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Irradiation of Polystyrene and Polypropylene to study NIH 3T3 fibroblasts adhesion

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ABSTRACT

When polymers are irradiated with heavy ions new chemical groups are created in a few microns of the material. The irradiation changed the polarity and wettability on the surface so that could enhance the biocompatibility of the modified polymer. The study of chemistry and nanoscale topography of the biomaterial is important in determining its potential applications in medicine and biotechnology, because their strong influence on cell function, adhesion and proliferation. In this study, thin films of Polystyrene and Polypropylene samples were modified by irradiation with low energy ion beams (30–150 keV) and swift heavy ions both with various fluences and energies. The changes were evaluated with different methods. Adhesion of NIH 3T3 fibroblasts onto unirradiated and irradiated surfaces has been studied by in vitro techniques. The correlations between physicochemical properties as a function of different irradiations parameters were compared with cell adhesion on the modified polymer surface.

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1. Introduction

The interaction between cells and polymers plays an important role in biotechnology and biomedical applications. Around the world, the increasing use of polymeric materials in these applications ranging from substrates for cell and tissue growth to vascular protheses. Materials for tissue or cell culture are not inert polymers without biological relevance, as it is known, the cellular response is driven by the polymer surface characteristics such as their topography, hydrophobic/hydrophilic relation and functional groups. As a result, a complex substrate, from the physicochemical standpoint, is thus obtained with important effects on adhesion, morphology, and cellular growth. *Improvement in biocompatibility due to surface modification is mainly based on incorporating new chemical groups, changes in polarization and surface free energy, as well as changes in topography.*

Diverse techniques, including nanolithography [1], laser machining [2], laser holography [3] and electrospinning [4] are typically applied in polymers used in implants or reconstructive surgery. They induce modifications in the surface nanocharacteristics such as cracks, ripples, points and fibrilar networks. The irradiation of polymers is yet an alternative way to modify the physicochemical properties of the surface such as: structure and chemical composition [5], surface free energy, morphology and surface charge properties [6] without disturbing the bulk material properties. In particular, ion beam irradiation is an interesting technique to induce bio and citocompatibility in controlled conditions and in selected areas of the polymer surface. In recent years, several studies indicated that cellular adhesion can be improved by this method [7–9]. Irradiation of nonpolar polyolefines such us Polystyrene (PS) and Polypropylene (PP) results in the creation of polar groups on the polymer surface, thus improving the interaction with biological components. Polystyrene is the most often used material in cell culture applications because of its non-toxicity and low production cost. For this reason represents a polymer widely applied in studies of mechanisms of cell–artificial material interaction. Polypropylene (PP) has become one of the most developed polymers because of its abundant resources and easy synthesis. Moreover, its chemical compositions contain only carbon and hydrogen which result in its hydrophobicity and limit its potential applications like biomaterial.

The cellular adhesion on the surface constitutes the first step in the cell–biomaterial interaction, then it is relevant a better understanding of this process for the study and improvement of the biocompatibility of these materials. This gives us information about how modifications in structure and composition at the surface of

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materials affects such interaction. The present work begin this project analyzing the changes in PS and PP upon irradiation with low (Ar, 30 keV) and high (Ag, 180 MeV and S at 110 MeV) energies and different fluences. To this end the adhesion of NIH 3T3 line fibroblasts was investigated, both on irradiated and non-irradiated surfaces. The different effects on the cellular response are discussed in terms of the induced changes due to irradiation in each case, particularly those related to hydrophilic properties and the functional groups present on the surface.

2. Experimental procedure

2.1. Polymer irradiation

We used films of PS and PP manufactured by Goodfellow (England), with a thickness of 25 and 8 μm, respectively. The samples were irradiated with S (110 MeV) and Ag (180 MeV) in the Tandar accelerator CAC, Buenos Aires, Argentina. They were placed in a mechanical device that rotates in front of the beam with the purpose of produce an homogeneous modification. The actual ion fluence was determined by collecting ion current with a Faraday Cup during short times when the samples are out of the beam. The surface irradiated was a disk with a diameter of 1 cm. On the other hand the 30 keV Ar beam were provided by the ion implanter (Instituto de Física, UFRGS, Porto Alegre, Brazil) under usual conditions of implantation. Both irradiation was done at a vacuum of 10^{-6}–10^{-7} Pa with perpendicular incidence to the sample surfaces. Table 1 lists the ions, fluences, and energies used in the different experiments. Irradiation times are a function of the current intensity and required fluence. In order to prevent excessive heating of the samples currents density between 15 and 150 nA/cm^{-2} were used.

