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Superhydrophilic poly(L-lactic acid) electrospun membranes for biomedical applications obtained by argon and oxygen plasma treatment

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ABSTRACT

Poly(L-lactic acid), PLLA, electrospun membranes and films were plasma treated at different times and power with argon (Ar) and oxygen (O_2), independently, in order to modify the hydrophobic nature of the PLLA membranes. Both Ar and O_2 plasma treatments promote an increase in fiber average size of the electrospun membranes from 830 ± 282 nm to 866 ± 361 and 1179 ± 397 nm, respectively, for the maximum exposure time (970 s) and power (100 W). No influence of plasma treatment was detected in the physical–chemical characteristics of PLLA, such as chemical structure, polymer phase or degree of crystallinity. On the other hand, an increase in the roughness of the films was obtained both with argon and oxygen plasma treatments. Surface wettability studies revealed a decrease in the contact angle with increasing plasma treatment time for a given power and with increasing power for a given time in membranes and films and superhydrophilic electrospun fiber membranes were obtained. Results showed that the argon and oxygen plasma treatments can be used to tailor hydrophilicity of PLLA membranes for biomedical applications. MTT assay results indicated that plasma treatments under Ar and O_2 do not influence the metabolic activity of MC3T3-E1 pre-osteoblast cells.

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1. Introduction

Biomaterials used as extracellular matrixes in tissue engineering applications play a key role in supporting cells and/or biologically active molecules to promote the repair of damaged tissue and/or tissue regeneration [1]. In particular, extracellular matrixes based on biodegradable polymers such as poly(L-lactic acid) (PLLA) have been extensively studied since they can be easily processed in a variety of forms [2]. PLLA is an aliphatic semi-crystalline polyester that can be produced from renewable sources, such as corn or sugar cane [3]. Due to its biocompatibility and biodegradability through hydrolytic scission of the ester groups into lactic acid, with a degradation rate that matches the healing time of damaged human tissues, low toxicity and proper mechanical characteristics, this polymer has been attracting strong interest for uses in tissue repair and regeneration [4,5]. Moreover, its piezoelectric properties (d_{25} = 10 pC/N) are also an advantage that can be used to stimulate tissue by mechano-electrical transduction [6,7] in order to implement active scaffolds [7,8], not just acting as passive supports for cells.

Fibrous membranes based on PLLA are particularly appropriate for tissue engineering applications due to their large surface area, small fiber diameters and porous structure [2]. It was demonstrated that PLLA fibers promote cell adhesion and proliferation [2]. Electrospinning is the most common and versatile method to produce those nanofibres membranes, by applying a high electrical field to a droplet of polymer solution or melt. By controlling the polymer solution and processing parameters, a stable process

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can be achieved allowing to obtain large fiber mats with tailored fiber dimension and orientation [9]. The major drawback of these fibrous membranes for tissue engineering is associated to the PLLA hydrophobicity, limiting their applicability, due to hindered cell–material interactions [10]. Several methods can be used to modify the surface properties of materials [11]. Amongst them, plasma treatment is one of the most extensively used methods to modify the chemical and physical properties of polymers without affecting their bulk characteristics. Plasma treatment can be used to tailor surface adhesion and wetting properties by changing the surface chemical composition of the polymers by a reactive chemical environment in which different plasma-surface reactions occur [11,12]. In this sense, plasma treatment allows to introduce functional groups, to control surface roughness and crosslinking, graft polymerization and thin film coating adhesion [11]. The modification of the surface chemical composition is important to promote covalent immobilization of different components onto the polymer surface. The success of plasma treatment is associated to the selection of plasma source type (oxygen, argon, nitrogen and acetylene, among others), treatment time and power [11–13]. Oxygen is one of the most reactive elements and can generate carboxyl groups on polymer surfaces, leading to suitable characteristics to promote cell growth due to the incorporation of hydrophilic functional groups [11]. Moreover, plasma treatments can be used to eliminate surface contaminants [13].

The surface of PLLA can be modified by several gases such as argon (Ar), Ar-NH_3/H_2, oxygen (O_2) and nitrogen (N_2) [14–16]. It has been shown that a significant decrease in contact angle occurs after Ar plasma treatment in PLLA films due to the development of oxygen-containing groups on the modified surface [17] and after Ar-NH_3/H_2 gas plasma treatment through the introduction of nitrogen-containing surface groups, such as amines (—NH_2) [18]. Also O_2 and N_2 can be used to improve the hydrophilicity of PLLA surfaces, with a significant decrease (75%) in contact angle due to the increase of the number of polar groups formed during the plasma treatment [19]. However, to the best of our knowledge, there is no work reported on O_2 and Ar plasma treatment for PLLA in which superhydrophilic surfaces have been achieved, which is a relevant issue for many applications [18]. In this work, the effects of O_2 and Ar plasma treatments on the surface wettability of PLLA electrospun membranes and films are investigated. Furthermore, the influence of plasma treatment on fiber morphology, surface composition and degree of crystallinity is also be evaluated.

