Immobilization of Biologically Active Species on PA-6 Foils Treated by a Dielectric Barrier Discharge

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ABSTRACT: In the field of biomaterials and biomedical devices, surface activation has been focused on creating functional groups capable of preferential adsorption of biologically active species (proteins, enzymes, cells, drugs, etc.). In this way an interface can be created between the synthetic material and the biological medium, with the aim of increasing the compatibility of the implant with the human organism. In our experiments a dielectric barrier discharge (DBD), in helium at atmospheric pressure, was used as the source of energy capable of creating active centers that render the functionalized surface favorable to immobilization of biological molecules. Retention of immunoglobulin (IgG) and heparin biomolecules on polyamide-6 (PA-6) surfaces after treatment by the DBD was analyzed by atomic force microscopy, adhesion evaluation, and measurement of the contact angle titration in order to assess this incorporation on the treated surfaces. The marked adsorption of the biomolecules on the active sites created by DBD on the exposed surfaces also was related to a complex set of processes, such as enhanced roughness, increased surface wettability, and modified distribution of cationic and anionic groups on the treated surfaces. All these factors could promote interfacial interactions between the specific groups of the biomolecules existing in the biological medium and the type of cationic and/or anionic groups present on the surface. The efficiency of the DBD treatment showed that the DBD technique is useful for preactivation of the polymer surface for immobilization of other biologically active species (such as drugs and enzymes). © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1985–1990, 2003

Key words: dielectric barrier discharge; functionalization; heparin and immunoglobulin immobilization; atomic force microscopy (AFM); contact angle titration

INTRODUCTION

Biological liquids, such as blood, tears, milk, and water contain such components as proteins, lipids, carbohydrates, salts, and cellular elements that interact not only with each other but also with an implant surface and require stability between the fluid and the biomaterial. This interfacial contact between the human organism and an implant implies several complex processes that can generate undesired effects such as toxic and adverse allergic reactions, deposition of the biological liquid components, thrombus formation (in the case of blood–implant contact), and cell interaction.[1–3]

One of the solutions for preventing and/or controlling possible adverse effects on blood–biomaterial interface is to create "a new surface" that could make the implant more human compatible by immobilization of certain biologically active species.[4,5] For example, deposition of albumin and immunoglobulin (IgG) layers could prevent cell attachment from the blood on the surface,[6,7] whereas heparin immobilization could enhance the inactivation of clotting factors at the implant surfaces.[5,8,9]

This coverage of the surface with biomolecules (e.g., heparin, IgG, collagen, drugs, enzymes, cells etc.) could be accomplished by preactivation/functionalization of the polymeric surface,[2–4,10] using various techniques such as chemicals,[11] UV radiation, electron and ion beams, glow discharge treatments,[2–4,10,12] and plasma deposition.[13,14]

Among plasma technologies, the dielectric barrier discharge (DBD) provides a processing method capable of inducing the desired surface modification and optimizing certain interfacial phenomena such as wettability, hemocompatibility, cell and protein adhesion, and electrical properties.[10,15,16] The DBD can work at low or atmospheric pressure, with some specific advantages such as dry, rapid, sterile, and localized processing and keeping the bulk properties of the material unaffected.

DBD treatment allows the creation of active species on the polymer samples in order to form linkages with the biomolecules that must be immobilized. In these immobilization processes the surface topography and physicochemical properties are very important, yielding either good, stable retention from chemical reactions between specific backbone groups or else desorption of the immobilized biomolecules, which might occur frequently. In particular, the plasma treat-

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ments could modify the electrical properties of polymer surfaces by creating ionized groups on the surface. For implants the presence of some types of ionized groups must be associated with blood compatibility, knowing that the electrostatic interactions between the charged vessel walls and the components of blood (a strong electrolyte) enhance thromboembolic processes. Thus, it was found that positively charged prosthetic materials are thrombogenic, whereas negatively charged surfaces tend to be nonthrombogenic.[17]

The principal aim of this study was to create active centers, using DBD treatments in helium at atmospheric pressure, for the incorporation/immobilization of some biological molecules that are of interest in medical applications. The molecules studied were heparin (a biomolecule with known anticoagulant properties) and IgG (a protein in antibody activity, in antigen–antibody interactions, and in immune complexes).

