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IMPROVEMENT OF POLYURETHANE SURFACE BIOCOMPATIBILITY BY PLASMA AND ION BEAM TECHNIQUES

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A wide variety of gas phase excited species and ions with kinetic energies from 1–10^7 eV increasingly are being used for the growth and modification of polymer interfaces. Ions can be used to deposit thin layers or to expose fresh interfaces by sputtering; induce specific chemical functionalities to a surface and create micron- and nanometer-scale interface structures. In our work we used surface treatments by helium plasma at atmospheric pressure and Ar^+ ion implantation in order to increase biocompatibility of a new biodegradable polyurethane with lactate segment, poly(lactaturethane), films surface suitable for tissue replacement The results obtained for poly(lactaturethane) films was compared with that obtained for the films of poly(esterurethane) precursor. The *in vitro* tests refers to the films structure and surface properties by: differential scanning calorimetry (DSC), wide-angle X-Ray diffraction (WAXRD), attenuated total reflectance Fourier Transform InfraRed (ATR-FTIR), atomic force microscopy (AFM); and thermodynamics of energy adhesion by contact angle measurements. The *in vivo* biocompatibility tests of polyurethanes were performed by intradermic implants in lumbosacral region on adult rabbits. Histological tests reveals an important increase of poly(lactaturethane) surface activation by Ar^+ ion beam irradiation confirmed by formation of a high functionalized surface which may increase the endothelial cell adhesion.

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Keywords: Ar^+ ion implantation, Helium plasma, Poly(lactaturethane), Poly(esterurethane), Films characterization, Biocompatibility

1. Introduction

Polyurethanes are polymers with very good mechanical performance and biocompatibility that make them a versatile class of synthetic polymers widely used in medical applications like vascular prostheses [1], biocompatible coatings for ceramic or metal medical devices, ventricular assist blood pumps, intra-aorta balloon pumps, blood bags and compensatory chambers, implantable devices [2], separation applications [3], controlled release system for insulin in artificial pancreas [4], orthopedic surgery [5], artificial hearts, pacemaker lead insulators and vascular grafts [6].

Polyurethanes are also used in the reconstruction of soft tissue and organ covers. For soft tissue replacement, synthetic membranes should be thin to allow gas exchange and transport of biomolecules, in order to assess the cell activity beneath the membrane-tissue interface, and good mechanical properties [7].

Recent advances in surface-modification technology allow the development of biologically compatible surfaces of synthetic polymer films. These methods include carbon deposition, plasma treatments, ultraviolet irradiation and ion implantation [8].

Previous studies demonstrated that the surface of segmented polyurethane, in which endothelial cells are not usually able to proliferate, after modification by surface treatment, allowed selectively cell adhesion and proliferation on the subjected region [8].

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Our study refers to the surface films improvements, by DBD and Ar^+ ion beam treatments, of new polyurethane with lactate segment, poly(lactateurethane) (BP), comparatively with poly(esterurethane) (PU) precursor, in order to increase endothelial cell attachment and to enhance long-term haemocompatibility.

2. Experimental

Thermoplastic polyurethanes PU and BP, was kindly supplied by Professor C. Ciobanu, "Petru Poni" Institute of Macromolecular Chemistry; the synthesis was presented elsewhere [9]. The primary structures of polyurethane units are presented in Fig. 1.

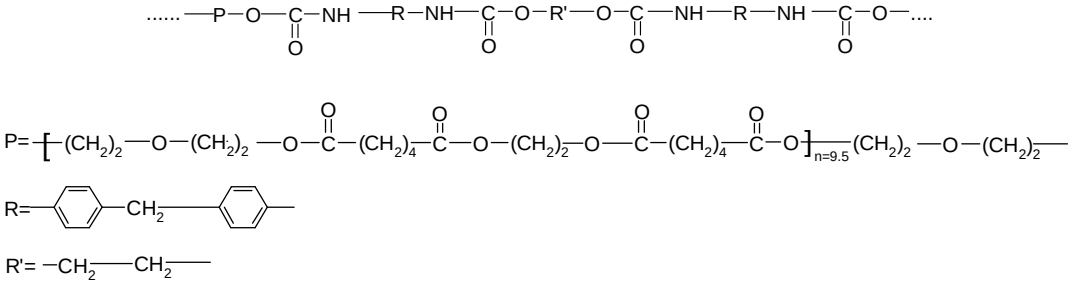

Fig. 1. The primary structures of BP and PU units.

