Functionalization of Plasma-Treated Polymer Surfaces with Glycidol

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Received 12 July 2010; accepted 13 December 2010
DOI 10.1002/app.33971
Published online 23 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The ionic species created by the O_2-plasma treatment of poly(styrene) surfaces are able to initiate the polymerization of glycidol in the absence of any initiator. Consequently, thin hydrophilic films of poly(glycidol) are formed at the surface of oxygen plasma-treated polymer surfaces upon treatment with glycidol. These were characterized with the aid of X-ray photoelectron spectrometry, contact angle measurements, and scanning electron microscopy. A significant decrease of unspecific protein adsorption at the plasma-treated glycidol-modified surfaces was evidenced using fluorescence microscopy. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 2543–2550, 2011

Key words: plasma; polyesters; poly(styrene); proteins; surfaces

INTRODUCTION

One important goal in the surface modification of polymers is to suit various biological applications.[1,2] Ongoing work involves the development of different functionalities at the surface of polymeric bio-supports. The optimization of the surface functionality, in both nature and quantity, for the optimum covalent attachment of the bio-entities at the surface is of primary interest; however, the nature of the subsurface itself plays a preponderant role in the behavior of biomolecules subsequently attached to or coming in contact with the surface. In biological and biomedical applications,[3] polyethylene glycol (PEG) is frequently used as a spacer[4–11] to limit protein adsorption at the surface. This is because of the most favorable properties of PEG, which is nontoxic, nonimmunogenic, nonantigenic, and highly soluble in water. Particularly the latter property of PEG allows hydrophobic molecules, for example, drugs and catalysts, to become water soluble upon PEGylation.

Several articles have been published on the synthesis of various hyperbranched polyglycerols.[12–18] Polyglycerol are the molecules of choice as their structure is deduced from PEG[17] and therefore can be used, not only to distance biomolecules from the supporting surface, but also to efficiently suppress their adsorption.[19] In terms of further modification, they provide multiple anchor points by simple modification of the numerous hydroxyl groups.[20–22] Polyglycerols are generally prepared via cationic[23] or anionic polymerization[12–15,18,24] of glycidol in the presence of initiators such as Lewis acids,[23,25,26] protonic acids,[23,25] alkoxides, and other bases.[12–14,16,18,24,27] Some recent articles have shown the possibility of attaching polyglycerol at the surface of gold samples[19] or to grow polyglycerol from the functionalities introduced at the surface of a silicone wafer.[28]

To circumvent multistep surface functionalization procedures, we developed a novel and simple plasma-based approach that allows for the surface-grafting of glycidol to polymeric surfaces such as poly(styrene) (PS) and polyethylene terephthalate (PET), respectively. Thus, by applying an oxygen plasma (most probably anionic), surface-localized initiating groups are created that allow for the *in situ* polymerization of glycidol from the polymer surface in the absence of any additional initiator. Mostly, linear and hardly any hyperbranched structures are

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Contract grant sponsors: AiF (German Federation of Industrial Research Associations), the Federal Republic of Germany, the Free State of Saxony.

formed. This approach thus represents an attractive alternative to the potassium methylate triggered grafting-from as described by Khan and Huck.[28]

EXPERIMENTAL

Materials

PS samples were cut out from Greiner Petri dishes. PET samples were cut out from a commercial PET sheet ~100 μm in thickness. Before plasma treatment, the samples surfaces were cleaned with ethanol and carefully dried. Glycidol, potassium methylate (25 wt % solution in methanol) albumin-fluorescein isothiocyanate conjugate albumin bovine (FITC-BSA, Sigma A9771), myoglobin from equine skeletal muscle 95–100% and Tween® 20 were obtained from Sigma-Aldrich (Germany). Albumin bovine (BSA) (fraction V pH = 5) was obtained from Acros (Germany). Phosphate buffered saline (PBS) 10× concentrate obtained from either Sigma-Aldrich Germany or Carl-Roth GmbH + Co. KG, was used at pH 7.4 after dilution. Distilled water Millipore Grade and absolute ethanol were used to rinse the samples. NMR spectra were recorded in D_2O on a Bruker Avence 600 spectrometer (600.25 MHz for proton and 150.93 MHz for carbon) at room temperature.

Plasma treatment

PS and PET samples, cut out to fit the dimensions required for surface analysis, were exposed to oxygen plasma using a FEMTO 2 L laboratory plasma system (AC generator 40 kHz, 100 W) from Diener Electronic GmbH + Co. KG (Germany) for 5 min.

