

Characterization of biodegradable polymers irradiated with swift heavy ions

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ABSTRACT

In view of their application as biomaterials, there is an increasing interest in developing new methods to induce controlled cell adhesion onto polymeric materials. The critical step in all these methods involves the modification of polymer surfaces, to induce cell adhesion, without changing their degradation and biocompatibility properties. In this work two biodegradable polymers, polyhydroxybutyrate (PHB) and poly-L-lactide acid (PLLA) were irradiated using carbon and sulfur beams with different energies and fluences. Pristine and irradiated samples were degraded by immersion in a phosphate buffer at pH 7.0 and then characterized. The analysis after irradiation and degradation showed a decrease in the contact angle values and changes in their crystallinity properties.

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1. Introduction

Biopolymers, which present a well defined biocompatibility and in vivo biodegradability, provide a suitable alternative to produce biocompatible and biodegradable scaffolds with specific physico-chemical, morphological and mechanical properties, appropriate for the intended medical applications. Scaffolds for tissue engineering are degradable matrices designed to support cell attachment, proliferation and growth and to finally provide functional living tissue. The structure and the surface morphology of the scaffolds have to meet general requirements which are specific for the targeted tissue. Since the scaffold is a temporary matrix for cell proliferation and differentiation, the degradation performance of the scaffold must correspond to the regenerative rate of the affected tissue in order to permit concomitant growth, replacement of tissue and matrix clearance. The material surface properties will influence the initial cellular events on the cell-material interphase. Out of a number of studies, it is clear that the surface properties will determine which biological molecules will adsorb. There are some aspects that will influence the material biocompatibilities, including surface roughness, surface chemistry, and surface en-

ergy. Some studies also showed that the surface properties of a biomaterial, especially hydrophilicity, influenced cell adhesion and proliferation on the materials [1]. Polyhydroxyalkanoates (PHA) are a family of biopolyesters and its application as biomaterials will add values to these polymers. In this work we used two types of PHA: polyhydroxybutyrate (PHB), a biomaterial that has been used in vitro and in vivo studies with various degree of biocompatibility and biodegradability, and poly-L-lactide (PLLA) that has been applied to a variety of biomedical products such as degradable sutures, temporary orthopedic fixtures and tissue engineering scaffolding materials. These polymers have been well documented [2] for their excellent biodegradability, biocompatibility, nontoxicity and their biocompatible degradation products. However, the poor hydrophilicity and the lack of natural recognition sites to promote cell attachment have limited their application and utility. Many surface modification techniques or methods have been used to modify polymer surfaces as Desmet et al. described in their work [3]. The irradiation of polymers is an alternative way to modify the physicochemical properties of the surface, including structure and chemical composition, surface free energy, morphology and surface charge properties, without disturbing the bulk material properties. In particular, swift heavy ion beam irradiation is an interesting technique to induce bio and citocompatibility in controlled conditions and in selected areas of the polymer surface. The energy deposited by the ions in the material is characteristic

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of the ion type and its energy. The kinetic energy transfer takes place in the so-called latent track in which cross-linking and scission phenomena occurs. Chain scission generally results in deterioration of polymer properties instead of crosslinking which in general improved the mechanical properties. In a previous work [4], we studied the enhancement of cell adhesion and proliferation, as a function of surface polarity increase. Through irradiation, we modified the hydrophilic grade of PLLA and PHB with ion type, energy and fluences that were chosen especially to promote and enhance cell adhesion and proliferation [5]. However, these modifications should not affect the degradation and biocompatibility of biomaterials. In this work, the irradiated and pristine PHB and PLLA films were degraded and characterized using different techniques to assess whether the polymer, which serves as a good substrate [5] for fibroblast culture, did not change their biodegradability properties.

2. Experimental procedure

2.1. Polymer manufacture and irradiation

PHB is a commercial polymer of natural origin elaborated by Biocycle. The polymer is a yellow/white solid with a molecular weight determined by gel permeation chromatography (GPC) of about 600,000, as stated in the material safety data sheet (MSDS). Films of PHB suitable for irradiation were prepared by dissolving the polymer in chloroform at 62 °C using a Soxhlet extractor and by casting the solution onto a Teflon plate. PLLA is a commercial polymer, with a thickness of 50 µm, manufactured by Goodfellow (England). Both polymers were irradiated with C and S in the Tandem accelerator at CNEA, Buenos Aires, Argentina, at a vacuum of 10^{-6} – 10^{-7} Pa with perpendicular incidence to the sample surfaces. Ions, fluences, and energies used in the different experiments are listed in Table 1. Irradiation times are a function of the current intensity and required fluence, measured in situ with a Faraday cup. In order to prevent excessive heating of the samples, beam currents were used between 1 and 50 nA.