Films were prepared by dry phase inversion method (casting - evaporation). The 30 wt% of BP or PU in DMF solutions were degassed by ultracentrifugation and casting onto glass plates. After casting, the solvent was removed by drying in air at room temperature for 24 hours. The resulted films were dense, transparent and very flexible [9]. Each polyurethane as prepared films have been treated by a dielectric barrier discharge (DBD) in He at atmospheric pressure and by Ar^+ ion beams.

The experimental arrangement of DBD system is described in detail elsewhere [10]. Discharge configuration consists of two electrodes separated by a dielectric barrier of 1mm thick glass plate. The distance between electrodes is adjusted to 20mm and the discharge is driven by a 28kV peak-to-peak high voltage pulsed, with a frequency of 13.5kHz and 40W integral dissipated power. Gas is introduced into the inter-electrode gap with 100cm^3/min flow rate by a gas shower placed near the active electrode. Treatments were performed in the DBD-filamentary regime, generated in a disc-to-plan geometry at atmospheric pressure in helium (spectral purity 99.99%), selected as the most efficient inert gas in the functionalization and crosslinking of the polymer surface. Discharge voltage and current intensity time evolution are monitored by IEE488 interface protocol and visualised with a METRIX oscilloscope.

Surface treatments with Ar^+ ion beam were performed at 100 KeV energy at room temperature using the RIKEN 200KV low current implanter, Tokyo, Japan [11]. The beam current density was 0.1 μA/cm^2, at a fluency of 10^{15} ions/cm^2.

The films microphase mezostructure was investigated by DSC with an EXSTAR 6000 Differential Scanning Calorimeter, WAXRD with Philips X'pert XRD System and surface structure by ATR-FTIR spectra were analysed with a BOMEM MB-104 spectrometer, at 4 cm^{-1} resolution in the range 4000-500 cm^{-1}.

The film surfaces morphology was investigated by AFM in the taping mode with standard silicon nitride cantilever NSC21 having a force constant of 17.5 N/m, 210kHz resonance frequency and tip with radius of curvature less than 10 nm. The AFM measurements were performed at room temperature and ambient pressure. Scanned samples area were from 20 x 20 to 5 x 5μm with a 256 x 256 pixels image resolution.

The adhesion work, W_a, was chosen as a relevant thermodynamic parameter for the energy activity of polymer surfaces. Therefore, W_a was calculated using the Dupré-Young equation [12]:

$$W_a = \gamma_{lv}(1+\cos\theta), \quad (1)$$

where θ is the contact angle between the water (a test biological liquid) and the polymeric surface and γ_{lv} the surface tension of the liquid. Contact angles were measured by the sessile drop technique, immediately after the DBD treatments, at 23^0C, in a room with controlled humidity and averaging the contact angles over ten measurements.

The *in vivo* test biocompatibility was accomplished using twelve adult rabbits of age 2 by intradermic implants performed in lumbosacral region. According to ISO 10993 Guideline (corresponds to EN 30993), local anaesthesia of rabbits have been made with Neurotranc. The implants were plates of 1.5x2 cm^2 with one face subjected to Ar^+ ion beam implantation. After 16 days the samples have been extracted together with the adjacent attached tissue, for macro and microscopic investigations. The specimens have been included in paraffin and from the resulted blocks slides of 4μm have been cut perpendicular to the plate surface. The slides have been coloured by haematoxylin-and-eosin (H.E.) and van Gieson methods. The optical microscopy investigation of slides was performed using a Nikon E600 optical microscope; images were recorded with a Coolpix 950 camera.

3. Results

The presence in BP of lactate segment show important changes, comparatively with PU, of the transition temperatures in the DSC curves (Fig. 2) and in the microphase organization structure done by X-ray diffractograms (Fig.3) [13].

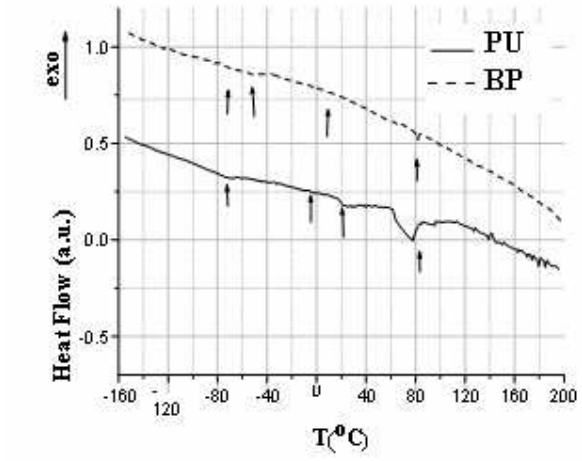

Fig. 2. The DSC traces of BP and PU films.

