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Attachment of horseradish peroxidase to polytetrafluorethylene (teflon) after plasma immersion ion implantation

Alexey Kondyurin *, Neil J. Nosworthy, Marcela M.M. Bilek

Applied and Plasma Physics, School of Physics (A28), University of Sydney, Sydney, NSW 2006, Australia

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Abstract

The aim of this work was to investigate the potential of polytetrafluorethylene (PTFE) as a surface for biologically active protein attachment. A plasma immersion ion implantation (PIII) treatment was applied to PTFE to produce an activated surface for the functional attachment of the enzyme, horseradish peroxidase (HRP). Fourier transform infrared–attenuated total reflectance spectra show oxidation and carbonization of the surface layer as a function of ion fluence. The PIII treatment increases by threefold the amount of attached HRP and the activity of HRP on the modified surface is about seven times higher than that on an untreated PTFE surface. This result indicates that the PIII surface modification improves both the polymer's protein binding capacity and its ability to retain the protein in a bioactive state.

Keywords: Polytetrafluorethylene; Plasma immersion ion implantation; Horseradish peroxidase; Protein attachment; Enzyme activity

1. Introduction

Diagnostic assays using biosensors and micro-arrays require immobilization of proteins and retention of their activity [1]. Polymers have properties which make them attractive for use as structural supports in biomedical applications, such as light weight, ease of forming, low cost and corrosion resistance. However, their interactions with biological systems are often compromised by their highly inert hydrophobic surfaces. Surface activation treatments for improving their biocompatibility are therefore of great interest.

Polymer surface activation can be achieved by several methods: chemical treatment of the polymer surface by active substances [2], mechanical treatment (roughening) [3], ultraviolet (UV) irradiation [4], plasma treatment [5], ion beam implantation [6] and its variant, plasma immersion ion beam implantation (PIII). Such methods of polymer surface modification have been shown to affect protein attachment to the surface. High-temperature oxidation of polyethylene was found not to affect the attachment of proteins but did appear to affect their conformation on the surface resulting in improved cell spreading [7]. UV light irradiation in ozone decreased the amount of irreversibly adsorbed albumin on the polystyrene surface [8] but appeared to better preserve the conformation of the bound protein. Ion beam treatment was found to increase the amount of attached albumin on siloxane and polyethylene terephthalate surfaces [9]. In some cases, the attachment of proteins on polymer surfaces can destroy their conformation [8] and cause a loss of biological activity, as observed for the enzyme horseradish peroxidase (HRP) attached on untreated polystyrene [10].

Recently, we used the PIII method for activation of polyethylene and polystyrene surfaces and studied the attachment of the enzymes HRP and catalase. Some advantages of PIII method modification of polymers are considered in Ref. [11]. After PIII treatment the protein covered the polymer surface [12]. It was attached covalently without the use of linker molecules [13] and showed improved bioactivity [13,14]. The chemical bond between

* Corresponding author. Tel.: +61 293515962.
E-mail address: kond@mailcity.com (A. Kondyurin).

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the polymer macromolecule and the protein molecule was stable in the presence of protein detergents like sodium dodecyl sulfate (SDS) and NaOH solutions. The attached protein remained in a biologically active conformation and catalytic activity was retained.

Polytetrafluorethylene (PTFE) is of interest for many biomedical applications because it is stable in organism media and does not have any toxic effects. The low adhesion of protein to its surface may be useful in the fabrication of biosensors and micro-arrays.

PIII surface modification as well as plasma and ion beam implantation methods have previously been applied to PTFE surfaces. Ion beam implantation of PTFE results in the formation of new CF_3, CF, C–O groups and elemental carbon bonds, as well as in etching of the PTFE [6,15].

In this paper, we present the effects of PIII surface modification on protein (HRP) attachment to PTFE and its porous analog ePTFE. As in the case of polystyrene and polyethylene, we observed covalent attachment of the enzyme to the surface and retention of bioactivity.

2. Experiment

HRP was purchased from Sigma (cat. No. P6782) and PTFE of 20 μm thickness was from Halogen (Perm, Russia). The nitrogen gas used for PIII was 99.99% pure.