2.2. Polymer characterization

Polymers characterization was performed by using the following techniques:

Environment scanning electron microscopic (ESEM) studies: The PHA matrices were mounted on copper stumps, followed by coating with gold. They were then examined under an ESEM environment scanning electron microscope Philips ESEM QUANTA 200.

FT-IR microspectroscopy: The IR spectra of PLLA were measured with a Shimadzu IR Prestige-21 with a DLATGS detector at a 4 cm^{-1} spectral resolution and 90 scans, by attenuated total reflection (ATR) mode. A ZnSe plane crystal was used with an incident angle of 45°. For PHB the measurement was carried on with the Micro-Ir Shimadzu AIM-8800 at specular reflection.

Thermal analysis: Differential scanning calorimetry (DSC). DSC measurements were performed on a Shimadzu DSC60 system over a temperature range from AT to 500 °C at heating and cooling rates

of 10 °C /min. Nitrogen was used as the purge gas at a flow rate of 10 ml/min. The analysis of DSC curves for both the heating and cooling processes was performed for the second run data.

XPS: XPS was carried out using an XR 50, SPECS GMBH equipped with an Mg K X-ray source operated at 300 W and an hemispheric energy analyzer PHOIBOS 100 MCD5, SPECS GMBH.

Contact angle measurement: Wettability was examined by measuring contact angles of water. The contact angles of distilled water were measured with a sessile drop method using a profilometer Praxiz Po400hd. Approximately 5 µl of distilled water was gently plated on the surface of the films. A contact angle value consists in the average of at least 20 independent measurement performed at different parts of the film at ambient temperature.

2.3. Hydrolysis of PLLA and PHB films

PLLA and PHB pristine and irradiated films were chosen for in vitro hydrolytic degradation studies. These films were placed in 10 ml screw-top bottles filled with phosphate buffer saline solution (PBS) at pH 7.0 and antibiotic (streptomycin and penicillin) to prevent bacterial contamination, then were incubated at 37 °C for 40 days. Samples were removed for characterization, and the pH of the solution was monitored and maintained at 7.0. Films were removed, rinsed with distilled water and dried in a vacuum oven at 38 °C (PLLA) and 50 °C (PHB), then the samples were characterized.

Weight loss analysis: The samples were washed with distilled water and allowed to dry in oven until reach a constant weight. For each polymer sample the degradation rate was determined by the ratio of weight loss to the initial weight of the samples. The polymers were weighed in a Mettler AE 163 scale with a sensitivity of 0.01 mg/30 g.

3. Results and discussion

In this section we will summarize the characterization studies of samples. Due to lack of space in this paper, we will not go into detail, but rather present only an overview of the results. Irradiation decreased the contact angle of PHA films, meaning that hydrophilicity of the PHA surface was increased after the treatment. In PLLA, irradiation led to further decrease of contact angle compared with that of PHB. The results changed with ion type and energy but not with the fluence (Table 2). The values of φ_i for PHB vary because the samples were not homogeneous, therefore we measured in the same sample, after (φ_i) and before irradiation (φ_f). After degradation the contact angle in all samples decreased, about 10%. This can be explained because the hydrolysis produced hydrophilic chain ends so the samples may absorb increasing amounts of water.

ESEM micrographs of the films before and after irradiation (a, b) and both of them after hydrolysis (c, d) are shown in Fig. 1. Under every irradiation condition, PHB did not show any substantial change after irradiation, instead PLLA films showed some changes in their surface roughness (Fig. 1a and b). Pristine and irradiated PHB exhibit a similar smooth surface after hydrolysis. After degradation, PLLA surface presented an increasing porosity. Pristine

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

Ion	Energy (MeV)	Fluence (10^{10} cm^{-2})	Range (µm)
C ³⁺	18	7–30–70	21
C ⁵⁺	45	1–10–50	73
S ⁵⁺	27	1–6–10	12
S ⁹⁺	75	1–5–10	28

Table 2
Measurement of contact angle in PLLA and PHB before φ_i and after φ_f irradiation. The estimated uncertainty was $\pm 1^\circ$.