Important differences of chemical surface structure between BP and PU samples were noticed in the ATR-FTIR spectra within 700-1900 cm^{-1} (Fig. 4).

The surface morphology and its roughness, energetic characteristics and the chemical structure, in particular, the presence of specific functional groups are the parameters with the main role in the surface adhesion properties of biomaterials.

In Figs. 5 and 6 are presented the AFM images of BP and PU film surfaces, as prepared (A), treated by DBD (B), and by ion implantation (C) [14].

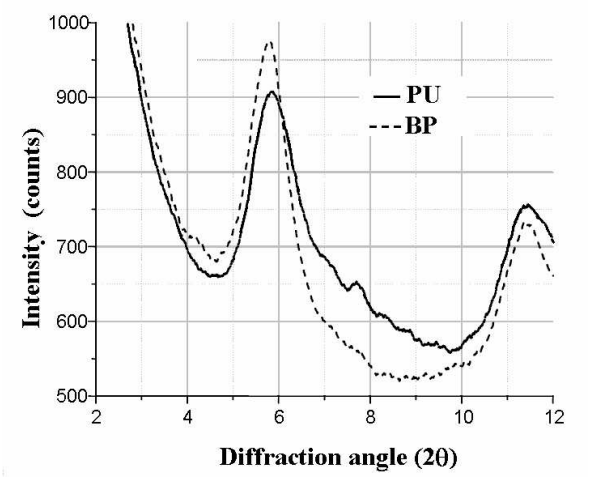

Fig. 3. X-ray diffractograms of BP and PU films.

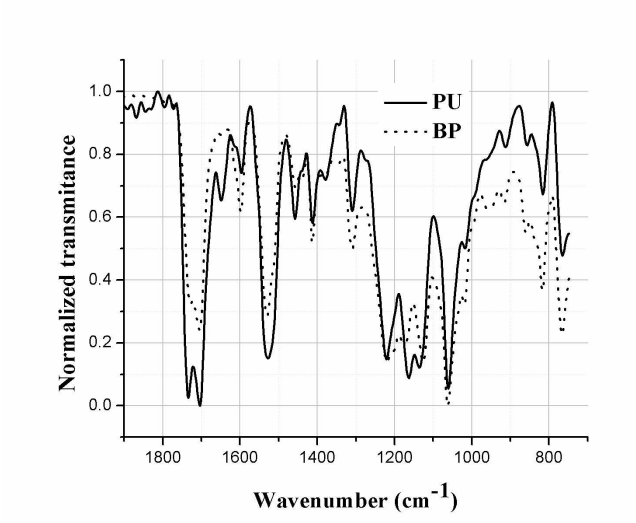

Fig. 4. The ATR - FTIR normalized transmittance spectra of BP and PU films within 700-1900 cm^{-1} range.

4. Discussions

In Table 1 are presented the DSC transition temperatures of BP and PU as shown in Fig.2. The α transition takes place at close temperatures in PU and BP membranes, around $T_g \cong 83^oC$. The following two transitions, β and γ, are very much affected by the lactate segment.

The δ transition around -71°C is due to modification of ether bridges inside the polyester chain of the polyurethane matrix. The γ transition is due to ester groups from poly(ethylene adipate)diol (PEA) and poly(ethylene diethylene adipate)diol (PEDA) affected by the presence of the lactate segment that shifts the transition temperature from $T_\gamma = -2.8^oC$ (PU) to $T_\gamma = -49.8^oC$ (BP).

An important shift of $\Delta T_\beta \approx 10K$ can be also observed for β transitions, due to changes in urethane-urethane-ester associations made through hydrogen bonds. The transition at $T_g \approx 81^oC$ is due to movements of the whole chain and is not affected by the presence of the lactate segment, being very close for both materials.