Sample modification

After plasma treatment, the samples were in contact with ambient air, within 30 min they were either dipped into a solution of potassium methylate in methanol, dried with hot air for 5 min, and placed in a solution of glycidol for 3 h or were directly placed into a solution of glycidol for 3 h at room temperature. After chemical modification, all samples were thoroughly rinsed three times with water, once with ethanol, and finally air dried in a dust-free environment. Samples are cited according to their acronyms and corresponding descriptions displayed in Table I.

TABLE I
List of Acronyms

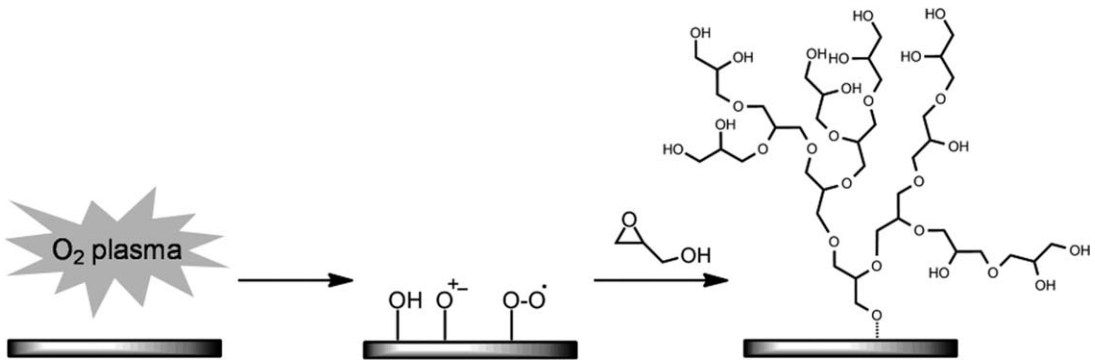
Pristine PS or PET	PS-0	PET-0
O_2 plasma-treated PS or PET	PS-1	PET-1
PS or PET treated with MeOK and glycidol after plasma treatment	PS-1-A	PET-1-A
PS or PET treated with glycidol after plasma treatment	PS-1-B	PET-1-B
Bare PS treated with MeOK and glycidol without plasma treatment	PS-0-A	–

Analytical methods

The surface composition of the samples was measured using either a SAGE 100 system (Specs GmbH, Berlin, Germany) X-ray photoelectron spectrometer (XPS) or a Kratos AXIS Ultra Spectrometer. The SAGE 100 system XPS was equipped with nonmonochromatized Al and Mg K_α excitation sources and was operated at 300 W (10 kV, 20 mA). The Kratos AXIS Ultra Spectrometer was equipped with a monochromatic Al $K\alpha$ excitation source and was operated at 150 W (15 kV, 10 mA). Binding energies were corrected for static charging of the samples by referring them to the C(1s) peak set at a binding energy of 285.0 eV. Scanning electron microscopy (SEM) was carried out on a Carl Zeiss SMT Ultra 55 (Oberkochen, Germany), the samples were preliminary coated with a 50-nm layer of Chrome to improve the conductivity. Static contact angles with water and diiodomethane were measured using the sessile drop method on a Krüss contact angle instrument (Krüss GmbH Hamburg, Germany) and the Drop Shape Analysis 2.1 software. All contact angles are the mean value of 10 measurements per 5 different locations on the sample's surface and present a standard deviation of < 3% throughout. Surface tension, as well as polar and disperse contributions to the surface tension were calculated using the Owens-Wendt-Rabel and Kaelble method.[29–32]

Protein adsorption studies

Protein adsorption experiments were conducted by treating substrates, with or without surface modification, with a 2 mg/mL FITC-BSA solution in PBS at room temperature for 2 h. Following the treatment, the substrates were rinsed twice with PBST (PBS with 0.05% (v/v) Tween 20); once with PBS solution, and finally with Millipore grade water to remove loosely adsorbed proteins. The samples were viewed and photographed with a Leica DM LM microscope equipped with a 100-W mercury lamp, appropriate filters and a cooled monochrome CCD camera (DFC 350 FX). BSA and myoglobin adsorption onto the polymeric surfaces were investigated using the bicinchoninic acid (BCA)-based assay.[33] Samples were cut to fit in the wells of a 48-well microtiter plate. To each sample, 200 mL of a protein solution (2.0 mg/mL) in PBS (pH 7.0) was added and the plates were shaken for 1 h at ambient temperature.