2.2. Surface characterization

Fourier transmission infrared spectroscopy (FTIR) was used to determine the changes in the functional groups of the polymeric materials after irradiation. All spectra were obtained with a Shimadzu IR Prestige-21 with a DLATGS detector, using a resolution of 4 cm^{-1}. Three independent measurements were made in each sample, for different points of the irradiated zone.

The surface hydrophillic properties was studied by means of contact angle measurements. With this purpose small drop of deionized water (volume of about 10 μl) was placed at the flat polymer surfaces using a micro-pipe, and the resulting advancing contact angle was evaluated with a profile analyzer (Prazis Po400 hd). All measurements where made at room temperature and the average of eight different measurements was recorded.

2.3. Cell culture

Adhesion of NIH 3T3 cells in modified polymers was studied in vitro. Cells were maintained routinely in Dulbecco's Modified Eagles's Medium (DMEM, GIBCO) supplemented with 10% FBS (Fetal Bovine Serum, Natocor, Argentina) and 1% penicillin/streptomycin used tissue culture Polystyrene (TCPS P100, Greiner Bio-one, Germany). After irradiation, PP and PS films were sterilized in 70% ethanol and placed on the bottom of a 24 wells TCPS, covered with a sterilized Viton ring to prevent floating. The cells were harvested using solution of 0.25% trypsin and 0.02% EDTA in PBS (Phosphate Buffered Saline). After that, cells were seeded onto 24-wells TCPS, covered with the studied polymers, with a density of 10,000 cells cm^{-2}. The TCPS culture wells were incubated at 37 °C with 5% CO_2/95% of air and at approximately 90% relative humidity. The viability of the adhered cell was determined by MTT assay, which is based on the metabolic reduction of 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma Inc.) by the succinate dehydrogenase mitochondrial enzyme in a colored blue compound (formazan). Once dissolved the intracellular formazan crystals, the absorbance of the solution thus obtained is measured in a spectrophotometer UV/VIS Perkin-Elmer at 555 nm. This test allows determining the mitochondrial functionality and therefore viability of the adhered cells. The cells morphology on the sample surface was evaluated on micro-photographs taken by an Olympus BX51 microscope.

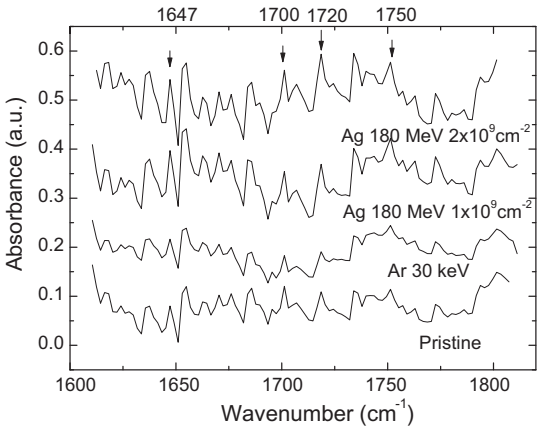

Fig. 1. FTIR spectra of the irradiated PS films with Ag and Ar are compared with the pristine samples. It show differences in the region between 1600 and 2000 cm^{-1}. The intensity of the carbonyl bands, C=O (1700, 1720 and 1750 cm^{-1}) and olefinic, C=C bond (1646 cm^{-1}), show an increase respect to pristine. For the samples irradiated with Ar its growth is weaker.

Table 1
Lists the ions, fluences and energies used in the different irradiation conditions.

