glass, soft silicone elastomer sheets (GoodFellow). In addition highly porous tricalcium phosphate (b-TCP) coated PES membranes, prepared at the Powder Technology Laboratory of EPFL in Lausanne using a Lina-Spark™ atomizer [8, 9], were used for surface fictionalisation.

Plasma polymerisation process was carried out in a glass reactor custom built in collaboration with ACR GmbH, Germany (power supply). Rf power (27.12 MHz) was capacitively coupled to the plasma using a ring electrode from outside the glass wall and earthed bottom metal base plate flange. The electrode arrangement and power supply allowed us to work in the both CW and pulsed modes. In this reactor the samples were placed outside of the plasma zone at variable distances in down stream direction. We focused on the variation of selected plasma parameters, mainly on the distance between the plasma zone (electrode gap) and the substrate position, on the input power and variable duty cycles. Argon was used as plasma gas and as carrier gas for the “afterglow-feed” of the monomers used.

The structure of the plasma coatings was examined using XPS on silicone wafers and FTIR-ATR analysis on all kind of substrates, mainly on highly porous b-TCP coated PES membranes and silicone elastomer. The coatings' morphology and thickness were measured by AFM on polished

glass pieces and by quartz microbalance thickness monitor.

The density of the functional groups on the substrate surfaces was determined as follows: 5-biotinpentylamine was quantitatively coupled on VAL functionalised Lotrafalcon A contact lenses and the amount of biotin present on the surface was detected using the avidin-HABA reagent (ImmunoPure, Pierce). When 2-(4'-hydroxyazobenzene) benzoic acid (HABA) is added in excess of avidin, an absorption band at 500 nm is observed and a change in colour occurred from yellow to red. This absorption decrease proportionately when biotin is added since biotin displaces the HABA dye due to its higher affinity for avidin. Several molecules of biotin can react with a plasma functionalised surface which in turn can each bind a molecule of avidin. This greatly increases the sensitivity of many assay procedures. Since the biotin is a relatively small molecule, the avidin-biotin interaction is the strongest known noncovalent, biological interaction ($K_a=10^{15} \text{ M}^{-1}$) between protein and ligand. The bond formation between biotin and avidin is very rapid and, once formed, is unaffected by wide extremes of pH, temperature, organic solvents and other denaturing agents [10].

A complementary method, implementing ESR, for surface functionality quantification was utilized after derivatising the functional groups with 4-amino-2,2,6,6-tetramethylpiperidinyloxy free radical (NH₂-TEMPO) in acetonitrile. For ESR experiments only contact lenses and soft silicon elastomer were used, the amount of detected functional groups was slightly higher in case of silicon elastomer because of its higher micro roughness in comparison to the contact lens surface.

Analogous to the experiments with NH₂-TEMPO, the coatings with various bio/polymers were performed in ultra pure water, again on contact lenses and soft silicon elastomers.

RESULTS & DISCUSSION: The plasma modified surfaces were first characterized by FTIR-ATR. The spectra showed that the structure of the deposited polymer chains is, to a large

extent, identical to the structure of the polymer obtained through a non-plasma radical solution polymerisation of the respective functional vinyl monomers. Absorption bands of the azlactone ring at $\sim 1820\text{ cm}^{-1}$ ($\text{C}=\text{O}$) and at $\sim 1673\text{ cm}^{-1}$ ($\text{C}=\text{N}$) were still present. The absorption band of the $\text{C}=\text{C}$ double bond in the monomer structure (vinylazlactone) at $\sim 1599\text{ cm}^{-1}$ disappeared after the plasma induced polymerisation similar as in case of the conventional radical polymerisation. Using isocyanatoethyl methacrylate (IEM) as a monomer gas we observed a very intensive absorption bands belonging to isocyanato group (NCO) at $\sim 2276\text{ cm}^{-1}$ and carbonyl from ester ($\text{C}=\text{O}$) at $\sim 1731\text{ cm}^{-1}$ (Fig. 1).

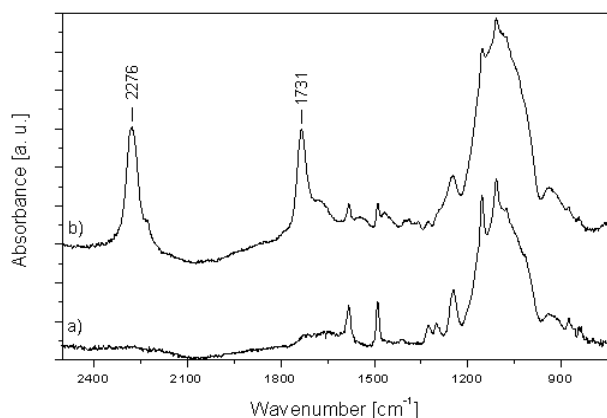


Figure 1. FTIR-ATR of IEM plasma polymer coated b-TCP a) in plasma zone, CW 40 W, b) 20 cm below plasma zone, CW 40 W.