Analysis of heparin and IgG retention on the treated PA-6 surfaces was achieved by atomic force microscopy (AFM) and of heparin and IgG adhesion on the surface by contact angle measurements. Special attention was devoted to measurement by contact angle titration, using buffer solutions of different pH values. Taken into account when making these measurements was the effect on the contact angle between a biological liquid and the surface of the number of these active and ionized surface sites and of the pH of the background electrolyte.[17] The pH of liquids is very important in interfacial processes, affecting the stability of membrane filtration properties[18] or the immobilization of active species on polymeric surfaces.[5,19] Dedicated software allowed for fitting the contact angle versus the pH and obtaining the surface density of the ionized groups, the acid–base dissociation constant, the effective dielectric constant at the polymer–water interface, and other values. Moreover, it was possible to correlate the ionized species on the surface after DBD treatment with the zeta (ζ) potential, an important quantity controlling the adhesion characteristics of materials and, particularly, the clotting sequence of blood.[17,19]

In our experiments the treated samples were polyamide-6 (PA-6) foils, which are used in medical applications for orthopedic implants, catheters, membranes for reverse osmosis, ultrafiltration, and electrodialysis or as monofilaments in nonabsorbable sutures.[20–22]

EXPERIMENTAL

DBD treatments

Figure 1 shows the schematic design of the homemade DBD equipment used for surface modification. The DBD configuration, which generally consists of two

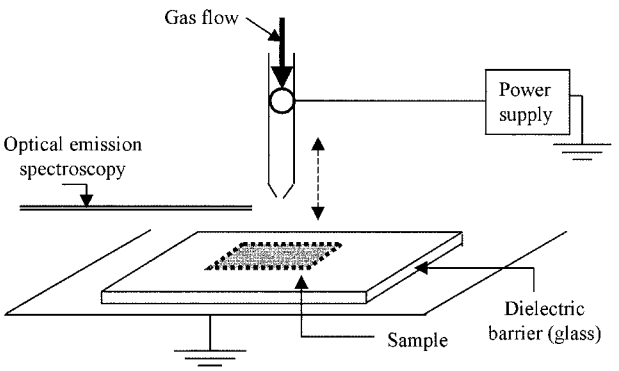

Figure 1 Experimental setup.

electrodes separated by a dielectric barrier, was the particular one in our experiments,[23] in which a pulsed corona system with one of the electrodes covered by a dielectric material could be used as a DBD.

The electrode geometry was the point-to-plane type, and the distance between electrodes could be modified. The HT electrode was spherically shaped (radius $\approx$1 mm), and the grounded electrode (GND) was a metallic plate of 100 $\times$ 100 mm^2 covered with a glass plate (dielectric barrier, $\varepsilon_r \leq 10$) 1 mm thick. In our treatments the distance between electrodes was adjusted to 20 mm. The samples to be treated were placed on the glass plate. The dielectric material distributed the conic shaped discharge uniformly over an area of about 2 cm^2 on the treated sample. The discharge was diffuse and glowlike, with no filamentary streamers in the discharge gap.

The discharge was generated between the electrodes by a pulsed high voltage (28 kV peak to peak), with a frequency of 13.5 kHz. Voltage and current signals through the circuit were monitored with a METRIX oscilloscope with a type IEE 488 acquisition system. The total electric power converted at the high-voltage electrode and dissipated on discharge can be controlled, and in our experiments it was 40 W.

A gas shower placed near the HT electrode introduced the working gas (helium, spectral purity 99.99%), the gas flow direction was orthogonal to the point electrode, and the flow rate, measured with a rotameter, had a constant value, 30 sccm, during experiments. Helium is well known for use in polymer surface treatments because it has a small degradation effect and high crosslinking and functionalization.[24]

In the experiments treatment time was varied between 10 s and 1 min.