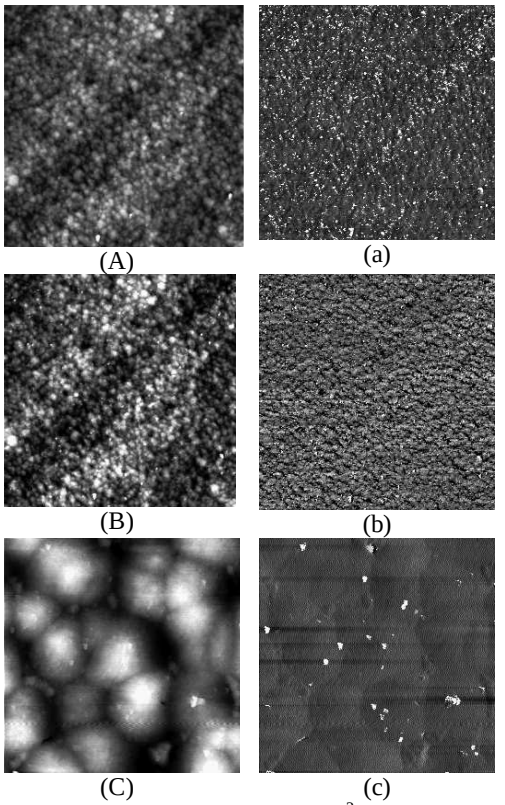

Fig. 5. AFM images ($10\times10\mu m^2$) of PU film surface: (a) as prepared, (b) plasma treated, (c) ion implanted. (X) topography and (x) phase.

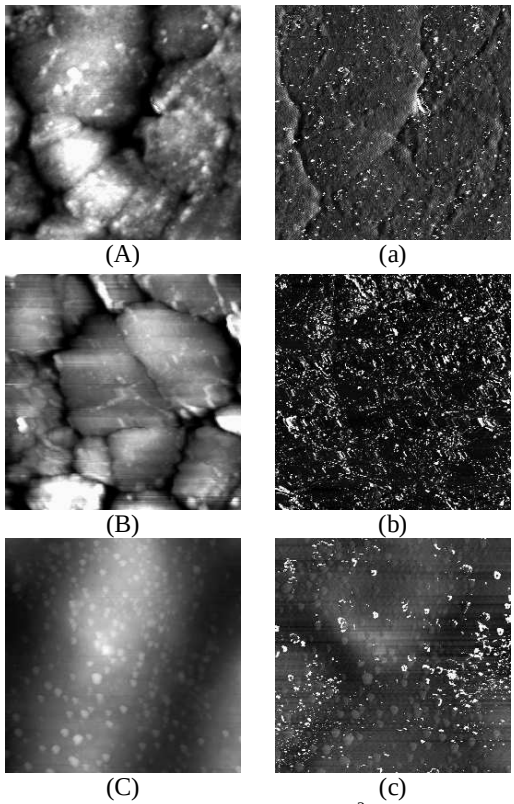

Fig. 6. AFM images ($10\times10\mu m^2$) of BP film surface: (a) as prepared, (b) plasma treated, (c) ion implanted. (X) topography and (x) phase.

The separation of the hard phase and the existence of a higher order degree in BP crystalline phase as compared with PU, are confirmed by X-ray diffractograms represented in Fig.3. This result is supported also by the ATR-FTIR spectra, showing that PU has a homogeneous medium crystallinity, while BP is formed from a soft amorphous phase with hard crystalline nuclei, with a higher order.

Table 1. DSC transition temperatures of BP and PU.

Sample	T_δ (^{0}C)	T_γ (^{0}C)	T_β (^{0}C)	T_α (^{0}C)
BP	-72.1	-49.8	10.2	83.3
PU	-71.2	-2.8	25.1	82.3

As Fig.4 shows, both BP and PU materials have the almost same lines but with important differences in peaks surface. Considering the surface ratio of $(C=O)_{ester}/(C=O)_{urethane}$ peaks at 1730.14cm^{-1} and 1705.07cm^{-1}, we observed a value close to one for BP and a value much smaller than one for PU. This result suggests that the structure of BP is more amorphous as compared with PU due to the lactate segment. Inside BP the crystalline phases due to urethane groups are separated from the amorphous phases including ester groups and form rigid structures connected by urethane-urethane bonds.

From AFM images of PU and BP film surfaces, as prepared, treated by DBD and by ion implantation (Figs. 5 and 6) one can see a surface roughness increased with 10-20 % of its original value, and a modified morphology due to the removal of amorphous regions with low molecular

weight (Figs. 5 and 6, B). Ion implantation determined an important recrystallisation, very much increased by the presence of the lactate segments (Fig. 6 C).

The phase images proved the changes in surface physicochemical properties, induced by He plasma treatment or Ar^+ ion implantation of polyurethanes.