2. Materials and methods

2.1. Materials

Purasorb PL18 PLLA, with an average molecular weight of 217.000–225.000 g mol^{-1}, was purchased from Purac; N,N-dimethylformamide (DMF) from Merck and dichloromethane (MC) from Sigma–Aldrich. Materials were used as provided.

2.2. Sample preparation

PLLA electrospun membranes were processed according to a previously reported method [4]. Briefly, a 10 wt% solution of PLLA in DMF/MC was prepared under magnetic stirring at room temperature until complete dissolution of the polymer. Then, the polymer solution was placed in a plastic syringe (10 mL) fitted with a steel needle with inner diameter of 0.5 mm. The electrospinning procedure was conducted at 20 kV with a high voltage power supply from Glassman (model PS/FC30P04) with a solution feed rate of 0.5 mL h^{-1} applied with a syringe pump (from Syringepump). The randomly oriented electrospun membranes were collected on aluminum plate. PLLA films were obtained by casting from the solution used for electrospinning and subjected to brief annealing, at 80 °C, during 30 min, to allow solvent evaporation.

2.3. Surface modification

Surface treatment was conducted in a Zepto plasma chamber Diener Electronics (Ø = 105 mm, L = 300 mm, V = 2.6 L) equipped with a 40 kHz radio frequency plasma generator. The base pressure of system (obtained before plasma ignition) was 20 Pa. Plasma treatments were performed independently with Ar and O_2 as working gases, for different times from 5 to 970 s and plasma powers from 10 to 100 W under a total pressure of 80 Pa.

2.4. Sample characterization

Fiber morphology was analyzed using scanning electron microscopy (SEM, Quanta 650, from FEI) with an accelerating voltage of 5 kV. The samples were previously coated with a thin gold layer using a sputter coater (Polaron, model SC502). Surface morphology and roughness of the PLLA films were studied by atomic force microscopy (AFM) using a VEECO CP II AFM working in tapping mode. A Si probe, RTES-PA-CP with a spring constant 20–80 N m^{-1} was used. The provided roughness value (Ra) represents the arithmetic average of the deviations from the center plane of the sample.

Wettability of the samples was determined by measuring the contact angle of distilled water (3 μL) at room temperature, using an OCA15 Dataphysics contact angle analyzer. Six measurements were carried out for each sample at different places and the average contact angle was reported.

X-ray photoelectron spectroscopy (XPS) was performed using a Kratos AXIS Ultra HSA, with VISION software for data acquisition and CASAXPS software for data analysis in order to evaluate the surface elemental composition and atomic concentration of the samples. The analysis was carried out with a monochromatic Al Kα X-ray source (1486.7 eV), operating at 15 kV (90 W), in fixed analyser transmission (FAT) mode, with a pass energy of 40 eV for regions of interest and 80 eV for survey. Data acquisition was performed with a pressure below than 1×10^{-6} Pa. A charge neutralization system was also used. The effect of the electric charge was corrected by the referencing all binding energies to the C1s hydrocarbon peak at 286.4 eV. Spectra were analyzed using CASAXPS software. Curve fitting of the high resolution spectra used 30% Gaussian/70% Lorentzian mixed line shapes for each component.

Fourier transformed infrared (FTIR) measurements were performed at room temperature in a Bruker alpha apparatus in attenuated total reflectance (ATR) mode from 4000 to 400 cm^{-1}, using 24 scans with a resolution of 4 cm^{-1}. Differential scanning calorimetry measurements (DSC) were performed in a Perkin–Elmer, model Pyris-1 acquiring data from 30 to 200 °C, with a heating rate of 10 °C/min, under nitrogen purge. The samples were cut in small pieces and placed into 40 μL aluminum pans. The degree of crystallinity (ΔX_c) was determined using Eq. (1) [4]:

$$\Delta X_c = \frac{\Delta H}{\Delta H_m^O} \quad (1)$$

where ΔH is the area under the thermogram between 65 and 160 °C, and ΔH_m^O is the melting enthalpy (93.1 J g^{-1}) [4].

2.5. Cytotoxicity assay

The indirect cytotoxicity evaluation of the samples was conducted adapting the ISO 10993-5 standard test method.