Scheme 1 Surface-modification of a polymeric substrate by O_2-plasma followed by the addition/surface-grafting of glycidol.

The samples were washed three times with 1 mL of PBS, respectively. Then, the BCA reagent was added to the samples and the plate was incubated for 25 min at 37°C. The plate was then shaken for 5 min at ambient temperature, the solution was transferred to a new microtiter plate and light adsorption at 562 nm was measured using a microtiter plate reader (Infinite M200, Tecan, Germany). For calibration, several protein concentrations ranging between zero and 30.00 mg/mL were used as described by the BCA test instructions.

RESULTS AND DISCUSSION

Gas plasma is a practical means for the modification of the uppermost layer of a polymer surface, introducing polar functionalities, ions and radicals, while reducing the use of solvents and the production of chemical waste at the same time.[1,33,34] We recently reported on the use of a nitrogen plasma to introduce predominantly *N*-based functionalities to the surface of PS-based devices and built up hyperbranched polymers at the surface for bio-medical applications applying a consecutive grafting-from approach.[11] In this study, we exposed PS samples to oxygen plasma to create oxygen-based functionalities at the surface, which can then be used as starting points for the growth of polyglycerol. After O_2-plasma modification, one set of PS samples was treated with an initiator, that is, MeOK followed by the addition of glycidol, while a second set of plasma-modified PS was directly treated with glycidol without the addition of any initiator (Scheme 1). All modified PS samples, which were found to be optically transparent, were analyzed by XPS. These measurements revealed that samples not treated with MeOK prior to the polymerization of glycidol also displayed high atomic oxygen content at their surface (Table II, Figs. 1 and 2). Thus, on both types of samples, the C1s regions of the XPS spectra (Figs. 1 and 2) clearly show peaks corresponding to the C—O bond of the ether segments at 286.7 eV. Measurements of the contact angles (Table III) demonstrate that upon treatment with glycidol, the polarity increased on both types of PS surfaces, that is, they became strongly hydrophilic. In case a nonplasma exposed PS (PS-0.A) surface was treated with MeOK/glycidol, no modification occurred at all, the oxygen content did not increase and the surface remained hydrophobic. This strongly suggests the

TABLE II
Surface Composition of Untreated, Plasma-Treated, and Chemically Treated PS and PET Samples, As Determined by XPS

	Atomic composition (%)				Ratio	C1s deconvolution			
						284.9 eV	286.5 eV	287-288 eV	289 eV
Sample	C1s	N1s	O1s	K2p	O/C	C—C, C—H	C—O	C=O	O=C—O
PS-0	98.4	–	1.6	–	0.02	100.0			
PS-1	77.4	1.9	20.7	–	0.27	73.2	18.9	3,6	4,3
PS-1.A	66.5	1.2	30.9	1.4	0.46	33.5	62.4	2.8	1.2
PS-1.B	63.4	0.8	35.9	–	0.57	32.8	61.3	4.2	1.9
PS-0.A	98.0	–	2.0	–	0.02	100.0			
PET-0	80.7	–	19.3	–	0.24	78.0	15.0		9.0
PET-1	63.6	1.9	34.6	–	0.54	53.7	25.5		20.8
PET-1.A	69.0	–	31.0	–	0.45	48.3	36.6		15.1
PET-1.B	64.6	2.2	33.2	–	0.51	43.5	30.2	9.8	16.5