Materials

The PA-6 samples, 10 $\times$ 10 mm in size and 0.015 and 0.25 mm thick (Goodfellow Co.). Before treatment they were washed in alcohol, rinsed in bidistilled water, and dried for 1 h in an oven at 50°C. Treated and

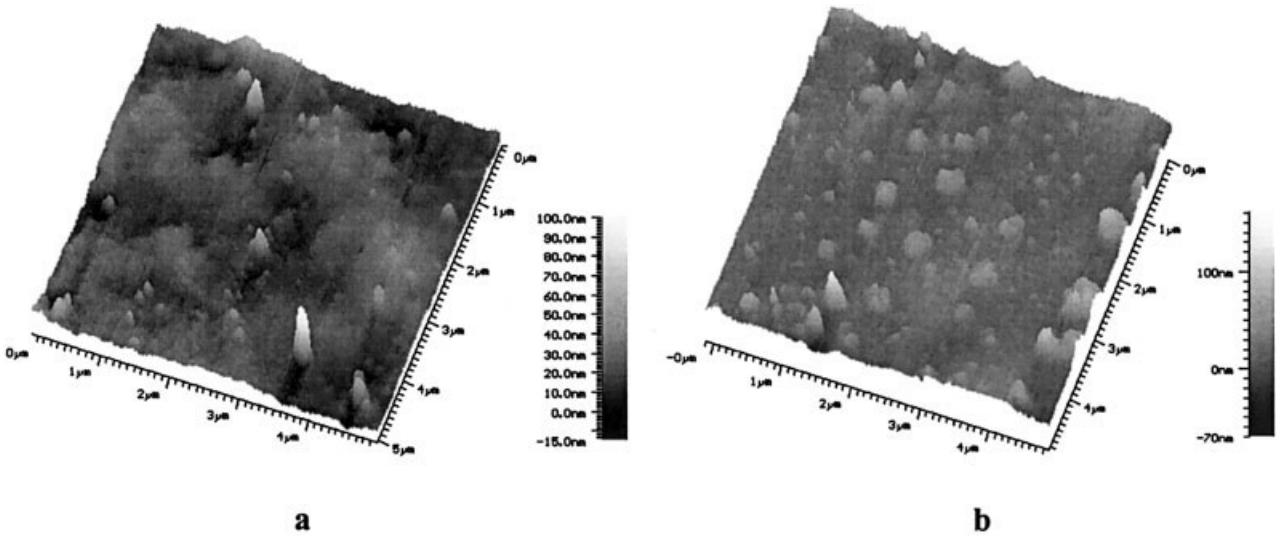

Figure 2 AFM images of (a) untreated and (b) 10 s–treated PA-6 surfaces, immersed for 1 h in human IgG (5 × 5 μm).

untreated samples were then immersed for 1 h at 20°C, in a solution of commercial human IgG and heparin (Fluka Chemie GmbH Co.), 0.5 mg/mL (pH 7.4), which was periodically mixed. Both shorter (1 h) and longer (up to 24 or 48 h) adsorption times were conveniently chosen in order to have information about the optimal conditions (i.e., minimum time of treatment and maximum efficiency) for immobilization. After adsorption the samples were rinsed again in bidistilled water and dried before surface analysis to avoid measurement errors related to intrinsic physical adsorption.

Relative amount of adsorbents (IgG and heparin) on the polymer foils were estimated by gravimetric measurement, using a torsion balance with 0.2 mg error, and were calculated in milligrams per square centimeter. Experimental data were obtained in static conditions and with low concentrations of biological liquids, as the exchange of molecules was readily observed.

AFM analysis

The AFM technique permitted a real-space visualization and comparison of the 3-D surface morphology of the PA-6 foils and of the adsorption of IgG and heparin on untreated and treated surfaces. AFM images were obtained in the tapping (noncontact) mode, which is nondestructive for the surface as the tip does not touch the surface and the biological layer is not damaged. Porosity (roughness) of the PA-6 surfaces, which was an essential parameter for this work, was verified by statistical AFM estimations. In our experiments the image covered various areas, from 70 × 70 μm to 1 × 1 μm, and AFM measurements were extended to different sites of the sample and repeated under the same temperature and pressure conditions, room temperature and ambient atmosphere.