PIII was carried out in an inductively coupled radio-frequency plasma powered at 13.56 MHz. The base pressure was 10^{-6} Torr (10^{-4} Pa) and the pressure of nitrogen during implantation was 2×10^{-3} Torr (4.4×10^{-2} Pa). The plasma power was 100 W with reverse power of 12 W when matched. The plasma density during treatment was continuously monitored by a Langmuir probe with rf block from Hiden Analytical Ltd. Acceleration of ions from the plasma was achieved by the application of high voltage (20 kV) bias pulses of 20 μs duration to the sample holder at a frequency of 50 Hz.

The samples were mounted on a stainless steel holder, with a stainless steel mesh, electrically connected to the holder, placed 45 mm in front of the sample surface. The samples were treated for durations of 20–800 s, corresponding to implantation ion fluences of 0.5–20×10^{15} ions cm^{-2}.

The ion fluence was calculated from the number of high voltage pulses multiplied by the fluence corresponding to one pulse. The fluence of one high voltage pulse was determined by comparing UV transmission spectra from polyethylene films implanted under conditions used here to samples implanted with known fluences in previous PIII and ion beam treatment experiments.

The wettability of PTFE was measured using the sessile drop method. Kruss contact angle equipment DS10 was employed to measure the contact angles. Deionized water, glycerol, formamide and diiodomethane were dropped on the sample and the angle between the edge of the drop and the surface was measured. Surface energy and its components (polar and dispersic parts) were calculated using the Rabel model with regression method.

After PIII treatment, the PTFE samples were stored for 3 days in air-filled closed containers at stabilized room temperature (23 °C). After 3 days the PTFE samples were incubated in HRP solution (50 μg ml^{-1} in 10 mM sodium phosphate buffer, pH 7) overnight at 23 °C. The incubation time was selected from previous experiments for UHMWPE and PS, which showed the protein absorption saturated after about 1 h. Overnight incubation was convenient and ensures saturation has been reached. After incubation, PTFE samples were washed six times (20 min each wash) in buffer (10 mM sodium phosphate buffer, pH 7). Samples for Fourier transform infrared (FTIR) spectral analysis were washed in deionized water for 10 s to remove buffer salts from the PTFE surface.

HRP activity was measured by clamping the PTFE samples (13 mm × 15 mm) between two stainless steel plates separated by an O-ring (inner diameter 8 mm, outer diameter 11 mm), which sealed to the plasma-treated surface. The top plate contained a 5 mm diameter hole. 3,3,5,5'-Tetramethylbenzidine (TMB; Sigma T0440) was added to the polymer surface. After 30 s 25 μl was removed and added to 50 μl of 2 M HCl followed by 25 μl of unreacted TMB to make the volume up to 100 μl. Optical density was then measured at a wavelength of 450 nm using a DU 530 Beckman spectrophotometer.

FTIR–attenuated total reflectance (ATR) spectra from the PTFE samples were recorded using a Digilab FTS7000 FTIR spectrometer fitted with an ATR accessory (Harrick, USA) with trapezium germanium crystal and incidence angle of 45°. To obtain sufficient signal/noise ratio and resolution of spectral bands we used 500 scans and a resolution of 1 cm^{-1}. Before recording, the surface of the PTFE was dried using a dry air flow. Differences, obtained by subtraction, between spectra of samples before and after PIII treatment as well as spectra of PIII-treated samples before and after HRP incubation were used to detect changes associated with the surface treatment and the attachment of protein, respectively.

To illuminate the attachment mechanism, the PTFE samples with attached protein were washed in 2% SDS detergent at 70 °C for 1 h and then washed with deionized water three times to remove the residual SDS. FTIR–ATR spectra were recorded before and after the SDS treatment. The difference spectra of protein incubated samples and buffer incubated sample were used for analysis. All spectral analysis was done using GRAMS software.

3. Results

The PTFE surfaces show color changes after PIII treatment. Films treated for a long time (400 s) become slightly gray in appearance, while lower dose treatments produced no visually observable color changes.

The wettability of the PTFE surface increased with PIII treatment (Fig. 1). The contact angles decreased for all liq-

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