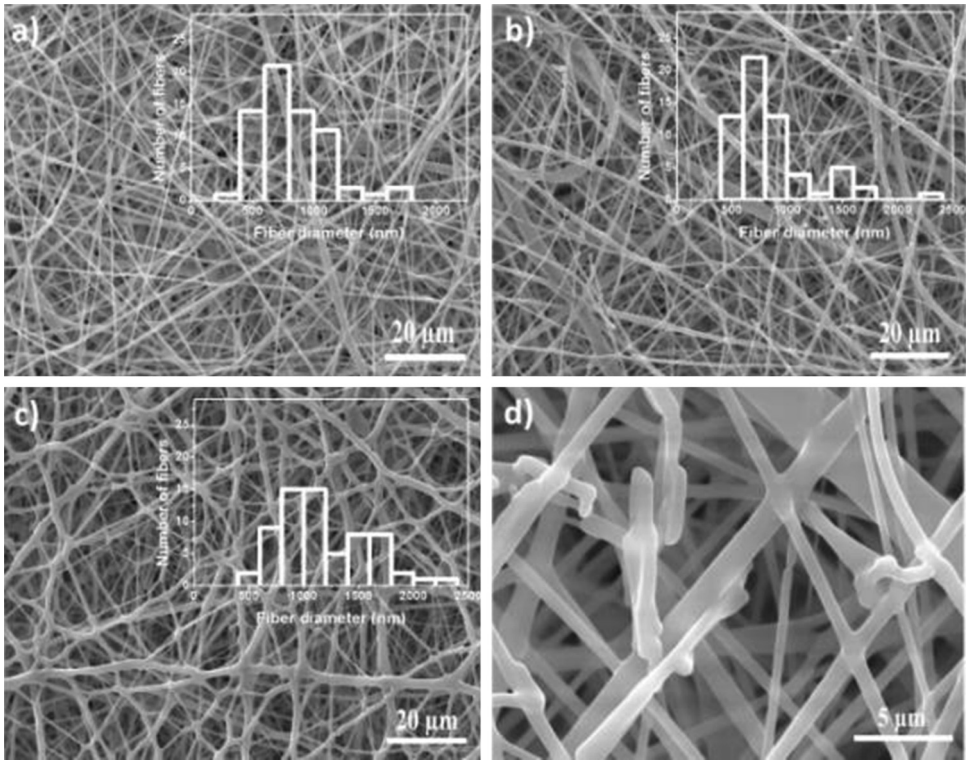


Fig. 1. SEM images of electrospun PLLA membranes: (a) untreated and plasma treated with (b) argon and (c) and (d) oxygen at $t = 970$ s and $P = 100$ W.

The extraction media was prepared by immersing the samples ($6\,cm^2\,mL^{-1}$) in a 24-well tissue culture polystyrene plate with Dulbecco's modified Eagle's medium (DMEM, Gibco) containing $1.0\,g\,L^{-1}$ glucose supplemented with 10% fetal bovine serum (FBS, Biochrom) and 1% penicillin/streptomycin (P/S, Biochrom), at 37 °C in a 95% humidified air containing 5% CO_2 and incubated for 24 h. A 20% of dimethylsulfoxide (DMSO, Sigma–Aldrich) was used as a positive control and the cell culture medium was employed as negative control.

Furthermore, MC3T3-E1 pre-osteoblast cells were seeded in the 96-well tissue culture polystyrene plate at the density of 2×10^4 cells mL^{-1} and incubated for 24 h to allow cell attachment on the plate. After 24 h, the culture medium from the 96-well tissue culture polystyrene plate was removed and the as-prepared extraction media were added to the wells (100 μL). Afterward, the cells were incubated for 72 h and the cell viability evaluation was quantified with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich) assay.

The MTT assay measures the mitochondrial activity of the cells, which reflects the viable cell number. After 72 h, the medium of every well was removed and fresh medium containing 10% MTT solution (stock solution of 5 mg MTT mL^{-1} in phosphate buffer solution (PBS)) was added. The viable cells with active metabolism convert MTT into a purple colored formazan product. After 2 h of incubation, the MTT crystals were dissolved with DMSO and the optical density was measured at 570 nm.

All quantitative results were obtained from four replicate samples and controls and analyzed as the average of viability ± standard deviation (SD).

The percentage of cell viability was calculated according to Eq. (2) [20]:

$$\text{Cell viability}\ (\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of negative control}} \times 100 \qquad (2)$$

3. Results and discussion

Plasma treatment was performed at different times (from 5 to 970 s) and powers (from 10 to 100 W for both Ar and O_2 gases). In the following, the results will be shown for those samples and conditions that better represent the observed variations.

3.1. Morphology

Morphology, topography and physico-chemical variations of the samples, if any, caused by the plasma treatment would be larger and more clearly quantified for the more aggressive plasma treatment conditions, achieving the desired hydrophilic membranes. Thus, those analysis were performed on the electrospun membranes and films with the largest plasma treatment time ($t = 970$ s) and power ($P = 100$ W).

Fig. 1 shows SEM pictures of the untreated (Fig. 1a) and treated membranes under Ar (Fig. 1b) and O_2 (Fig. 1c) – $t = 970$ s and $P = 100$ W – as well as the histogram of the fiber diameters. The histograms of the fiber diameters were determined from the corresponding SEM images with the ImageJ software and taking into account 50 fibers.

