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Dedicated to Professor Bogdan C. Simionescu on the occasion of his 65th anniversary

SURFACE CHARACTERIZATION OF AMINE FUNCTIONALIZED PET FILMS AFTER COLLAGEN IMMOBILIZATION

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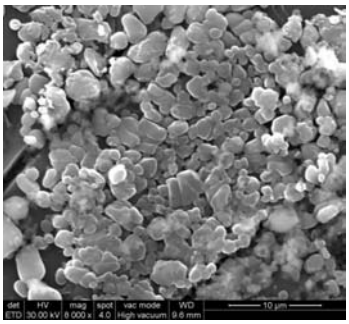
This article presents the experimental results concerning the plasma and chemical treatments influence on poly (ethylene terephthalate) (PET) films. The results of contact angle, atomic force microscopy (AFM) and scanning electron microscopy (SEM) measurements revealed the surface and structural changes induced by the treatments. The collagen immobilization was made on samples treated 30 min chemical and those who suffered plasma and 30 min chemical treatment. Optimal conditions for collagen immobilisation were evidenced.



INTRODUCTION

Research in the field of polymer surface modification for the immobilization of bioactive compounds has been led by the biomedical field.[1,2] When surface modification is a precursor to attaching a bioactive compound, these techniques must be tailored to introduce a specific functional group. The ideal surface modification techniques will be those that introduce as close to a monolayer as possible of a desired functional group without causing irregular etching or producing significant hazardous waste.[3,4]

Nowadays the application of plasma treated surfaces or deposited polymers in medicine and biotechnology is getting more important.[5,6] This evolution is due to the fact that plasma polymers have advantageous properties for such applications, as follows: conformal and pinhole free layers can be deposited; unique substrates with complex shape can be modified; good adhesion to the substrates; unique surface and film chemistries can be achieved; meanwhile a wide variety of characterization methods are available; plasma modified surfaces are sterile, after preparation.[7,8] Plasma treatments can be used as precursor for chemical methods.

Chemical treatments (wet-chemistry) improve wettability and different compounds adhesion, hydrolyze processes, aminolysis and alcoolysis leading to the new hydrophilic groups creation by a selective broken of estheric bonds. In PET case, which is a semicrystallin polymer, with an aromatic-aliphatic structure with a hydrophobic

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behavior, the scission reactions succession can determine a relative concentration of functionalization at the surface level.[9, 10]

This research presents optimal treatment conditions for collagen immobilisation on PET surfaces.

EXPERIMENTAL

For plasma treatments, biaxially drawn PET films samples (25 x 25 mm) were positioned vertically in a metal sample holder to allow treatment on both sides. The discharge system contains two semicylindrical electrodes connected to a 1.2 MHz high frequency, placed outside the discharge chamber (neutral Pyrex glass, 30 cm length and 5 cm diameter).[6] In each run, prior to the treatment, the reactor is evacuated down to a base pressure of 0.3 Torr. During the reaction, a vacuum gauge is used to measure the pressure inside the plasma reactor. The power of the glow discharge is kept at 200 W for 10 min. After plasma treatments, the sandwich-prepared samples were treated with triethylenetetramine (TETA) at 90 °C in oven at 30 min. Under the plasma action, the chains scindation will appear on the PET surface, generating polar groups such as COO-, OCO-, -OH. An important role in the modification of the PET surface is played by the atomic species and the UV radiation.[11-14] At or near the surface, the free radicals are interacting, leading to the formation of cross-linking structures and unsaturated groups, plasma removing low-molecular weight materials or converting them into high-molecular products by cross-linking reactions, as well. In addition, the stabilization of free radicals (favored by the aromatic groups) could contribute to the formation of oxygen groups such as C–O. The plasma-treated PET, having some deprotonated carboxylic acid functions, is interacting with the positively charged protonated amines from the collagen buffer solution to form an ionically crosslinked surface. Oxygen and nitrogen are always present in the discharge chamber, these atoms can originate from the air present in the device or from the gaseous products which the plasma desorbs (H2O, O2, N2) from the reactor walls or etches from the PET surfaces.[15] As a result, the oxygen incorporation during the plasma treatment occurs.