Polymer	Ion	Energy	Fluence (cm^{-2})
Polystyrene	Ag	180 MeV	1×10^{09}
			2×10^{09}
			3×10^{09}
			4×10^{09}
	Ar	30 keV	5×10^{12}
			1×10^{13}
			3×10^{13}
			1×10^{14}
			5×10^{14}
Polypropylene	S	110 MeV	1×10^{05}
			5×10^{05}
			1×10^{06}
	Ar	30 keV	5×10^{12}
			3×10^{13}
			5×10^{14}

Table 2
Contact angle measurements for PP and PS films. Ion type, energy and fluence are detailed in the irradiation conditions column.

Polymer	Irradiation conditions			Contact angle (°)
PS	Pristine			85
	Ag	180 MeV	1×10^{09} cm^{-2}	76
			2×10^{09} cm^{-2}	76
	Ar	30 keV	3×10^{13} cm^{-2}	80
			1×10^{14} cm^{-2}	81
PP	Pristine			89
	S	110 MeV	Every fluence	~86
	Ar	30 keV	Every fluence	~87

3. Results and discussion

3.1. Surface characterization

The FTIR spectra for irradiated and non-irradiated polymers were analyzed. In PS, differences were found for absorbances in the 3600–1200 cm^{-1} range. Nevertheless no differences are observed in PP. Fig. 1 shows that in the samples irradiated with Ag the intensity of the carbonyl bands, C=O (1700, 1720 and 1750 cm^{-1}) and olefinic, C=C bond (1646 cm^{-1}) have an increase respect to pristine. PS films irradiated with Ar, also show a weak increase.

The measured contact angles are shown in Table 2. The standard deviation was ±2° for each angle. The pristine PS surface was relatively hydrophobic but after irradiation, the contact angle decreases in all cases depending on irradiation conditions, showing a more hydrophilic surface. Contact angles were measured under all conditions, and differences were found for different ion ener-

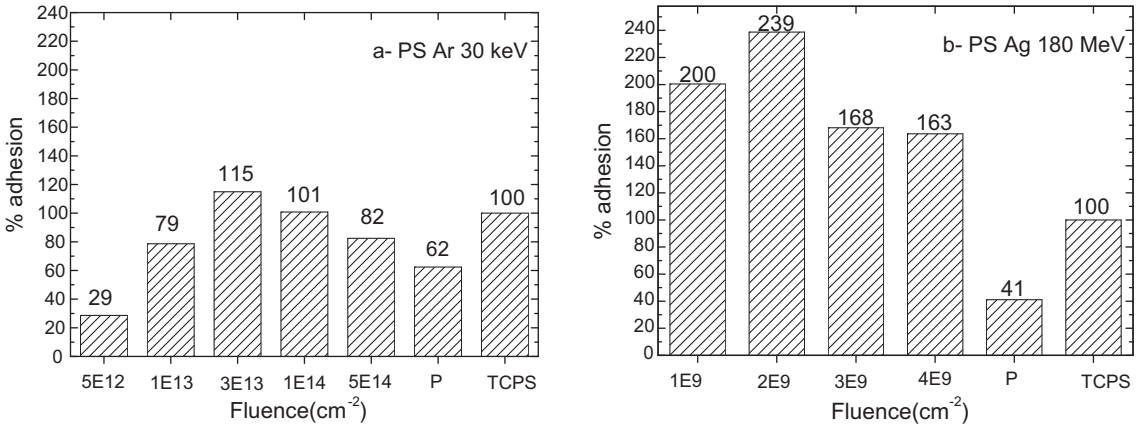

Fig. 2. NIH 3T3 adhesion vs TCPS control in irradiated PS films for different fluence and energies with a dispersion of 3%. P symbolizes pristine samples.