Ion	Energy (MeV)	PLLA		PHB	
		φ_i (°)	φ_f (°)	φ_i (°)	φ_f (°)
C ³⁺	18	70	65	69	65
C ⁵⁺	45	70	65	73	70
S ⁵⁺	27	70	63	72	70
S ⁹⁺	75	70	63	69	67

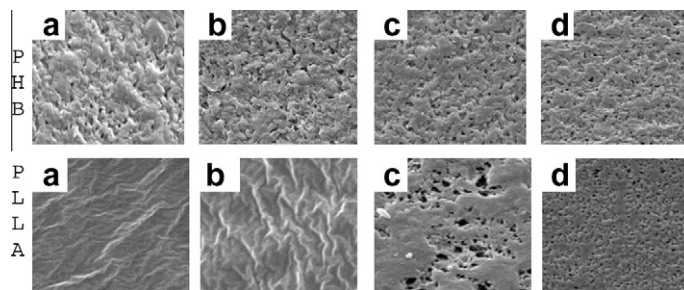


Fig. 1. SEM micrographs of the films before (a) and after irradiation (S 75 MeV 1×10^{11} cm⁻²) (b), degraded pristine (c) and irradiated and degraded (d) for both PHB and PLLA polymers at a magnification of 8000X.

Table 3

Weight loss in PHB after 40 days of degradation by hydrolysis. The estimated uncertainty was 0.1%.

Ion	Energy (MeV)	WL (%)
C ³⁺	18	1.5
C ⁵⁺	45	1.0
S ⁵⁺	27	1.0
S ⁹⁺	75	1.1
Pristine		1.1

samples showed a design of highly porous islands embedded in an unmodified matrix. On the other hand, the irradiated samples presented small holes covering the full surface.

Weight loss (WL) of PHB films is presented in (Table 3). Both polymers showed a low WL during the whole incubation time but the PLLA was almost negligible. The weight loss yield is defined: $WL(\%) = (m_d - m_0)/m_0$ where m_d and m_0 are the dry and initial mass, respectively. The low weight loss value may be justified considering the micro-porosity of the polymer that reduces the water adsorption rate inside the matrix and at the same time represents a barrier for the flow of the degradation products to the surrounding buffer [6].

In order to analyze the chemical changes on the PHA surface XPS technique was used. Fig. 2 shows the C_{1s} spectra of a pristine, irradiated and degraded PLLA and PHB sample, respectively. The spectra were fitted using a combination of Gaussian and Lorentzian peak shapes to characterize the change in the chemical bond of the C atom. The 1s state of carbon (C_{1s}) spectrum of both polymers can be resolved into three components with different bonding state: the first at energy of 285.0 eV assigned to C–C or C–H bond; the second at energy of 286.6 eV assigned to C–O bond; the third at energy of 289.1 eV assigned to C=O bond. The corresponding relative peak areas of the fitted peaks are shown in the figure.

The C_{1s} spectra of irradiated polymers show some changes in the chemical state of C. After irradiation and after degradation C–O peak bands of both polymers have decreased, with respect to pristine. The results show that chains were broken by hydrolysis.

In this study, the spectra of PHB (Fig. 3) and PLLA (Fig. 3) as a function of different parameters were measured by FTIR spectroscopy. After irradiation and degradation PHB spectra did not present new absorbance peaks compared with the pristine sample [7]. But in the spectra of PLLA bands at 2916, 2846, 1595 and 1535 cm⁻¹ appeared with degradation. The 2916 and 2846 cm⁻¹ are related with the stretching mode of CH₃ while 1595 and 1535 cm⁻¹ may be attributed to water adsorbed in the polymer during degradation. It can be seen that the absorbance of certain bands change with irradiation and degradation. In PLLA (Fig. 3), the bands at 895 and 871 cm⁻¹ become weaker when polymer is irradiated and degraded. On the contrary the band at 921 cm⁻¹ increases in intensity with degradation. The crystallinity measured as the ratio between the peaks intensity at 921 and 956 cm⁻¹ [8] decrease with irradiation, amorphization, and increased with degradation. After irradiation with higher energy, namely C 45 and S 75 MeV, the only absorbance band that grows with the fluence was the 1750 cm⁻¹ related with the carbonyl groups. The PHB spectra, measured with Micro-IR, showed no significant changes, but if we made the ratio between the peaks intensity at 1380 and 1186 cm⁻¹ [9] we can appreciate that this increased with degradation, so the crystallinity increased too.

The effect of irradiation and hydrolysis on the thermal properties of polymers was evaluated by performing calorimetric scans on samples taken from the buffer solution and dried in an oven. The results of the DSC heating scans on samples before and after irradiation and degradation were reported in Table 4.