Special attention was devoted to phase detection. This information is complementary to the topography images, reflecting changes in surface adhesion properties.

Surface energy characterization and contact angle titration

Measurement of the contact angle between the biological liquids and the polymeric surface allowed the investigation of the surface energetic properties, particularly the work of adhesion, the surface free energy, and their components, before and after DBD treatment. Contact angles were measured by the sessile drop technique immediately after the DBD treatment.

The pH of 10 m*M* NaCl was adjusted, adding small quantities of concentrated solutions of either HCl or NaOH, and the experiments were conducted at different pH values above, below, and at the isoelectric point (pI). While the contact angle measurements were being made, the room's temperature and humidity were controlled, and the response of the material to various pH solutions was monitored at 1 h after the DBD treatment. We must mention here that a limitation of this approach is the heterogeneity of the surface active and ionized sites, which may not be uniformly distributed on the surface.

RESULTS AND DISCUSSION

The influence of DBD treatment on the adsorption phenomena at the interface of the biological liquid with the sample of PA-6 is illustrated by the AFM images, shown in Figure 2(a,b) for IgG and in Figure

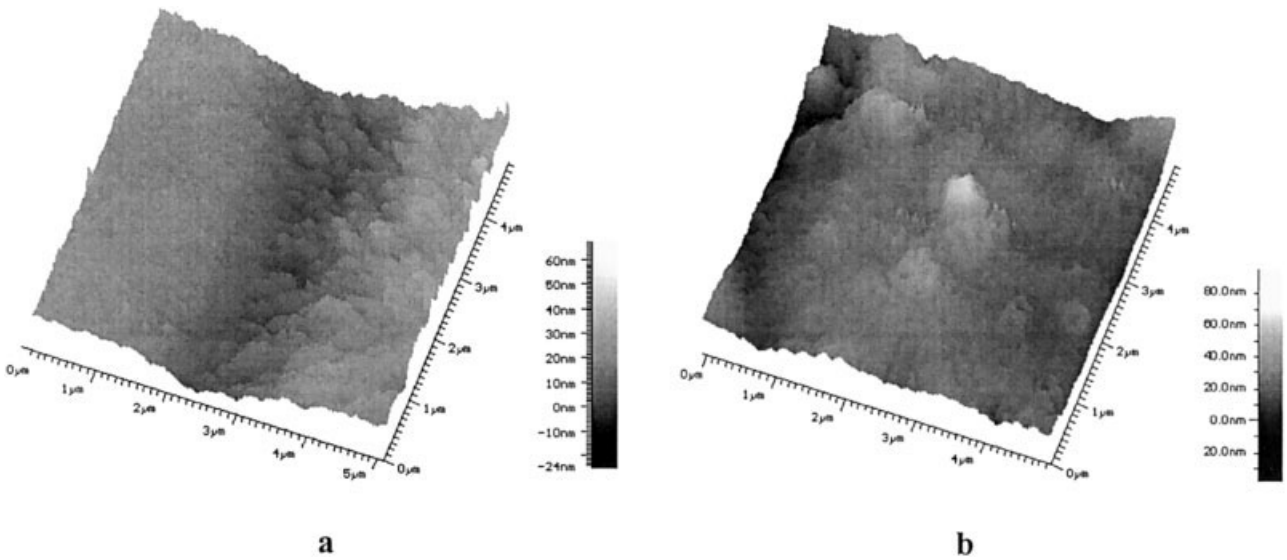

Figure 3 AFM images of (a) untreated and (b) 10 s–treated PA-6 surfaces, immersed for 1 h in heparin (5 × 5 μm).

3(a,b) for heparin adsorption. There was a readily observed density increase in adsorbed biomolecules on the treated surfaces compared to the untreated ones. The IgG molecules can clearly be seen on the surface [Fig. 2(b)], as they are larger than the heparin molecules in AFM images represented on the same scale.