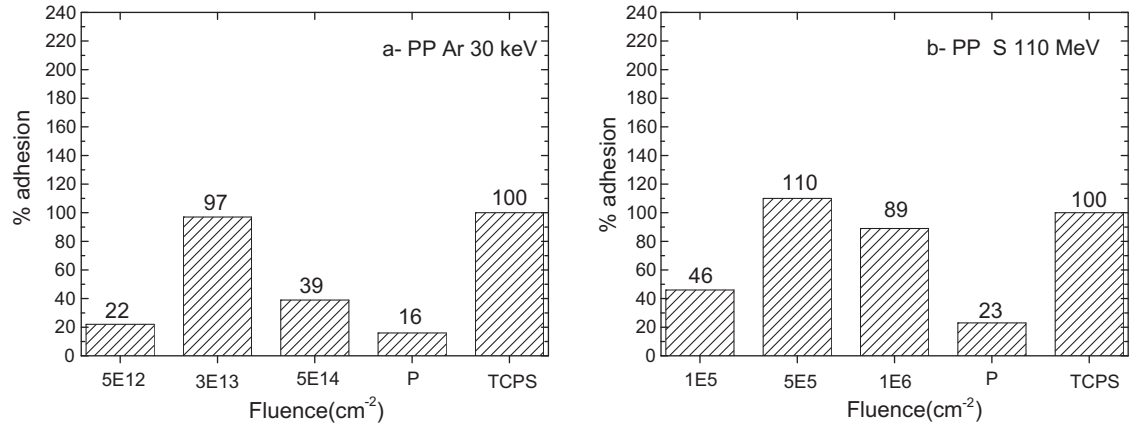

Fig. 3. NIH 3T3 adhesion vs TCPS control in irradiated PP films for different fluence and energies with a dispersion of 4%. P symbolizes pristine samples.

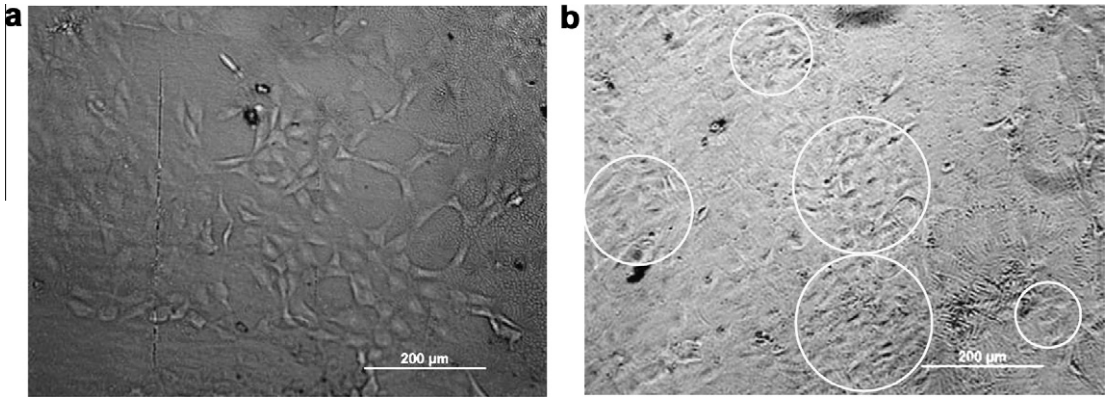

Fig. 4. (a) PP irradiated with S 110 MeV and 5×10^5 ion/cm^2 (adhesion vs TCPS control = 110.00%). Fibroblast exhibited a high degree of spreading and their morphology was lengthened and fusiforme corresponding to normal fibroblasts morphology. A similar morphology is obtained on TCPS control. (b) PS irradiated with Ag 180 MeV and 2×10^9 ion/cm^2 (adhesion vs TCPS control = 238.76%). The circles mark the sites where the cells were mostly rounded and conglomerate.

gies, but no so for different fluences. The pristine PP was hydrophobic too ($\theta = 89°$), but after irradiation that value decreased only to 86° for all the irradiation conditions. For both polymers, the decrease of the contact angle can be correlated with the intensity increase of the polar functional groups, such as C=O bands, measured by FTIR spectroscopy.

3.2. Adhesion, morphology and growth of cells

Adhesion of fibroblast onto PS and PP substrates was studied in vitro. Figs. 2 and 3 show the percentage of adhering cells at 24 h using TCPS 24-wells as control, using wells in equivalent position to a significative comparison. Fig. 2a and b correspond to PS irradiated at different fluences with Ar (30 keV) and Ag (180 MeV), respectively.