The general reaction scheme for the aminolysis of PET is presented in Fig. 1.[3]

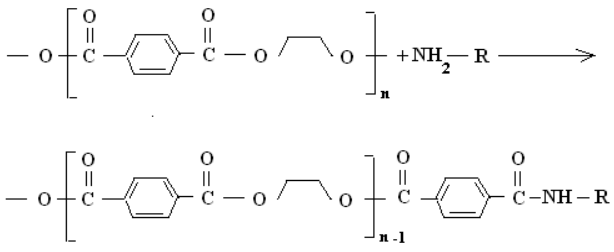


Fig. 1 – General reaction scheme for the aminolysis of PET.

Collagen molecules were immobilized chemically on functionalized films surfaces via glutaraldehyde. First, the aminolyzed PET (Fig. 1) films were immersed in 1 wt % glutaraldehyde (GA) solution for 3 h at room temperature, followed by rinsing with a large amount of de-ionized water for another 24 h to remove free GA. The films were then incubated in 3 mg/ml collagen/phosphate buffered solution (pH = 3.4) for 24 h at 2- 4° C. The collagen immobilized films were rinsed with 1 % acid acetic solution and then rinsed with de-ionized water for 24 h to remove free collagen. The reaction between NH_2 and OHC - $(CH)_3$ – CHO yielded a bonding via – N = CH - $(CH)_3$ – CHO, and one free aldehyde group could react with NH_2 groups existing in collagen. Collagen was covalent conjugated to the aminated PET surface via glutaraldehyde crosslinking.

RESULTS AND DISCUSSION

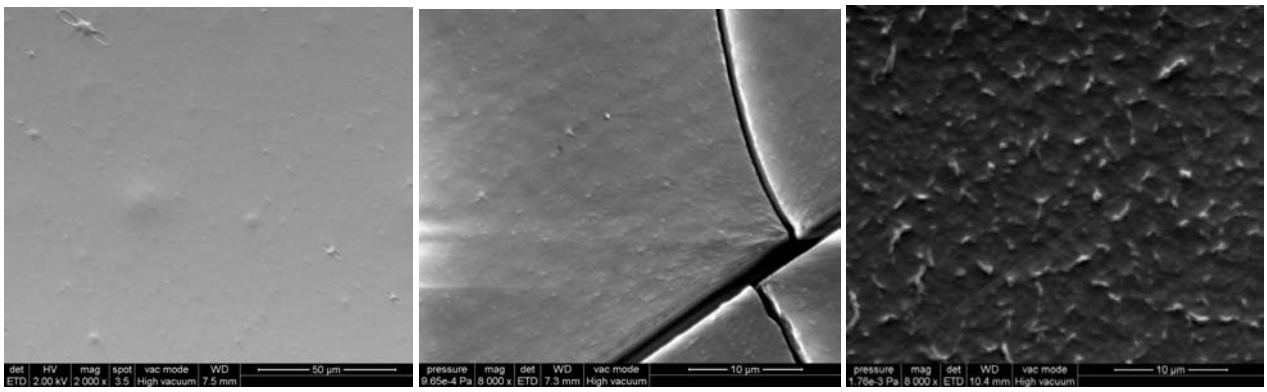
The changes in surface hydrophility were studied with a CAM 101 Contact Angle Goniometric System, KSV Instruments LTD, Finland. In Fig. 2 the contact angle values decreasing from 64° for untreated PET (Fig. 2a), to 45° for simple chemical treatments (Fig. 2b) and 20° for plasma and chemical treatments (Fig. 2c).

SEM investigations of the treated PET samples were performed with a QUANTA 200 instrument. Fig. 3 highlights smooth surfaces for the untreated PET (Fig. 3a) and the plasma 10 min followed by chemical 30min treatments (Fig. 3b), while in the case of the chemical treatments 30 min, the surface became rough (Fig. 3c).