As can be seen, PLLA fibers were successfully electrospun into a non-woven mesh with smooth fiber surface without the presence of beads (Fig. 1a). After Ar plasma treatment (Fig. 1b) the appearance of fiber mesh is similar to the pristine ones. Some fiber joining can be observed in Fig. 1c and d (higher magnification) on the fibrils surface treated by O_2 probably related to surface polymer melting of the smaller fibers due to the high energy supplied by the plasma source and to etching associated with the reactions between radical oxygen species and the polymeric surface [11,21]. Furthermore, fibers get a flat configuration after O_2 plasma. This important fiber modification, occurring at a larger scale, will superimpose any surface roughness variation, as it will be shown to occur in the film samples, and will determine the wettability observed. However,

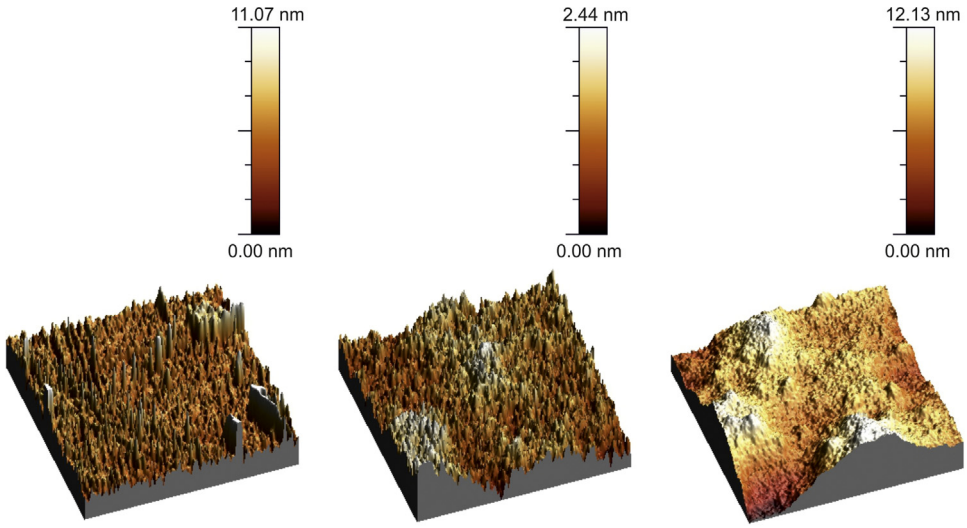


Fig. 2. AFM images of the surface microstructure (2 μm × 2 μm scans) of: (a) untreated PLLA films and PLLA plasma treated films (970 s and 100 W) with (b) Ar and (c) O_2 gas plasma. The average R_a roughness of the samples is: (a) 0.78 nm, (b) 0.85 nm and (c) 3.4 nm.

no complete fiber melting was detected showing the still open microstructure due to the space between the membrane fibers.

The influence of Ar and O_2 plasma treatments on fiber mean diameter was determined. Pristine PLLA mats present a mean average size of ~830 ± 282 nm (Fig. 2a). After plasma treatments an average size of ~ 866 ± 361 nm and 1179 ± 397 nm were observed for Ar and O_2, (Fig. 1c and d, respectively). Thus, Ar treatment does not induce a significant variation in fiber diameter, in contrast, O_2 plasma treatment leads to an increase in average fiber diameter as a result of fiber joining.

The influence of plasma treatment on the surface morphology and roughness of the PLLA polymer films was studied by AFM in pristine and plasma treated PLLA films, due to the small size of the fibers. Fig. 2 shows a 3D plot representation of PLLA surface (2 μm × 2 μm) obtained by AFM. It is shown that both Ar and O_2 plasma treatments induce changes in the surface morphology of PLLA that depends on the type of gas used in the plasma treatment. In general, ion etching during plasma treatment usually leads to a roughness increase in the polymer surface [18]. The surface of the untreated PLLA is characterized by a mean surface roughness (R_a) of