There are differences in the percentage of adhered fibroblasts between irradiated PS and pristine for both energies. High adhesion is observed for PS samples irradiated with Ar and fluence of 3×10^{13} cm^{-2} but the highest was obtained for PS irradiated with Ag at 180 MeV at a fluence of 2×10^{9} cm^{-2}. On the other hand, for PP (Fig. 3a and b) the adhesion had an improvement in comparison to the pristine films but there were no significant differences for the irradiated PP surfaces and TCPS control.

Cell morphology was observed with a confocal microscope, showing two dissimilar behaviors that can be appreciated in Fig. 4a and b. Fibroblasts exhibited a high degree of spreading and their morphology was lengthened and fusiform corresponding to normal fibroblast morphology (Fig. 4a). Whereas in the other case the cells were mostly rounded and conglomerate as can be observed within the circular domains shown in Fig. 4b.

4. Conclusion

This work examines the changes in PP and PS polymers due to low and high energy ion irradiation. The FTIR spectra obtained vary depending on irradiation conditions, in particular for functional groups such as C=O. This changes are correlated with that measured in the contact angle value. Preliminary results show that surface characteristics have an important effect on the initial adhesion of fibroblasts NIH 3T3 when these polymers are used as culture layer. For certain induced changes, the observed adherence is greater than that observed when growing cells on standard TCPS, a fact that could be related to the improvements in biocompatibility of the irradiated polymers.

The enhancement of cell adhesion is considered in connection with the polarity increase in the polymer surface. Nevertheless, these cells showed marked differences in their morphology and spreading, indicating active changes in the cytoskeleton and membrane surface of the cells as a response to physicochemical changes in surface properties. The surface presents a high degree of adhesion, but for NIH 3T3 fibroblasts this does not mean that the surface is adequate as a substrate for functional cells. If this kind of substrate possesses surface characteristics such that inhibit the migration after mitosis, then it can induce cell agglomeration and contact inhibition. This is an inadequate cellular response in functional NIH 3T3 cell type because fibroblasts are placed separated from one another, surrounded by their extracellular matrix in their native tissue.

In the present study, the discussion of the results about cellular adhesion was based on an analysis of the physicochemical changes induced on the surface. Further studies are necessary in order to determine the cellular mechanisms relevant to the fibroblast adhesion to the irradiated surfaces, such as conformation of cytoskeleton proteins in the adhered cells, conformation of plasmatic membrane molecules, biosynthesis of membrane proteins and production of an extracellular matrix.

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References

[1] M. Arnold, E.A. Cavalcanti-Adam, R. Glass, J. Blümmel, W. Eck, M. Kantlehner, ChemPhys 5 (2004) 383–388.
[2] B. Zhu, Q. Lu, J. Yin, J. Hu, Z. Wang, J. Biomed. Mater. Res. B 70B (2004) 43–48.
[3] P. Clark, G.A. Dunn, A. Knibbs, M. Peckhamc, Int. J. Biochem. Cell Biol. 34 (2002) 816–825.
[4] M. Schindler, I. Ahmed, J. Kamal, A. Nur-E-Kamal, T.H. Grafe, H.Y. Chung, Biomaterials 26 (2005) 5624–5631.
[5] J.-L. Dewez, A. Doren, Y.J. Schneider, P.G. Rouxhet, Biomaterials 20 (1999) 547–559.
[6] K. Webb, V. Hlady, P.A. Tresco, J. Biomed. Mater. Res. 49 (2000) 362–368.
[7] K. Rockov-Hlavckov, V. Svorck, L. Backov, B. Dvornkov, J. Heitz, V. Hnatowicz, Nucl. Instrum. Meth. Phys. Res. B 225 (2004) 275–282.
[8] V. Svorck, P. Tomsov, B. Dvornkov, V. Hnatowicz, R. Ochsner, H. Ryssel, Nucl. Instrum. Meth. Phys. Res. B 215 (2004) 366–372.
[9] K.-H. Kim, J.-S. Cho, D.-J. Choi, S.-K. Koh, Nucl. Instrum. Meth. Phys. Res. B 175 (2001) 542–547.