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THROMBOGENICITY TESTS ON AR-IRRADIATED POLYCARBONATE FOILS

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ABSTRACT

Understanding polymer surface properties is extremely important for the most wide range of their applications, from basic coating to the most complex composites and biomaterials. Low energy ion beam irradiation of polymer can improve such surface properties. By modifying its surface biocompatibility, polymers are excellent candidates for biomaterials, due to its malleability and low weight, when compared to metals. In this work, we irradiated 30-μm Bisphenol-A Polycarbonate foils with 23-keV Argon ion beam at six different doses. Aluminium foils were simultaneously irradiated in order to measure the doses by Rutherford Backscattering Spectroscopy. The surface modifications after the argon ion beam irradiation were analyzed by water contact angle measurements and atomic force microscopy. Platelet adhesion tests were used in order to investigate thrombogenicity, showing a growing tendency with the irradiated Argon dose.

1. INTRODUCTION

Any material, natural or synthetic, which realizes, increases or substitutes a natural function is considered a biomaterial. The main surface properties for a material to be investigated in order to find a biomaterial candidate are: corrosive properties, morphology and roughness, surface crystallinity, surface chemical composition, wettability and cell adhesion [1]. Polymer, as a class of materials, are important potential biomaterials due to their low molecular weight and high flexibility.

When a polymer comes into contact with blood, its first response is the protein absorption, followed by interactions with platelets, white cells and red cells. The living species will

adhere to the materials surface if there is any attractive force between them. The great obstacle on implanting artificial polymer prostheses in cardiology is the blood clotting when in contact to the material. Usually, the problem is managed by the administration of anti-clotting drugs, which can cause collateral effects when taken for long periods. For this reason, a number of chemical and biological surface modification procedures were developed in order to prevent the activation of the clotting factor cascade. However, such chemical and biological treatments present risks of pathogenic transfers, liberation of cytotoxic agents and immune reactions, making the use of ion beam irradiation techniques more adequate for achieving better surface properties.[2].

In the past, the radiation effects on polymers were considered to be only depending on the material structure and not on the kind of radiation. However, the response of such materials is considerable different among gamma rays, electrons and ions. Polymers can become either cross-linked or chain-scissioned under ion beam irradiation. Additionally, the different chemical compounds present in polymers (*e.g.* C, N, O, H, F) can interact independently with the incident particle and become excited or ionized. The activated species thermalize, recombining or leaving the surface, resulting in changes of the polymer surface chemical and physical properties. Because of such features and the possibility of controlling precisely beam species, energies and doses, materials scientists are using ion beam irradiation to modify polymer surface properties. [3].

2. EXPERIMENTAL

The pristine sample is a 30 μm commercial polycarbonate (PC) foil (Makrofol DE,*Bayer MaterialScience AG* - Leverkusen, Germany). Its nominal composition is polycarbonate with initializer Bisphenol-A ($[C_{16}H_{14}O_3]_n$). Five PC foils were irradiated at the Laboratory of Ion Implantation - IFUSP with a 23 keV Ar^+ ion beam with doses ranging from 1 to 50×10^{15} ions/cm^2. (as shown in Table 1). The current density was kept under 10 μA/cm^2. The doses were determined by Rutherford Backscattering Spectroscopy at the Laboratory of Ion Beam Analysis of Materials - IFUSP using a 2.2 MeV He^+ beam with normal incidence, 50 nA current and 40 μC integrated charge for all samples at 170° backscattering angle.

Table 1: Argon beam dose irradiated for each PC sample.

Sample	Dose (10^{15} ions/cm^2)
PC	-
PC1	1.000(18)
PC2	5.000(90)
PC3	10.00(18)
PC4	33.00(59)
PC5	50.00(90)

Water contact angle measurements were done by dropping 30 μL drops of distilled water on the samples' surface. The images were captured by a CCD camera coupled to a magnifying lens system. The analysis were made using the snake plugin [4] for the Image-J software [5].

Thrombogenicity tests were performed at the Biotechnology Center Laboratory following the ISO 10993 standards. Positive and negative control samples (natural Latex and polypropylene, respectively) were also tested. The procedure is as follows: the samples are firstly fragmented and distributed over the surface of a microscope coverglass and then they are lined and capped in a Petri dish humidified by distilled water and placed in a 37°C bath for fifteen minutes. After the bath, fresh blood from healthy donors is added to the plates system, submerging all the samples. The new system is then kept five minutes in a 37°C bath. The samples are washed in 0.9% saline solution followed by glutaraldehyde 2.5% addition, covering all the samples for 10 minutes. After removing the glutaraldehyde solution, the samples are dehydrated with alcohol 50%, 75% and 95% solutions. Finally, the Petri dish is transferred to a dryer for 24 hours. The samples are evaluated with Scanning Electron Microscopy, carried out at the Analytical Central of the Chemistry Institute of the University of Sao Paulo.