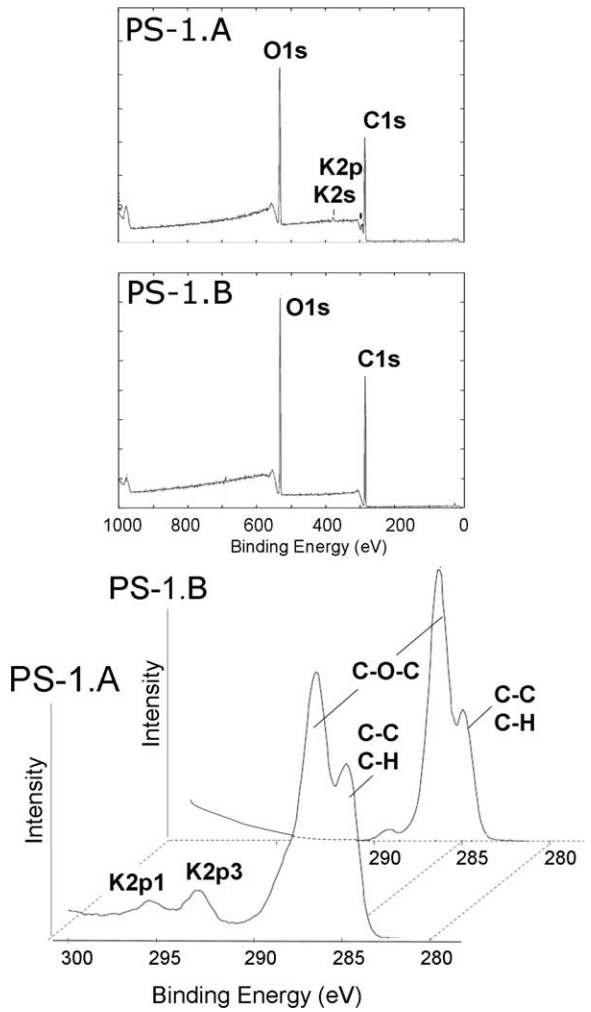


Figure 1 XPS survey spectra and C1s region (bottom) of modified PS surfaces with MeOK treatment prior to the glycidol polymerization (PS-1.A) and with direct addition of the glycidol on the surface (PS-1.B).

possibility to directly use the O_2 plasma-derived ions at the surface of the polymer to start the ring-opening polymerization of glycidol in the absence of any initiator.

To find out whether other polymers could be modified in the same way, we turned to poly(ethylene terephthalate) (PET) samples and proceeded as described above. Table II summarizes the atomic composition at the surface of bare PET (PET-0), O_2 plasma-treated PET (PET-1) and glycidol modified, O_2 plasma-treated PET (PET-1.A and PET-1.B) as determined by XPS. Compared to PET-0, the oxygen content at the surface increased with all other samples. A comparison of the deconvoluted C1s peaks (Table II and Fig. 3) of the surface of the various samples indicates that the C1s peak at 286.7 eV of PET-1A and PET-1.B samples, corresponding to the C—O ether bond had greatly increased, even though the overall oxygen concentration at the surface does not drastically change. In Figure 3, the C1s deconvolution peaks corresponding to glycidol-modified PET surface (PET-1.B) exhibits an additional peak at 287.2 eV that can be attributed to simple C=O bonds contribution. The contact angle values (Table III), confirm that the PET-1.A and PET-1.B surfaces were in fact modified with glycidol resulting in a hydrophilic surface. For both PS and PET modifications the contact angle values, surface composition determined by XPS, and the observation of the C1s deconvolution peaks, indicate that the thickness of the glycidol surface layer is in the range of the XPS sampling depth.

Some further surface analysis using SEM shows interesting differences between the various surfaces (Figs. 4 and 5). Although O_2 plasma-treated PS surfaces reacted either with MeOK/glycidol (PS-1.A)

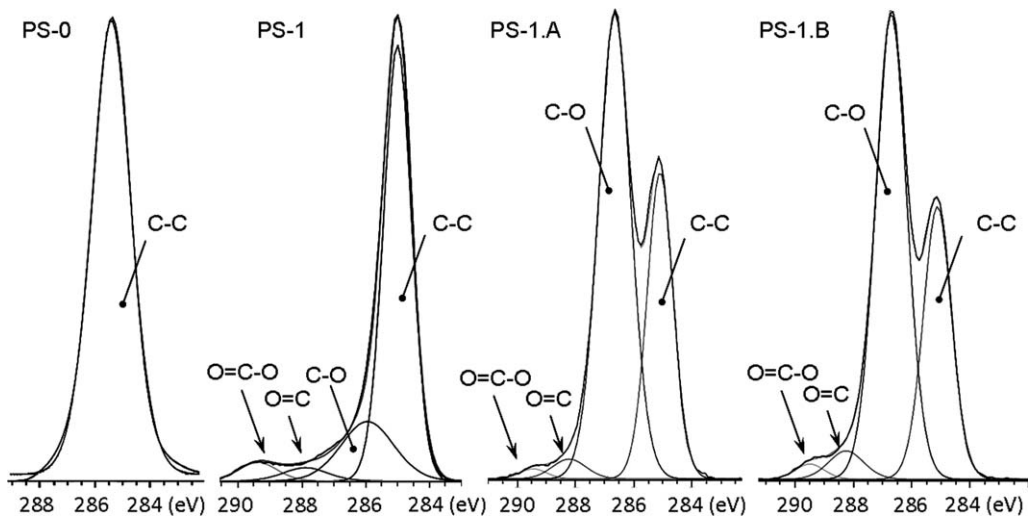


Figure 2 XPS C1s region of unmodified PS (PS-0), O_2 plasma-modified PS (PS-1), O_2 plasma-modified PS treated with MeOK prior to glycidol polymerization (PS-1.A), and O_2 plasma-modified PS directly treated with glycidol in the absence of MeOK (PS-1.B).