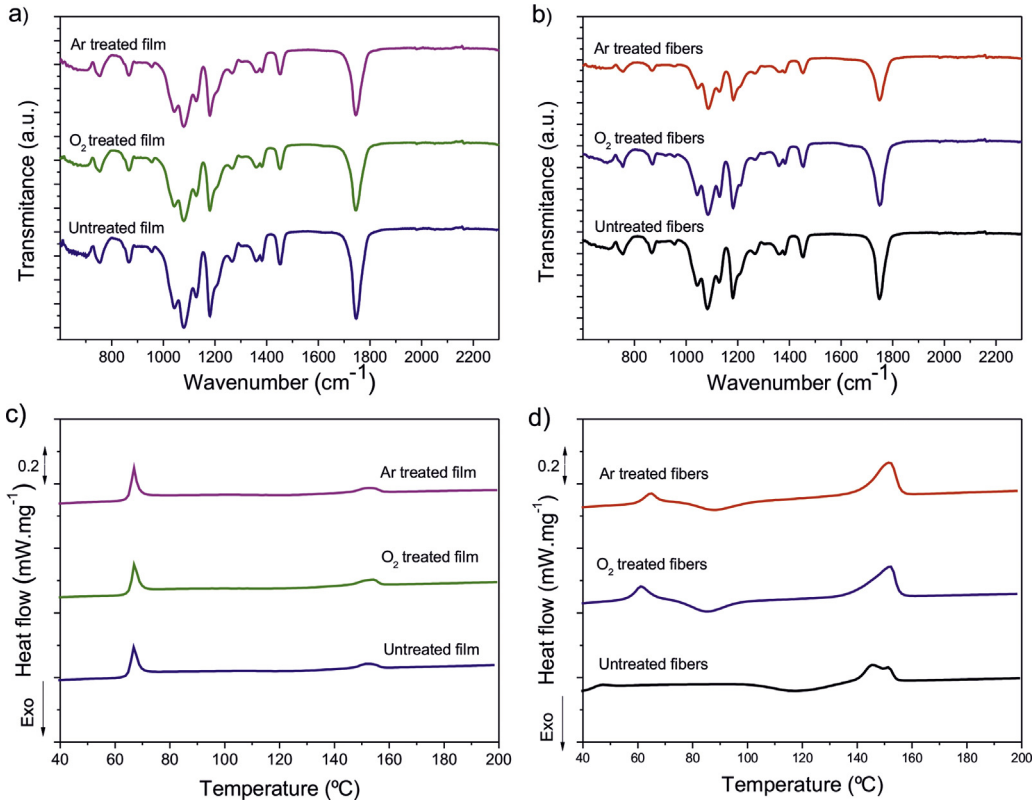


Fig. 3. Physical–chemical characterization of electrospun PLLA membranes and films: (a) and (b) FTIR-ATR spectra; and (c) and (d) DSC curves of untreated and treated PLLA electrospun membranes and films for a plasma treatment for 970 s at 100 W with Ar and O_2.