The collagen immobilization was made on samples treated 30 min chemical and those who suffered plasma and 30 min chemical treatment. Following collagen immobilization at PET surface, the SEM images emphasize the distribution of collagen molecules, evidencing an irregular sphere-like appearance, due to the occasional overlapping of some collagen molecules to each other (Fig. 4). For smooth hydrophilic substrates, collagen forms homogeneous layers with small surface features (Fig. 4a), while elongated structures attributed to collagen aggregates are found on hydrophobic ones (Fig. 4b). The glycines, prolines and hydroxyprolines are mainly responsible for the triple helix structure of the collagen. The repulsion of the prolines gives the helical structure and turns the H-side chain of the glycines to the inside of the helix, therefore the self-assembled collagen molecules with intra- and intermolecular cross-links are stabilizing the structure.

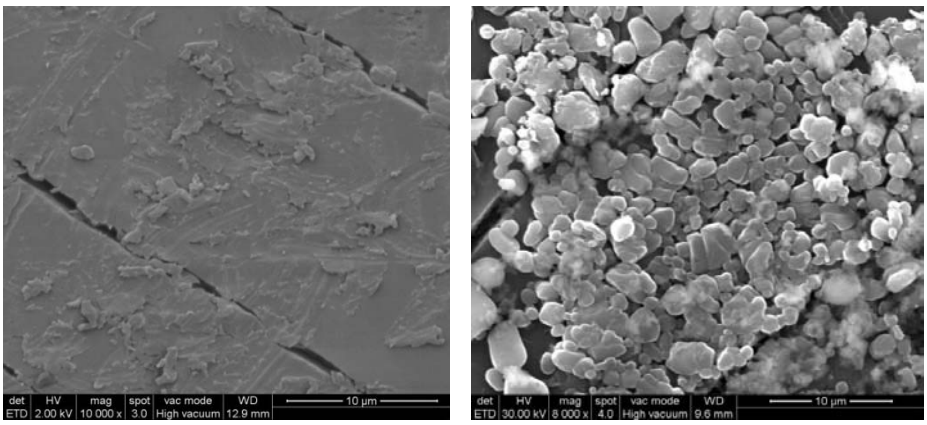
(a) (b) (c)

Fig. 2 – Contact angle images for (a) untreated PET, (b) simple chemical treatments, (c) plasma and chemical treatments.



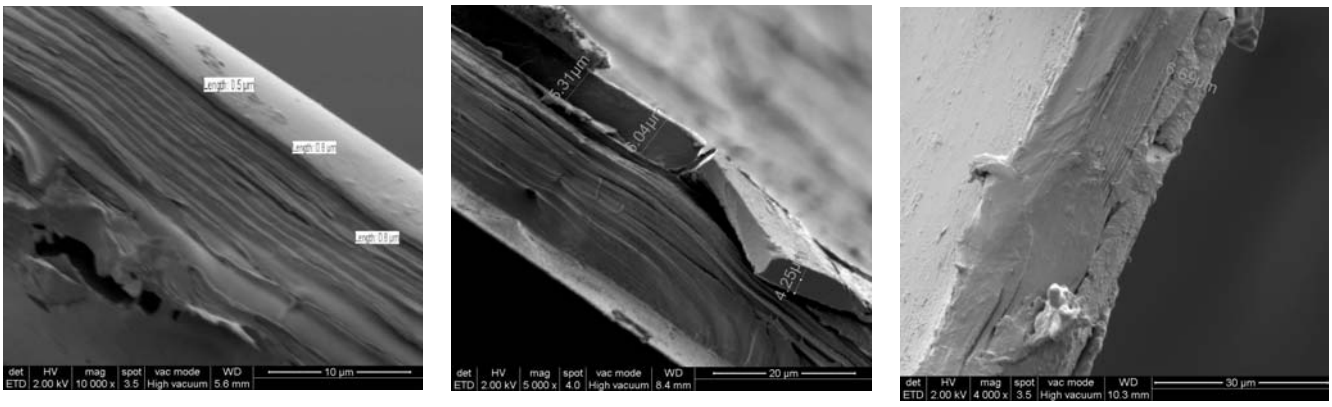
(a) (b) (c)

Fig. 3 – SEM images of (a) Untreated PET, (b) Plasma 10 min followed by chemical 30 min treatments,
(c) Chemical treatments 30 min.