TABLE III
Water (W) and Diiodomethane (D) Contact Angles of Untreated, Plasma-Treated, and Chemically Treated PS Samples

	Contact angle		Surface tension	Dispersive polar contributions	
	W (°)	D (°)	(mN/m)	(mN/m)	(mN/m)
PS-0	89.2	16.6	48.8	48.8	0
PS-1	N/A	N/A	–	–	–
PS-1.A	44.0	41.6	59.1	38.8	20.3
PS-1.B	43.4	44.1	58.9	37.5	21.4
PS-0.A	88.1	48.4	36.3	35.2	1.2
PET-0	78.6	45.4	40.2	36.8	3.4
PET-1	N/A	N/A	–	–	–
PET-1.A	43.0	41.7	59.7	38.8	20.9
PET-1.B	29.1[a]	34.7	68.5	42.2	26.3

[a] Water contact angle was difficult to measure as the water spread on the surface.

or only with glycidol (PS-1.B) had a globular surface structure (Fig. 4), these globular shapes were much more pronounced with the MeOK-treated surfaces. Since these globular structures most likely correspond to the nucleation sites at the plasma-treated polymer surface, it appears that treatment with MeOK results in a different glycidol polymerization process; however, without any beneficial impact in terms of surface polarity or protein repulsion (*vide infra*). Similar observations have been made by Ford et al.,[34] who reported on the growth of nanostructures derived from the grafting of hyperbranched poly(ester amines) brushes on polymeric surfaces, which developed into "nano-fruits" type textures of different sizes.

Likewise, when comparing the SEM of both poly(-glycidol)-modified PET surfaces in the absence and presence of MeOK (Fig. 5), the final MeOK-treated coatings possessed a more regular texture as compared to those prepared without the addition of any initiator. This suggests again that the mechanism of polymerization of glycidol at the surface treated with MeOK differs from the one without any additional initiator, thus producing this different surface organization. To elucidate the structure of the poly(-glycidol) formed at the surface in the absence of MeOK, the polymer was subjected to ^{13}C-NMR analysis (Fig. 6). As can be seen, mostly linear, 1,4-derived structures (L) as well as the corresponding terminal $T_{1,2}$ groups were observed in the range of $\delta = 74–75$ and at $\delta = 65.3$ ppm. The by far less prominent signals were assigned to the $T_{1,3}$, $L_{1,3}$ as well as (tentatively) to the D-structure.

Finally, to test the protein adsorption at the surfaces of glycidol-modified surfaces, we exposed both a PS Petri dish and a flat PET sample to the O_2 plasma and then treated the surface with glycidol, as described above, without any additional initiator. These modified samples and the corresponding untreated pristine samples were treated with a drop