The adsorption process depends on DBD treatment duration, duration of immersion of the PA-6 foils, and oncentration of the biological liquids. Taking into account the results of a series of tests of these adsorption parameters, we chose the shortest times—a 10-s treatment duration and a 1-h immersion of a sample in the biological liquid—in order to have measurable and reproducible adhesion.

The IgG and heparin immobilization on the PA-6 samples had to be correlated with the DBD treatments, which induce different types of surface alterations: mechanical and physical modification by removal of specific functional groups from the material surface, functionalization by generation of active sites, chemical modification by etching or grafting, and creation of a new chemical layer on the surface.[2,6,10,15] Thus, it is difficult to assess which of these processes might have been primarily responsible for the immobilization of biomolecules at the surface of DBD-treated samples and also to separate the physicochemical mechanisms at the interface between the treated sample and the biological liquid.

This immobilization could be correlated with new roughness of the surfaces and with surface-modified energetic characteristics, all of which are modifications induced by the DBD treatments.

Although an absolute roughness cannot be estimated, the root mean square roughness (R_{rms}), the average diameter, and the density of grains on the surface were verified systematically before and after the treatments. The results of all sample measurements showed smooth surfaces, with about 5–7 nm of R_{rms} before DBD treatments [Fig. 4(a)]. After DBD treatments there was an increase in surface R_{rms} roughness [for example, from 7 to 12.6 nm for a 10-s treatment, Fig. 4(b)], with changes in the size and distribution of the grains. A rearrangement and/or preferential orientation of the grains on the treated surfaces also was observed [Fig. 4(b)].

Nonetheless, this adsorption on the treated surface cannot be correlated only with the modified surface roughness. Here we emphasize that the immersed samples were washed before surface analysis to minimize any temporary physical effect. Moreover, the marked biomolecules' immobilization was stable with time. Thus, the DBD treatments, as shown by the contact angle measurements, enhanced the hydrophilicity of the polymers, leading to high adsorption of IgG and heparin on the treated surfaces of PA-6. After the treatments the degree of hydration of the PA-6 samples increased, being favorable to interfacial adsorption phenomena (Fig. 5). The stability of this new interface was reached within a duration of 1–3 days, which ensured a mutual rearrangement of molecules in the interfacial layer (Fig. 5). The increased wettability can be attributed to the formation and/or introduction of polar groups and scission products, which enhanced new and specific interactions across the interface.

The presence of polar sites after DBD treatments was confirmed by contact angle titration measurements of acid and basic test solutions (NaCl). Figure 6 presents the dependence of the contact angle between the surface of the PA-6 foils and NaCl solution at various pHs. The contact angle showed no depen-