In comparison to the IEM plasma polymer coated in the down-stream position 20 cm below the plasma zone, IEM plasma polymer prepared in the plasma zone contains no functional groups that would be available for further chemical reactions. XPS analysis showed full surface coverage at all distances from 10 to 30 cm downwards from the plasma zone (Fig. 2). After spin-labelling of the reactive groups with $\text{NH}_2\text{-TEMPO}$, ESR spectroscopy indicated a correlation between the substrates "downstream" distance and the density of the functional groups achieved. This was confirmed through the avidin-biotin coupling procedure, where 5-biotinpentylamine was covalently linked to the lens surface and then detected using the modified avidin-HABA ImmunoPure test (Fig. 3). ESR spectroscopy showed an increase of the number of reactive groups from the lower edge of the plasma zone towards distance of 20 cm to 30 cm. Beyond 30 cm the amount of functional groups started slowly to decrease. The HABA test confirmed this trend a platform from 10 cm up to 30 cm, then a decrease of functionality was observed.

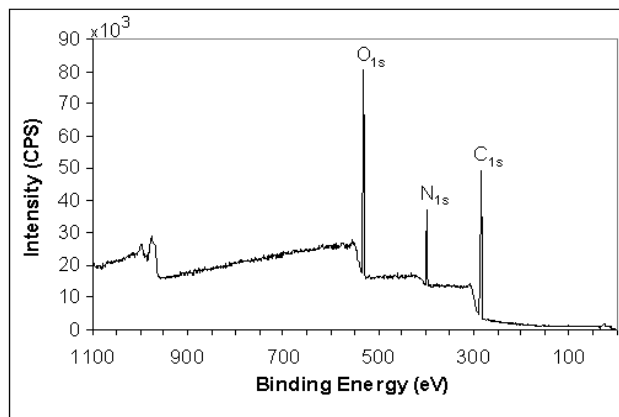


Fig. 2 Survey XPS spectrum of plasma polymerised IEM on Si wafer, Distance = 30 cm

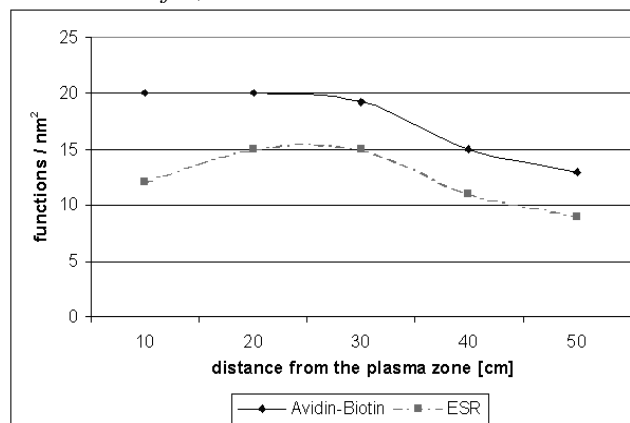


Fig. 3 Results from avidin-biotin assay compared to the ESR analysis of VAL plasma coated contact lens at various distances (macroscopic area)

High resolution XPS spectra and IR spectra showed that when the sample was prepared directly in the plasma zone a considerable part of the original monomer molecule was destroyed by energetic particles such as positive ions (mostly Ar^+) that directly had impact on the surface of the growing layer (Fig. 1a). At higher distances the photons still could reach the film, however energetic particles not. This could explain the disordered structure of the film grown in the plasma zone. ESR spectroscopy confirmed that the amount of accessible reactive groups on the surface prepared in the plasma zone is quite low. These observations indicated that heavy fragmentation of organic monomer species, usually occurring under conventional plasma conditions, was largely avoided at higher distances. Radical polymerisation of 4,4-dimethyl-2-vinyl-oxazolinone (often called vinylazlactone, VAL) or of isocyanatoethyl methacrylate (IEM) has been identified as the predominant process under the conditions used.

The optimal substrate distance downstream from the middle of the plasma zone for VAL and IEM plasma induced polymerisation was found to be between 20 and 30 cm. At this distance we did not

observe any delamination of the plasma generated polymer. At greater distances 100% coverage was not achieved as proven by XPS analysis. On one hand shortening the distance between the plasma zone and the position of samples and/or increase of plasma power increased the likelihood of covalent binding of the deposited polymeric chains. On the other hand, as indicated from FTIR-ATR spectra, decrease in sample - plasma distance or a further increase of the plasma power caused some structural changes of the deposited VAL or IEM polymer layers.

Various binding experiments were performed on contact lenses or silicon elastomer, functionalised either with VAL or IEM, which led to highly hydrophilic secondary coatings. Thus, secondary coatings made with 10% aqueous solutions of poly-(allylamine- co -N-allyl-gluconamide 1:1) ($M_w \sim 150000$) showed excellent surface wettability; the dynamic water/air contact angles were 0° [11]. The coating thickness significantly increased over 300 nm, as determined by AFM. Sterilization by autoclaving did not cause any detrimental effects. Other polymers which were successfully coated on lens surfaces were Jeffamine M-2070, polyethyleneimine, albumin (human) and elastin (5% aqueous solutions). The coating with elastin revealed a good water film break-up time, and the contact angles were 28° adv. / 18° rec. We did not observe any difference between the use of contact lenses and soft silicone elastomer as a substrate for preparation of secondary coatings.

CONCLUSIONS: Downstream plasma polymerisation of vinylazlactone and isocyanatoethyl methacrylate proofed to give a good control of substrate surface properties. The new highly functional coatings showed firm adherence, appropriate stability as well as a high potential for the covalent attachment of a large number of polymers, biopolymers and bioactive principles. The results obtained confirm that the down-stream plasma technique provides a valuable extension of conventional methods for surface fictionalisation.

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