Atomic Force Microscopy (AFM) was performed at the Laboratory of Thin Films - IFUSP, carried out in a NanoScope IIIA from Digital Instruments. The samples analysed were samples PC and PC4 in order to check morphology and roughness changes after high argon dose irradiation. Images rastering 4x4 μm areas of the samples were obtained.

3. RESULTS

3.1. Water Contact Angle Measurements

The measured contact angle for the pristine sample (PC) was 79.6(47)°. The Figure 1 presents the contact angle values obtained for the PC samples in function of the argon doses irradiated. Up to the dose 1x10^{16} at/cm^2, the tendency is of decreasing of the contact angle value with the increasing of the dose. For the two highest dose values (3.2x10^{16} and 5x10^{16} at/cm^2), an increase of the contact angle value is observed, tending to reach the same value as the one for the pristine sample.

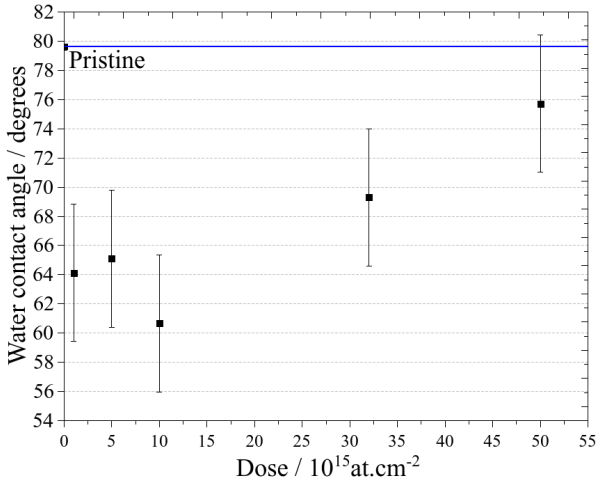
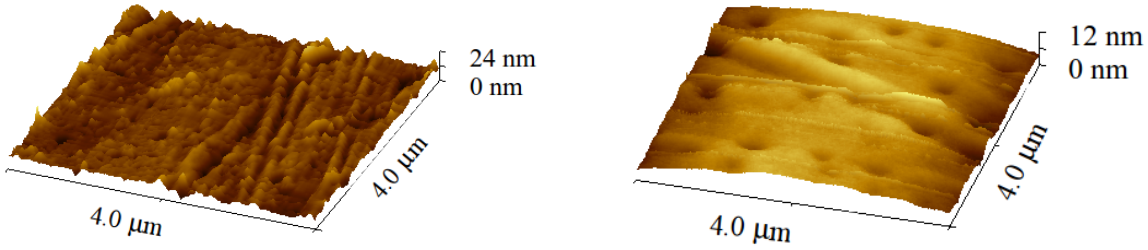


Figure 1: Contact angle values obtained for the PC samples in function of the irradiated argon doses.

3.2. Atomic Force Microscopy

Figure 2 show the AFM images obtained for samples PC and PC4 rastering 4x4 μm areas. The Figure 2(a) presents some tip effects which are not presented in Figure 2(b), where it can be observed the appearance of cavities.

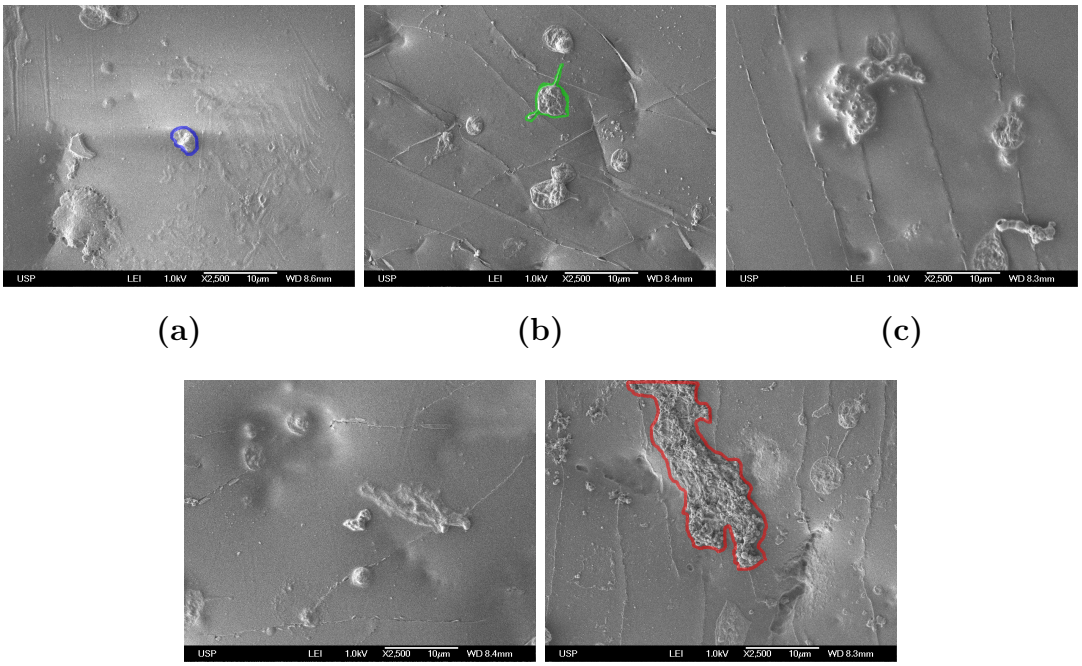


(a) (b)