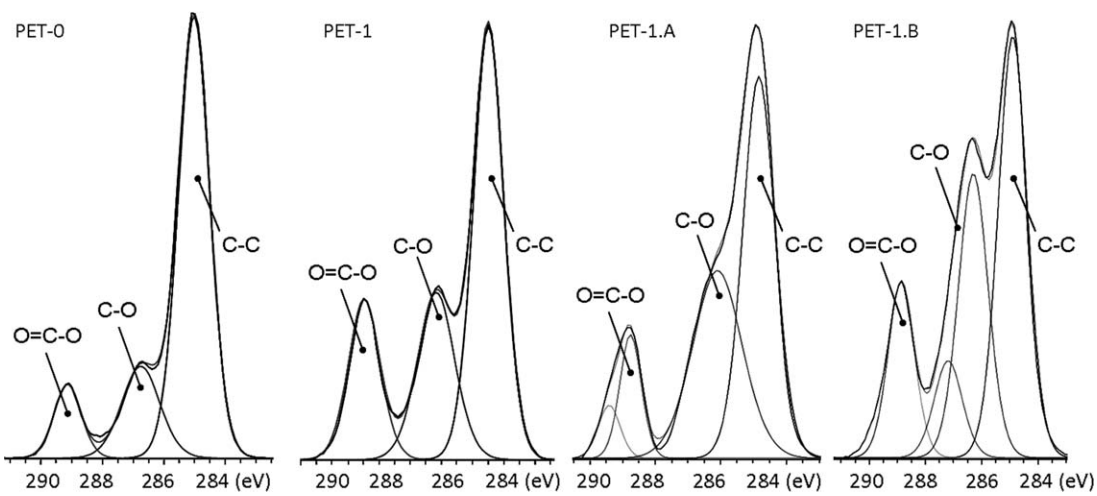


Figure 3 XPS C1s region of unmodified PET (PET-0), O_2 plasma-modified PET (PET-1), O_2 plasma-modified PET treated with MeOK prior to glycidol polymerization (PET-1.A), and O_2 plasma-modified PET directly treated with glycidol in the absence of MeOK (PET-1.B).

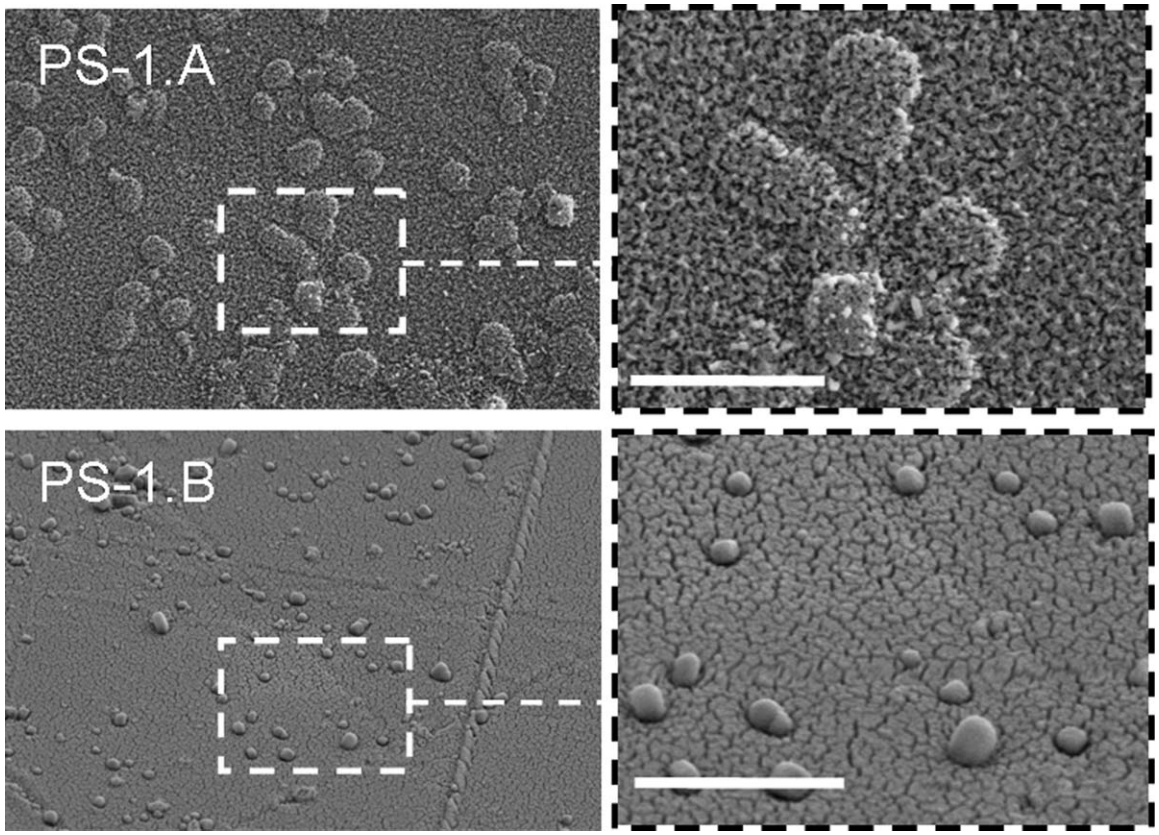


Figure 4 SEM of modified PS surfaces, after exposure to an O_2-plasma and treatment with potassium methylate followed by glycidol polymerization (PS-1.A) and after exposure to an O_2-plasma and glycidol treatment (PS-1.B). In each figure, the white scale bar corresponds to 1 μm.