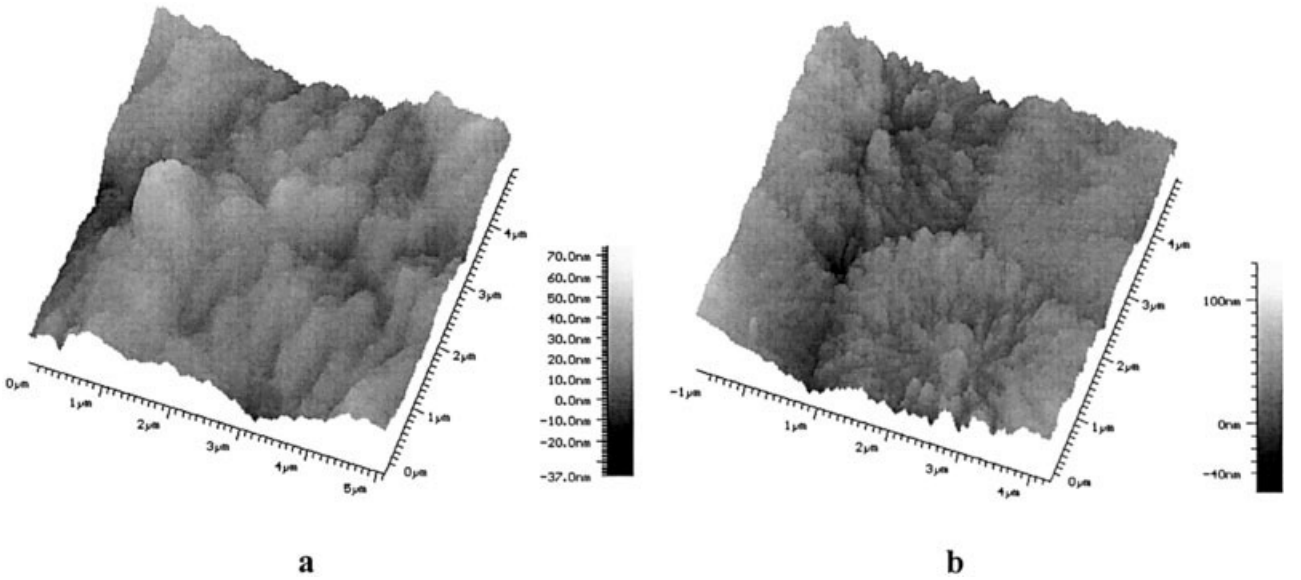

Figure 4 AFM images of (a) untreated (R_{rms} = 7 nm) and (b) 10 s–treated (R_{rms} = 12.6 nm) PA-6 foils (5 × 5 μm).

dence on the pH on the untreated surfaces, whereas for the treated surfaces, a decrease in the contact angle with an increase in pH was observed. This diminution in contact angle values indicates a higher hydrophilicity of the treated surfaces at a pH of 10–12 because of strong electrostatic interactions at the interface. We suppose that acid groups were created or introduced within the surface layer by the treatments and that the Cl^- and OH^- anions present in NaCl electrolyte were preferably adsorbed by these treated surfaces.

It is known that most polymers have a certain degree of both an acid and a basic character, but in our investigation, that is, with PA-6 foils, the cationic groups led to a decrease in the contact angle at a high pH. In the particular case of heparin adsorption, isolated groups of anionic heparin were linked to the cationic sites that the DBD treatment produced on the surface.

These results can be correlated with the ζ potential, as reported in the literature.[17] The ζ potential, defined as the boundary electrostatic potential between a liquid phase and a solid, enables the characterization of the long-distance interactions within an implant–human environment interface. Thus, it was reported that a decrease in ζ potential while pH was increasing suggests that at a high pH, ζ potential is more negative and, finally, that the material could be less thrombogenic.[17]

The presence of new groups within the surface layer was confirmed by the AFM phase images of the sur-

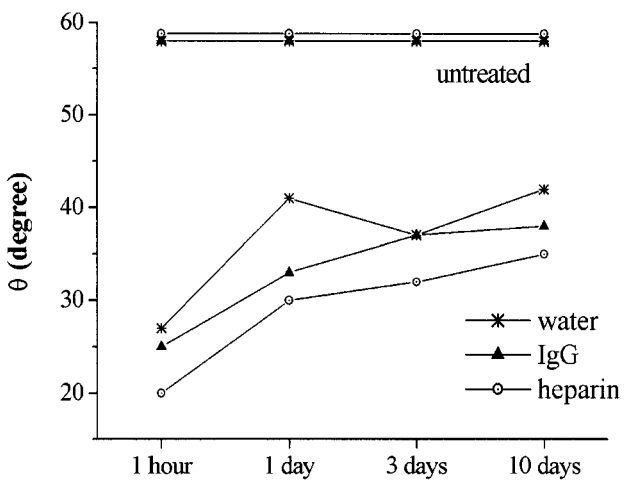

Figure 5 Adhesion properties of water, IgG, and heparin on the untreated and 10 s–treated PA-6 samples.