Figure 2: AFM Morphological images for samples PC (a) and PC4 (b) rastering 4 μm areas.

3.3. Thrombogenicity Tests

The electron microscopy images for samples PC, PC1, PC2, PC4 and PC5 after the platelet adhesion procedures are shown in Figure 3. The images for sample PC3 did not bring any valuable result. A Non-activated platelet (blue contour), an activated platelet (green contour) and a thrombus (red contour) are identified in Figure 3 as examples.



(a) (b) (c)

(d) (e)

Figure 3: Electron microscopy images for samples PC (a), PC1 (b), PC2 (c), PC4 (d) and PC5 (e) after the platelet adhesion procedures.

4. DISCUSSION & CONCLUSIONS

In the contact angle results, a possible explanation for the tendency to reach the same value as the one for the pristine sample observed is the fact that the samples irradiated with high argon dose became rougher, and the contact angle measure is a reflex of such roughness increase. The samples irradiated with lower doses do not present high roughness value, making possible the contact angle measurement to reflex only chemical changes in the surface, revealing a decrease of hydrophobicity with the increase of the irradiated dose.

In the AFM image of the argon beam irradiated sample (Figure 2(b)), it is possible to observe the appearance of cavities with mean diameter measured as 0.358(12) μm. Such cavities are possibly generated by thermal spike and argon atoms blistering effects. Values root mean square (RMS) roughness were calculated for both AFM images. In accordance to the contact angle measurements, the pristine sample presented a higher roughness value (2.05 RMS) than the sample PC4 (1.01 RMS). For higher doses (sample PC5) It is expected an increasing of the cavities density which would lead to an increasing of roughness, reflected on the contact angle measurements.

The adherence of platelets is characterized by their activation and subsequent agglomeration (thrombi formation). Activated platelets present pseudopods, which are important for the thrombi formation by increasing the collision frequency and minimizing the electrostatic repulsion between two platelets. Based on literature values for geometrical sizes of human platelets [6], the number of platelets non-activated, activated and thrombi was counted for each sample. The thrombi number was calculated as the ratio of a thrombus length by a non-activated platelet mean diameter. As all the images were obtained rastering the same size areas, the values are representative densities of the samples. The Figure 4 presents the counts in function of the irradiated argon doses on the polycarbonate samples. (the lines do not represent a fit and are just for guiding the eyes).

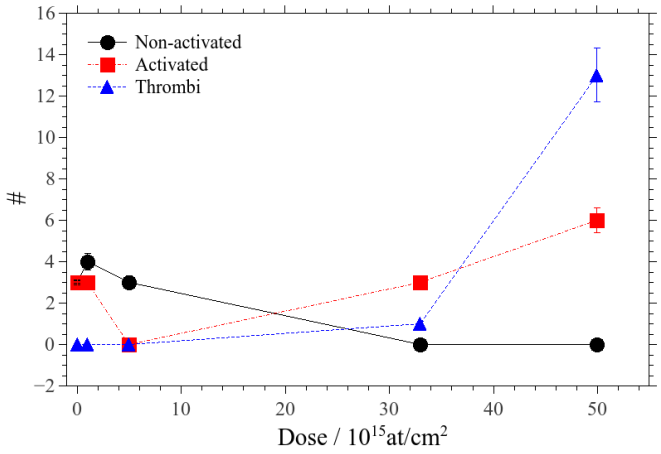


Figure 4: Representative densities of non-activated platelets, activated platelets and thrombi in function of the irradiated argon doses on the polycarbonate samples.

It can be observed that there is an increasing tendency of the activated platelets and thrombi formation with the increase of the irradiated dose. Such tendency is consistent with the decrease of non-activated platelets. The results indicate that the irradiation process changed the thrombogenicity of the polymer samples with significant increase for doses in the order of 10^{16} ions/cm^2.

The changes on thrombogenicity of the polymer samples' surfaces can be related only to the morphological changes, observed by the contact angle and atomic force microscopy measurements, however, different effects can also interfere on platelets behaviour, such as the activation of chemical species on the surfaces, removal of additives or hydrogen loss. Details on this effects and their influence on thrombogenicity were investigated by different spectroscopic techniques [7].

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