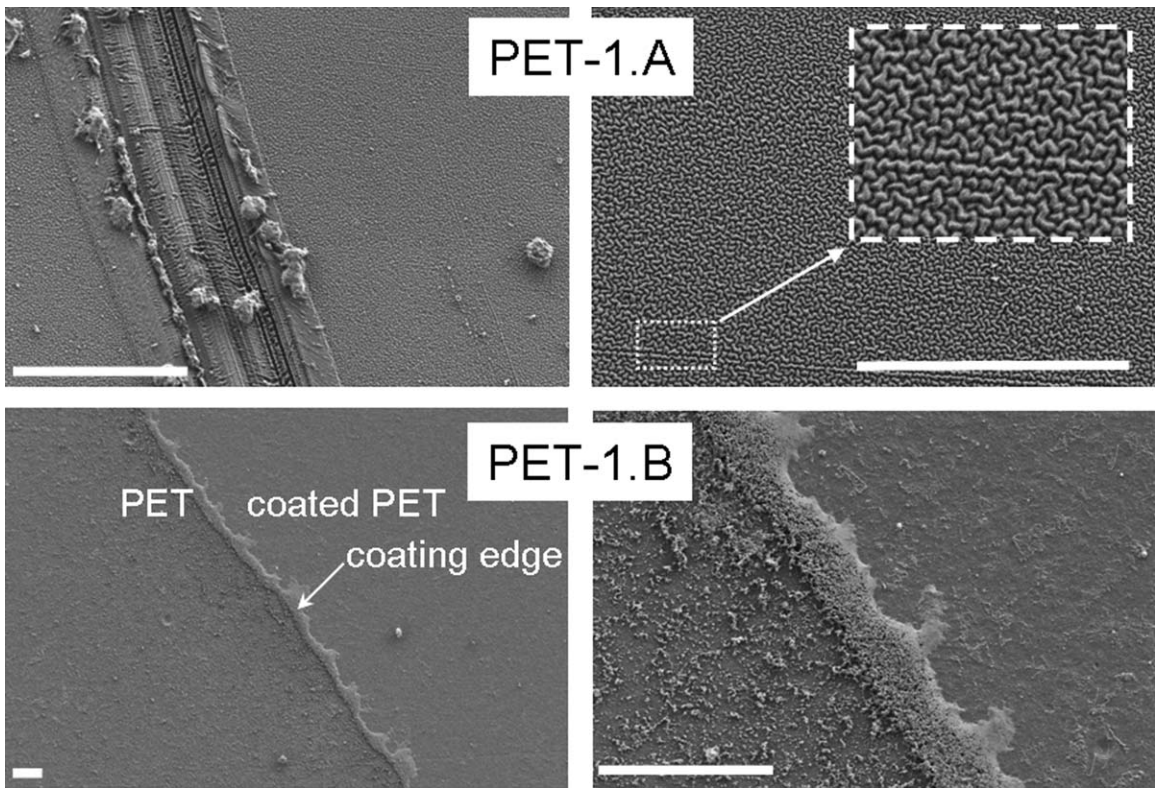


Figure 5 SEM of modified PET surfaces, after exposure to an O_2-plasma and treatment with potassium methylate followed by glycidol polymerization (PET-1.A) and after exposure to an O_2-plasma and glycidol treatment (PET-1.B). In each figure, the white scale bar corresponds to 10 μm.