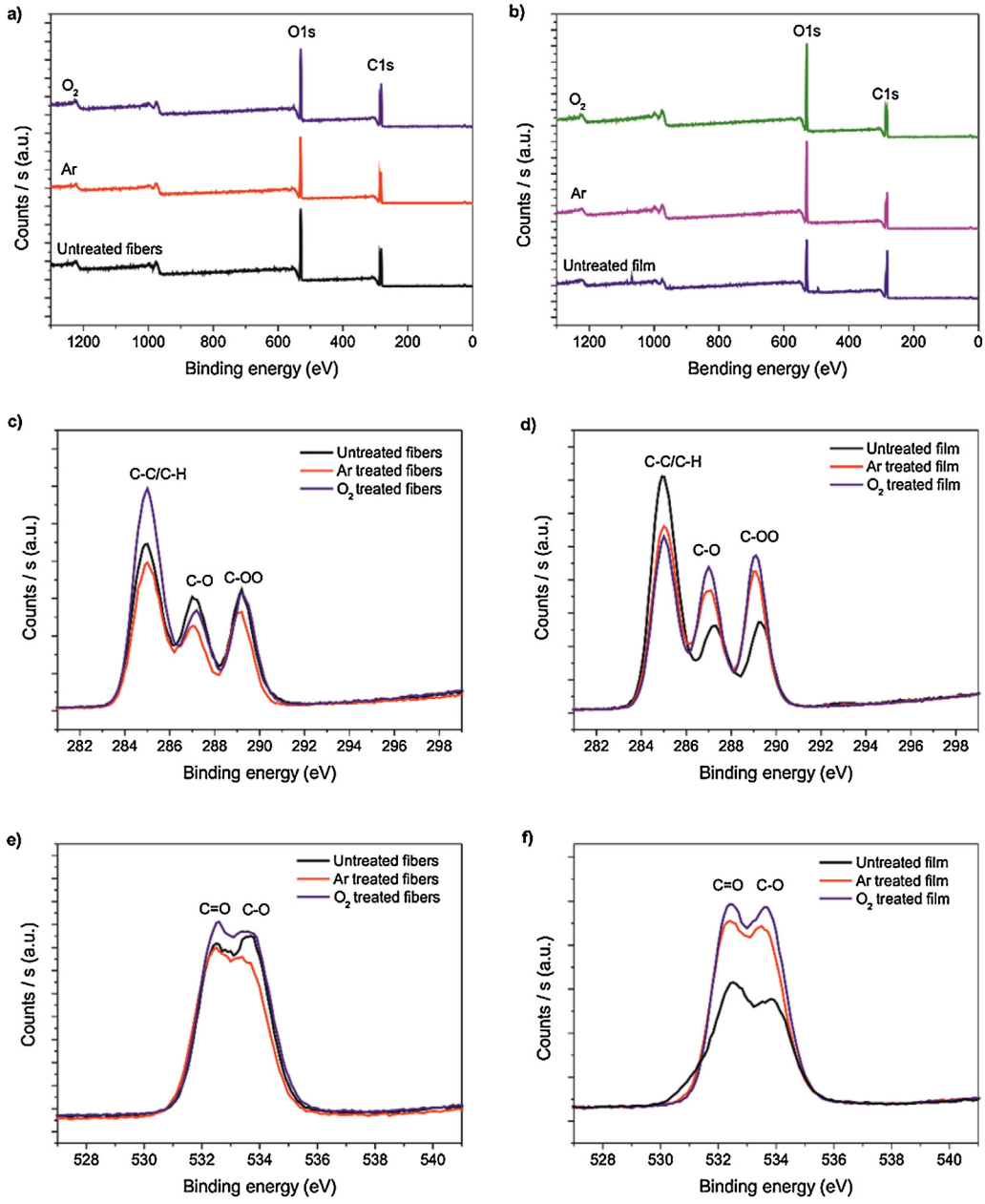


Fig. 4. XPS results of untreated and treated PLLA electrospun membranes and films under O_2 and Ar plasma for a plasma treatment of 970 s with a power of 100 W: (a) and (b) XPS survey scan; (c) and (d) C1s scan spectra; and (e) and (f) O1s spectra.

0.78 ± 0.10 nm (Fig. 2a). Ar plasma treatment does not induce significant changes in the R_a (0.85 ± 0.63 nm) (Fig. 2b). However, after O_2 plasma treatment, the surface roughness of PLLA increases to 3.4 ± 2.1 nm. The increase in roughness is associated to ion etching during plasma treatment that can be followed by mass loss related to bond scission of PLLA and reactions of the radicals generated in the PLLA chains upon plasma exposure [18,22]. When polymers are exposed to plasma, plasma cleaning and plasma etching occurs on the polymer surfaces [13]. As PLLA contains O_2 functionalities (ester groups), its surface presents high susceptibility to plasma etching [23]. Thus, it is concluded that a treatment time of 970 s, under O_2, promotes etching, which is not observed under Ar plasma.

3.2. Physical–chemical characterization

FTIR-ATR measurements were used to evaluate possible chemical changes in the PLLA polymer chain of electrospun membranes and films after a plasma exposure time of 970 s at 100 W. Fig. 3a shows the infrared spectra for PLLA electrospun membranes and films before and after plasma exposure. The chemical structure of PLLA is formed by the repetition unit of —CH—C=O—O— [24]. As it is observed, no noticeable variations occur in electrospun membranes and films before or after plasma treatment under Ar and O_2 gas. All samples are characterized by the bands at 755 cm^{-1} and 870 cm^{-1}, both characteristics of CH_3 stretching and rocking, assigned to the crystalline and amorphous phase, respectively [25]. The absorption band at 1044 cm^{-1} is attributed to the stretching vibration of the C—CH_3 bond. The bands at 1130, 1357 and 1450 cm^{-1} are associated to r_s (CH_3), δ(CH) and δ_{as}(CH) stretch, respectively. The 1083, 1185, 1271 and 1747 cm^{-1} absorption bands, characteristics of the presence of oxygen compounds, are ascribed to υ_s (C—O—C), ν_{as} (C—O—C) + r_{as} (CH_3), ν (CH) + ν (C—O—C) and ν (C=O), respectively [25]. As no differences can be observed in the PLLA FTIR spectra before and after plasma treatment for both fiber mats and films,