(a) (b)

Fig. 4 – SEM images of collagen immobilized on (a) PET films treated 10 min with plasma followed by 30 min chemical treatments,
(b) PET films 30min chemically treated.



(a) (b) (c)

Fig. 5 – SEM cryogenic section of (a) untreated PET; (b) PET films 10 min plasma treated followed by 30 min chemical treatments,
(c) PET films 30min chemically treated.

PET films are combinations of the tangled and disordered regions surrounding the crystalline areas (Fig. 5). During the aminolysis, the macromolecular scission occurs in the amorphous part resulting a compact crystalline surface of 0.5-0.6 μm thickness for the untreated PET (Fig. 5a), 4-6 μm for PET films 10 min plasma treated followed by 30 min chemical treatments (Fig. 5b) and 6.7 μm for PET films 30min chemical treated (Fig. 5c).

AFM images were recorded in air at room temperature, in the tapping mode using a Scanning Probe Microscope (Solver PRO-M, NT-MDT, Russia) with commercially available NSG10/Au Silicon cantilevers. The root-mean-square roughness RMS was calculated as the average value for the set of AFM frames of certain scales. The AFM image of the untreated PET is shown in Fig. 6a, with a RMS value of 1.39 nm. Due to the effect of the air-plasma treatment on the surface, a nanopatternation of the surface appears (Fig. 6b) with a RMS value of 1.78 nm. The plasma treatment gives a characteristic hill-valley structure in agreement with results obtained by others on PET fibers.[16] The surface is homogenous and "valleys" are predominant, the distance peak-to valley is 10nm. In the case of PET films treated 10 min with plasma followed by 30 min chemical treatments; RMS is increasing at a value of 3.30 nm.

The AFM images for collagen immobilization indicate a surface structure with small grains for PET films treated 10 min with plasma followed by 30 min chemical treatments (Fig. 7a) (with average diameter of 39 nm, height of 1.8 nm and a density of 1573 grains in 5x5 μm^2 area- Fig. 7b), and more randomly distributed large grains in PET films 30 min chemical treated case (Fig. 8a) (with average diameter of 98 nm, height of 14 nm and a density of 197 grains in 5x5 μm^2 area – Fig. 8b).

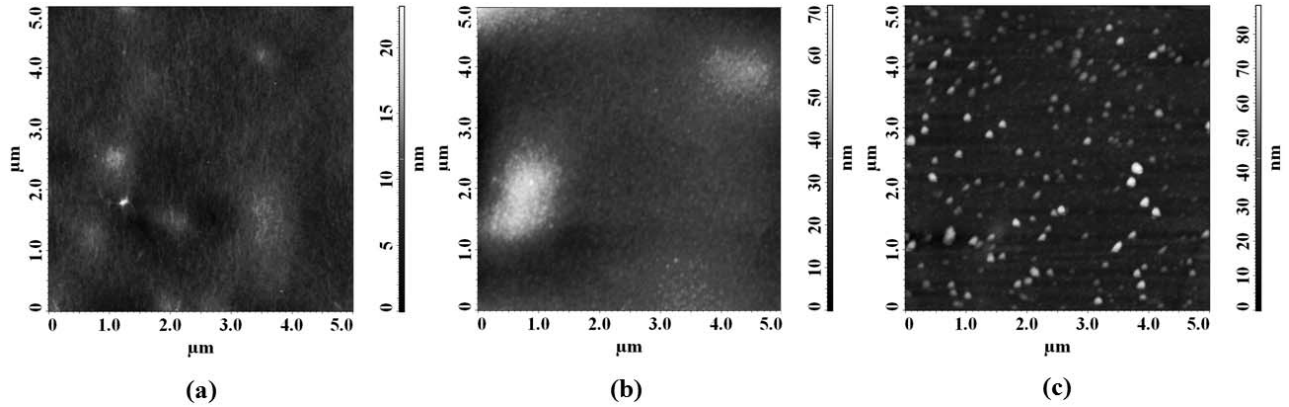


Fig. 6 – AFM images of (a) Untreated PET films; (b) PET films treated 10 min with plasma followed by 30 min chemical treatments, (c) PET films 30 min chemically treated.