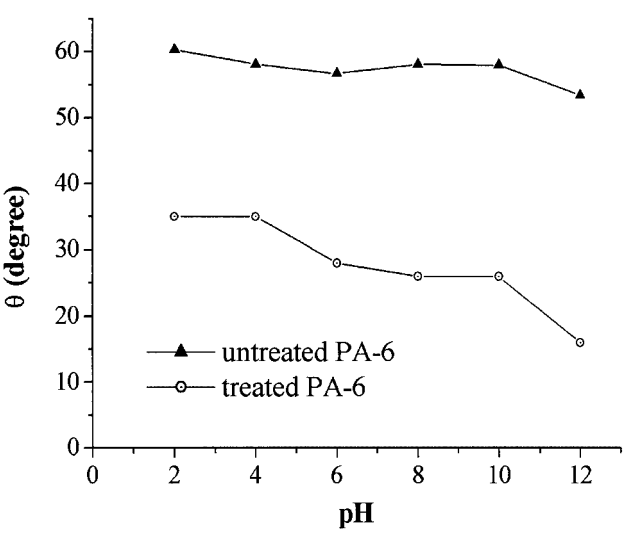

Figure 6 Contact angle between the surface of 10 s–treated PA-6 foils and NaCl solution at various pHs.

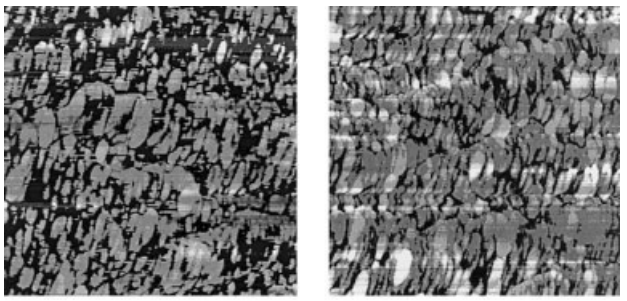

Figure 7 AFM phase images of (a) untreated and (b) 10 s–treated PA-6 foils (3 × 3 μm).

faces [Fig. 7(a,b)], which showed a modified chemical structure of the surface after the DBD treatments.

Normally, immobilization of certain biomolecules can be influenced by various factors such as a nonhomogeneous chemical composition of the polymer surface, instability of the surface properties because of desorption of impurities and additives, atmosphere irradiation, humidity, and oxygen, which may be favorable or unfavorable to biomolecule immobilization. On the other hand, at high pH values, at room temperature, and after a long exposure time, the polymer foils can be hydrolyzed. But in our measurements this partial degradation was not as possible, as the thermodynamic equilibrium of the contact angles was reached within a few minutes.

Gravimetric measurements allowed us to confirm the biomolecule immobilization on the treated PA-6 surfaces, with a 30% increase in weight shown on the treated surfaces.

Further investigations using surface spectroscopic techniques, IR and XPS, specifically, will permit us to discern among the interfacial phenomena (physical adsorption, chemical binding and/or chemical crosslinking) occurring in biomolecule adsorption. On the other hand, specific assays are required to investigate the activity of the immobilized biomolecules.

We mention also that marked adsorption of IgG and heparin has been obtained on PVC samples treated by DBD, these surfaces being more wettable after plasma treatment.[10,25]

CONCLUSIONS

DBD treatments had the ability to create active and ionized sites on the polymer surface of PA-6 foils, favoring interactions between the material and some biological liquids (IgG and heparin) of interest in medical applications. Short treatment times (10 s) allowed us to obtain important surface modifications, and the optimal reaction time for immobilization of IgG and heparin on the PA-6 surfaces was found to be 1–2 h after treatment. The marked adsorption of the biomolecules after the DBD treatments was related to a complex set of processes, including enhanced roughness, increased surface wettability, and modified distribution of cationic and anionic groups on the treated surfaces. All these factors could promote interfacial interactions between the specific groups of the biomolecules in the biological medium and a type of cationic and/or anionic group present on the surface.

The DBD technique has many advantages compared to chemical technologies, such as economics, reliability, and simplicity in developing for industrial implementation.

The efficiency of the DBD treatments for biomolecule immobilization makes this technique a useful method for activation of the polymer surface in order to immobilize such biologically active species as drugs and enzymes.

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