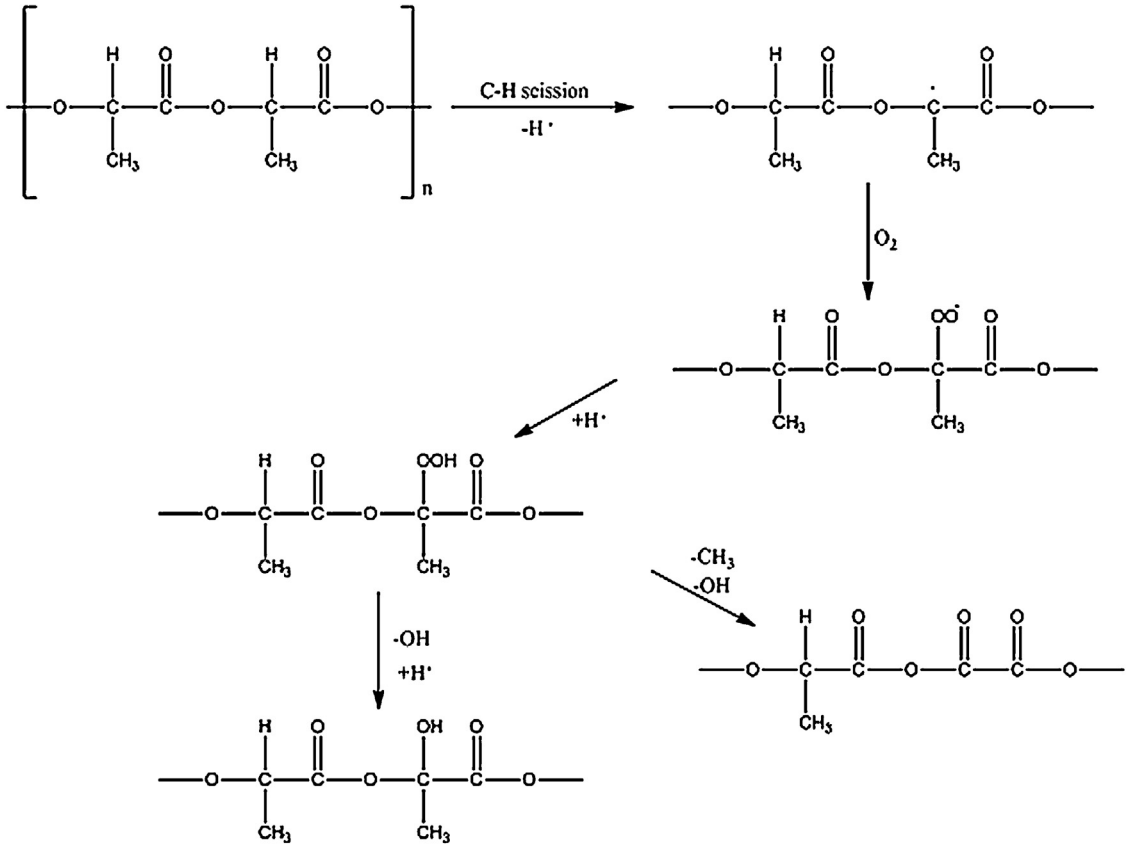


Fig. 5. Schematic representation of Ar and O_2 plasma treatments on PLLA electrospun membranes and films. Plasma treatment introduces free radicals that can react with oxygen.

chemical modifications (if present) should occur at the surface, which can be analyzed by XPS.

The thermal characterization of the PLLA samples was performed by DSC (Fig. 3c and d). All samples exhibit a strong endothermic peak at around 60 °C corresponding to the glass transition and a single melting peak in the region between 140 and 160 °C, in agreement to [4]. The melting peak in the PLLA films does not appear as clearly as in the fibers indicating a lower degree of crystallinity. Further, no exothermic features are detected in the films, leading to conclude that there is no polymer crystallization during the DSC heating scans. On the other hand, untreated and treated PLLA electrospun membranes crystallize during the DSC heating scan, showing a broad exotherm, immediately above the glass transition, in the range of 65–110 °C, with a cold crystallization peak around 85 °C. The cold crystallization behavior results from the ordering of PLLA molecular chains in a regular array above the glass transition, leading to the formation of a well-defined crystal structure. This fact shows that numerous crystal nuclei are present in the polymer as a result of the non-equilibrium chain conformations that result during the electrospinning process [4], which does not occur in the films. The degree of crystallinity of the samples was determined using Eq. (1). It was observed that Ar and O_2 plasma treatments do not promote changes in the degree of crystallinity of films and electrospun membranes compared to the untreated PLLA ones. Both, electrospun mats and films are nearly amorphous, the degree of crystallinity of the films being ~4% as evaluated by the area under the melting peak. The degree of crystallinity of the membranes is similar (as calculated by subtracting the cold crystallization from the total melting peak [4]) but reaches a maximum degree of crystallinity of ~32% after cold crystallization. Again, this is similar for plasma treated membranes, indicating that the plasma effects are just confined to the surface of the membranes.

3.3. Chemical surface variations

Plasma treatment generally leads to a surface modification process by the cleavage of the polymer molecular chains promoting the formation of free radicals that activates the surface [11]. To evaluate the quantitative elemental composition differences between the PLLA electrospun membranes and films before and after the plasma exposure, a XPS analysis was performed. The results obtained for untreated membranes and films and after the maximum plasma exposure time under Ar and O_2 are presented in Fig. 4.

Fig. 4a and b shows that plasma exposure treatment does not induce relevant chemical changes in the surface composition of the PLLA samples. In both pristine and plasma treated samples just carbon and oxygen were detected in the XPS spectra, due to the chemical structure of PLLA. Fig. 4 shows the individual components of C1s (4c and d) and O1s (4e and f). From Fig. 4c and d it is observed that PLLA fibers and films show three main C1s peaks at 284, 286 and 289 eV, characteristics of C—C/C—H, C—O and COO groups, respectively. The peaks at 532 and 533 eV in Fig. 4e and f for O1s scanning spectra are assigned to C=O and C—O groups, respectively. It can be observed a low level oxidation process in the PLLA film surface, while for PLLA fibers treated with O_2 plasma the variations are within the experimental error.