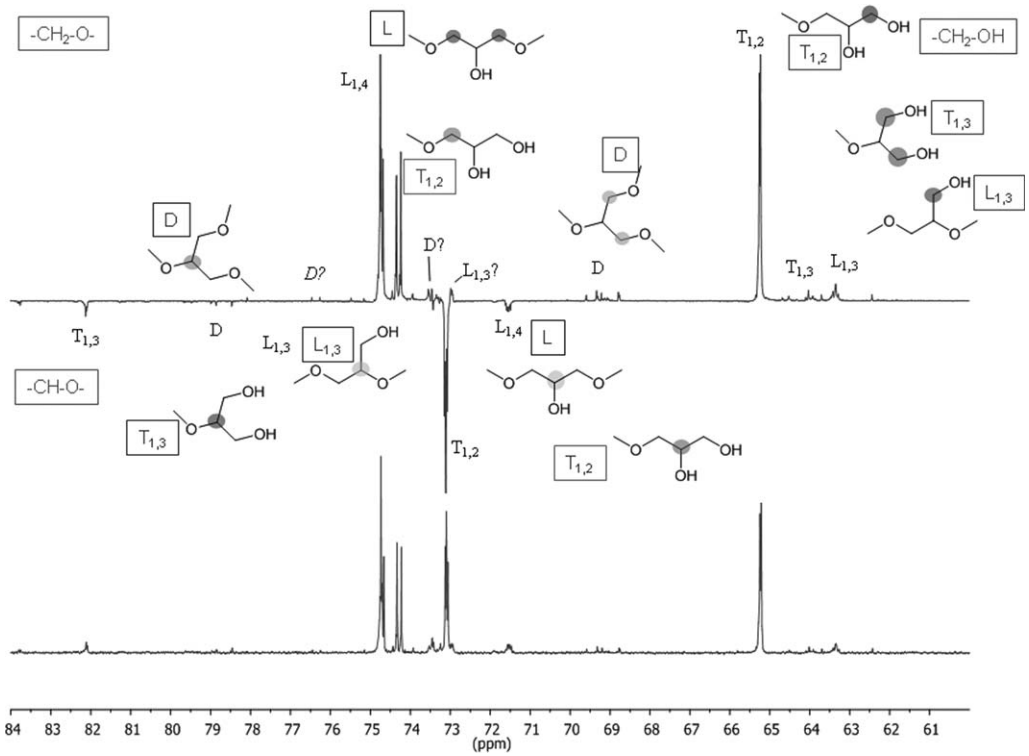


Figure 6 ^{13}C-NMR and DEPT spectrum (D_2O) of the poly(glycidol).

of FITC-BSA in a PBS solution for 2 h. After thorough rinsing, the surfaces were examined with a fluorescence microscope. Only scattered fluorescence spots indicative of little or no protein adsorption at the modified polymer surfaces were observed as compared to pristine samples exposed to FITC-BSA, where significant fluorescent staining indicated substantial protein adsorption. To quantify these results, PS samples were submitted to the BCA test for protein adsorption using two different proteins, that is, BSA and myoglobin. Details of the procedure are reported in the experimental part. Figure 7 represents the variation in protein adsorption onto native as well as different glycidol-treated PS surfaces. For both proteins, the lowest unspecific adsorption was observed for the O_2 plasma-treated surface that was directly reacted with glycidol without the addition of any initiator. These findings clearly illustrates the potential of this simple approach to poly(glycidol)-modified polymer surfaces.

CONCLUSIONS

Subsequent treatment of PS and PET surfaces with oxygen plasma and with glycidol allows for producing thin hydrophilic films at the surface of these polymers even in the absence of any additional initiator. This suggests that the oxygen plasma is capable of generating reactive species, most likely ions, at the surface of the treated polymer; these are stable and reactive enough to start the polymerization of glycidol, a compound well known to polymerize under anionic or cationic conditions. In the absence of any additional initiator, the film produced at the surface is transparent, while the surface becomes visually disrupted when an initiator, for example, MeOK, is added. Finally, we have shown that protein adsorption at PS and PET surfaces modified with poly(glycidol) upon plasma treatment can be significantly reduced. This straightforward approach

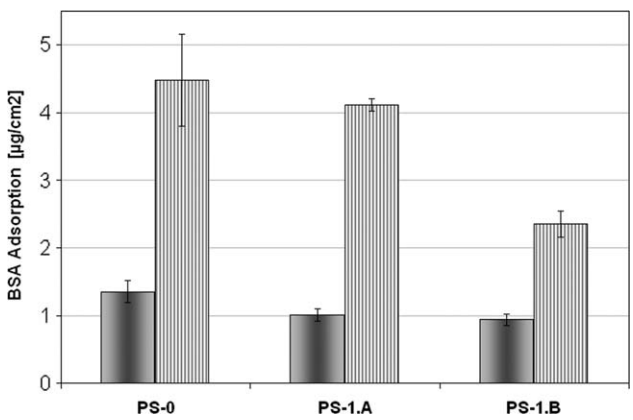


Figure 7 BSA (gray bars) and myoglobin (hatched bars) adsorption at the surface of bare PS (PS-0), after exposure to an O_2-plasma and treatment with potassium methylate followed by glycidol polymerization (PET-1.A) and after exposure to an O_2-plasma and glycidol treatment (PET-1.B).

is expected to lead to interesting applications in the biological and biomedical fields.

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