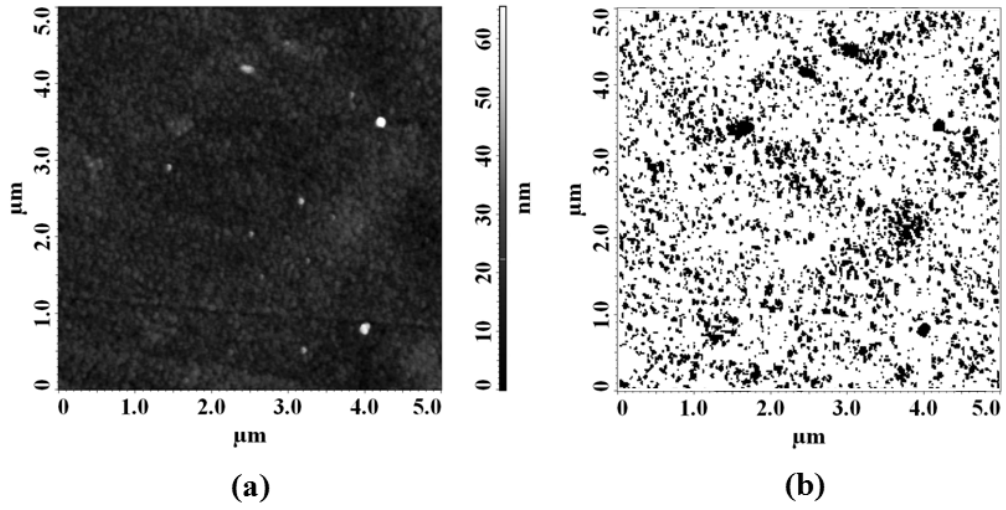


Fig. 7 – (a) AFM images and (b) grain analysis of collagen immobilized on PET films treated 10 min with plasma followed by 30 min chemically treatment.

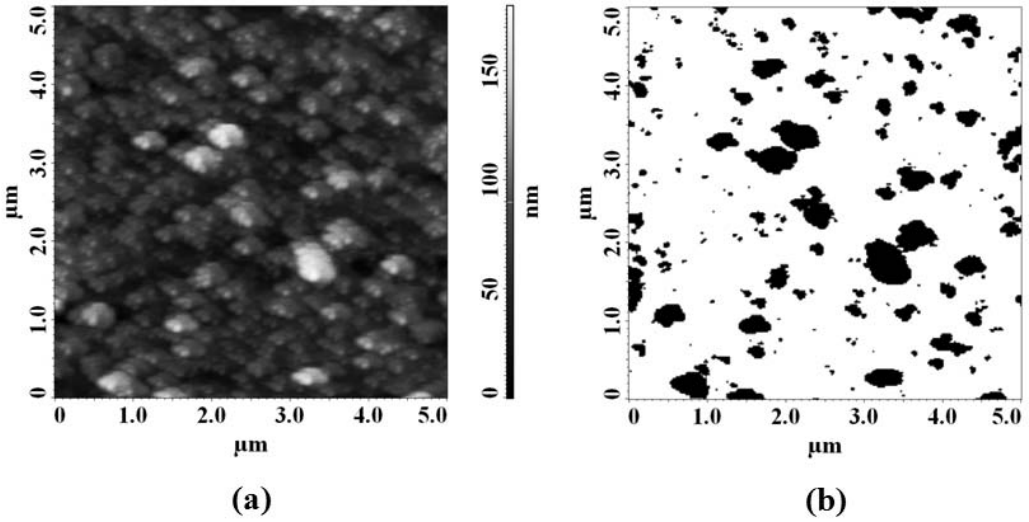


Fig. 8 – a) AFM images and (b) grain analysis of collagen immobilized on PET films 30 min chemically treated.

CONCLUSIONS

AFM images at nanoscale resolution suggest that the surfaces are fully populated with polymer chains. During the aminolysis the macromolecular scission occurs in the amorphous part, resulting a compact crystalline surface with thickness of micrometric scale. For surface activation and collagen immobilization, AFM and microscopic measurements demonstrated that plasma-precursor and chemical treatment was more efficient than simple chemical treatment.

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