The degree of crystallinity X_c was measured from the isothermal heat of crystallization ΔH_c as $X_c = \Delta H_c/H_f$ where H_f was the heat of fusion of the crystalline phase, for PLLA 81 J/g and for PHB 146 J/g

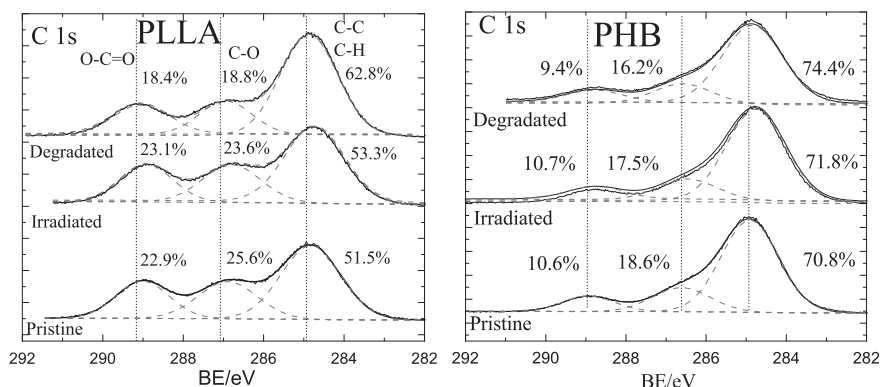


Fig. 2. C_{1s} spectra of PLLA samples and PHB samples.

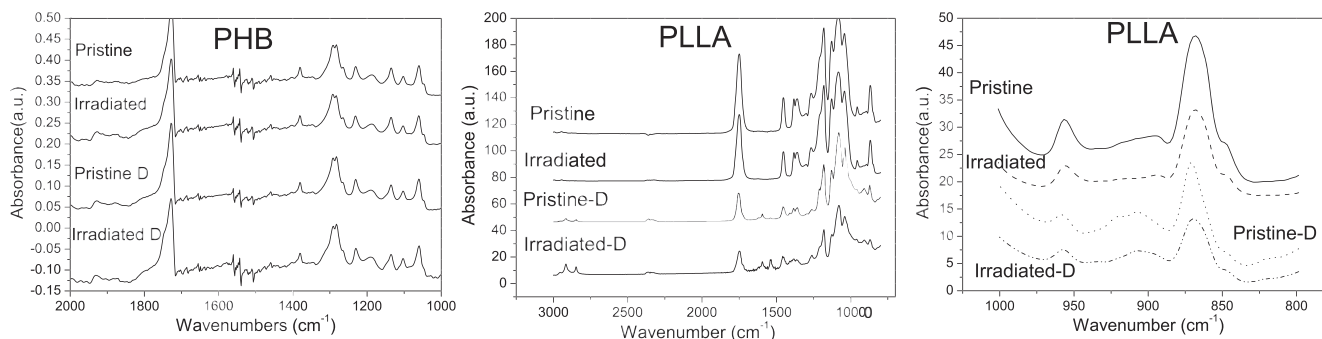


Fig. 3. FTIR spectra of PLLA and PHB for all the conditions.

Table 4

DSC measurement in PLLA and PHB before and after irradiation (I) ($S\ 75\ \text{MeV}\ 1 \times 10^{11}\ \text{cm}^{-2}$) and degradation (D).

Film	I	D	T_m (°C)	ΔH_m (J g ⁻¹)	T_g (°C)	X_c (%)
PLLA	n	n	166.1	29.0	55.3	36
PLLA	y	n	166.2	28.9	55.2	36
PLLA	n	y	166.1	28.9	52.3	36
PLLA	y	y	165.8	33.1	50.9	40
PHB	n	n	173.1	77.9	-1.0	53
PHB	y	n	172.7	76.3	-1.4	52
PHB	n	y	173.1	80.2	-0.9	55
PHB	y	y	170.8	79.1	-3.2	54

[10,11]. The calorimetric behaviour of irradiated samples is similar to the behaviour of unirradiated materials. The degraded samples shown a decrease on T_g values which means an increase of chain mobility at amorphous phase. By means of the other way X_c increased with degradation. The hydrolysis of the amorphous regions leads to an increase in the crystalline/amorphous ratio in accordance with FTIR measurement.

4. Conclusion

In this work we irradiated two biodegradable polymers (PLLA and PHB) with swift heavy ions to modify their surface properties and hydrophilicity and so enhance cell adhesion and proliferation. The effect of hydrolytic degradation at the various conditions of the samples were characterized by different techniques. After irradiation, the surface topography changed as could be seen at ESEM images and the contact angle was reduced, making a more hydro-

philic surface. FTIR measurements showed that the material became amorphous after irradiation and the C–O bands decreased according to XPS results. DSC results indicated that the T_g decreased slightly with degradation but had a greater decrease if the polymer was irradiated. Concluding, it is important to mention that the ion irradiation, applied in this work, does not change the degradation properties of the analyzed polymers. However the cellular response changes significantly with fluences [5].

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