Computed values of adhesion work data are given in Table 2. Plasma treated surfaces showed an increase of surface wettability. The Ar^+ implanted surfaces, showed a more intense effect and a longer remanence. This results is probably mainly due to the formation of surface dipoles such as –(C–O)–, –(C=O)–, –(C=O)–O– acting as electron acceptors and donors [11, 15].

Table 2. Adhesion work of water on the surfaces. Duration of DBD treatments was of 5 seconds.

Sample	Untreated Wa (mJ/m^2)	DBD Treated Wa (mJ/m^2)	Ar implanted Wa (mJ/m^2)
PU	66.5	123	135
BP	77	125.5	154

Macroscopic *in vivo* test biocompatibility investigations, reveals the missing of inflammatory reaction and a strong and uniform tissue adherence on the Ar^+ ion implanted surface relative to He plasma treated surface. Tissue adherence on the non-implanted surface was limited to few small areas.

The optical microscopy investigation of slides also revealed that inside the polymer pores there is a fibrins network, erythrocytes, few lymphocytes and fibroblasts with incipient blood vessels (Fig.7) [16]. The newly formed blood vessels form a dense network across the material.

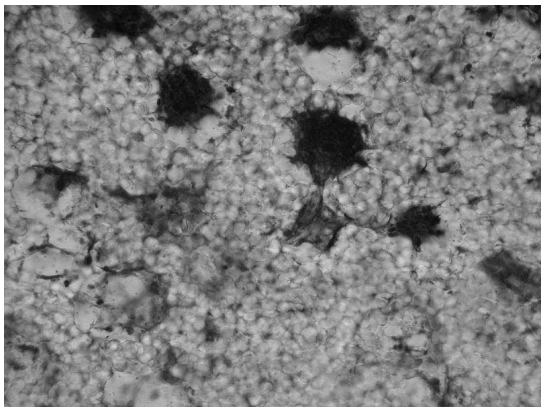

Fig. 7. Fibrin network, inflammatory elements, fibroblasts with incipient blood vessels inside the pores. Histological section (H. E. - Ob. 50×).

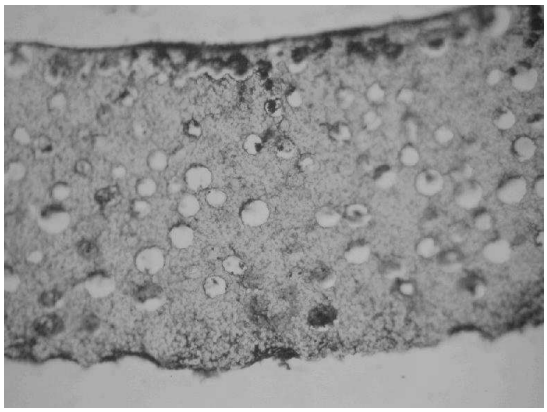

Fig. 8. The increased adherence of collagen material on the Ar^+ ion implanted surface (upper interface) as compared with not treated surface (bottom interface). Histological section. (H.E. - Ob. 20×).

On the material surface one observes fibroblasts adherence with a higher density in the ion implanted areas. Comparatively, one observes the presence of collagen fibbers with an increased adherence on the ion implanted surface (Fig.8, 9).

At small distance from the polymer implant surface one observes an inflammatory reaction with multinucleate giant cells accompanied by fibrosis, less intense on the Ar^+ ion-implanted surface (Fig. 10) comparatively with more intense inflammatory reaction on non-treated surface (Fig.11) and chronic inflammatory intense reaction with angiogenesis and fibroblasts on the non-treated surface (Fig. 12).

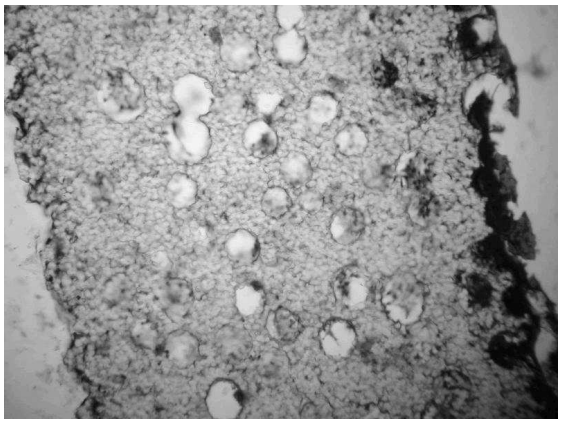

Fig. 9. Detailed representation of strong adherence between collagen material and the Ar^+ ion beam treated polymer surface. Histological section (H.E.- Ob. 40×).

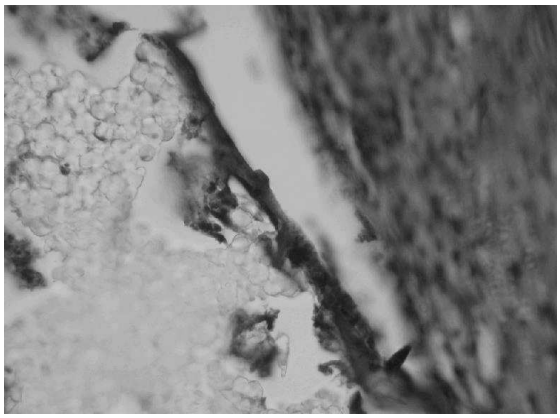

Fig. 10. Collagen fibbers adherent to the Ar^+ ion-implanted surface with lower intensity inflammatory reaction induced in the tissue. Histological section (van Gieson - ob. 60×).

This is in good agreement with previously reported consideration that pores in polymers are traps for antigens that stimulate the immune response [13]. The microscopic analysis of the histological sections revealed a larger numbers of pores occupied by cell aggregates as compared with the opposite surface (Figs.8, 9).

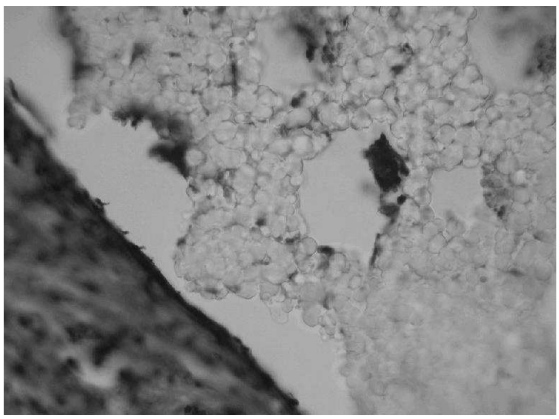

Fig. 11. Total absence of collagen adherence and inflammatory reaction on the non - treated surface. Histological section (van Gieson - Ob. 60×).

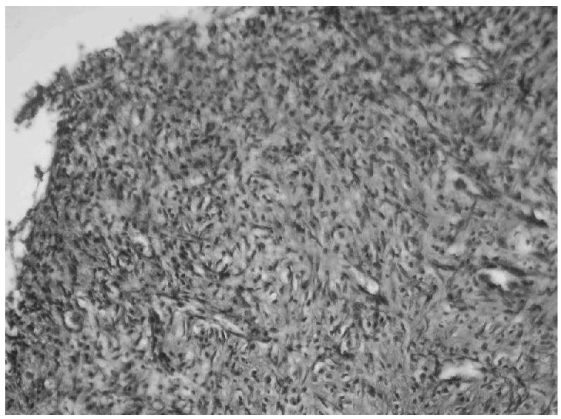

Fig. 12. Chronic inflammatory intense reaction with angiogenesis and fibroblasts on the non - treated surface. Histological section (van Gieson - Ob. 10×).

The ion beam treatment of polyurethanes diminishes the surface/tissues interaction and decrease the intensity of inflammatory reaction. On the other hand, the ion beam surface treatment promotes the fibroblasts adherence and collagen synthesis increasing the tissue adhesion.

5. Conclusions

Porous BP and PU films were prepared by dry phase inversion method from 30 wt% polymer in DMF solution.

Structural investigations by DSC, WAXRD and ATR-FTIR demonstrated that both BP and PU films are multiphase materials. Because of the lactate segment, inside BP chain phase separation

was favored and the crystalline phase, which coexists with the amorphous one, has an increased order degree.

The PU and BP films were subjected to He DBD and Ar^+ ion beam surface treatment techniques.

The treated surfaces have modified morphology and adhesion work.

Argon ion beam surface treatment intensified cell attachment and increased cell growth more than He DBD treatment.

Macroscopically it was observed that the tissue adheres on the ion beam treated surface.

Microscopically it was that on the treated surface there is an increased adherence of fibroblasts and a decreased intensity of inflammatory reaction as compared with the non-treated surface.

The above mentioned results led us to the conclusion that Ar^+ ion implantation on membranes improves the biocompatibility, poly(lactateurethane) being more useful in biomedical applications.

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