Table 1 summarizes the XPS analysis for the % of elemental composition and the oxygen to carbon ratio (O/C) before and after the plasma exposition.

The carbon and oxygen amounts present in PLLA untreated fibers are approximately 70 and 30%, respectively. The values obtained for untreated PLLA films are not so different being 68% and 32% of carbon and oxygen atoms, respectively.

After O_2 and Ar plasma treatments, the amount of carbon atoms slightly decrease while the oxygen content increases. The increase

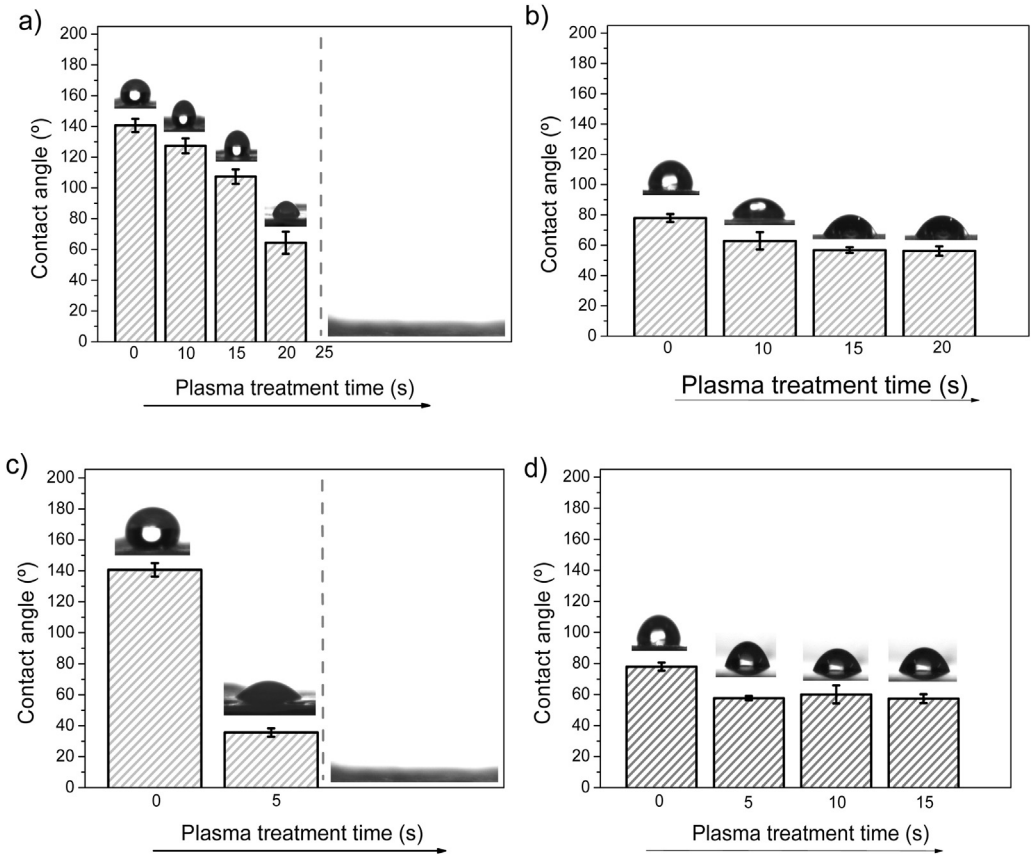


Fig. 6. Influence of Ar plasma treatment time in the contact angle value for (a) PLLA electrospun membranes and (b) PLLA films and the influence of O_2 plasma treatment time for (c) PLLA electrospun membranes and (d) PLLA films at a power of 100 W. The bars in the graph are the standard deviations.

in oxygen content is more noticeable for PLLA films after O_2 exposure and in the case of PLLA fibers after Ar treatment.

It was observed that the O/C ratio shows a small increase from 0.4 to approximately 0.5 in fibers and films, indicating that a small chemical reaction occur in the fibers and films during plasma exposure under Ar and O_2. This slight increase can be explained by the scission of the C—H bond from the polymer chains due to the interaction of the O_2 and Ar plasma with the PLLA surface. During plasma treatments, the scission of carbon–hydrogen bond (C—H) due to the strength of the C—H (410 kJ mol^{-1}) when compared to C=O (745 kJ mol^{-1}) bonds induces radicals formation on the surface. After the activation of the surface, the free radicals can interact with the oxygen from air exposure leading to the formation of hydroperoxides groups enriched in oxygen, increasing the amount of O1s (Fig. 4f). These compounds are highly unstable and can thermally decompose producing secondary radicals that subsequently are able to react with air exposure, originating newly formed C—O and C=O groups and therefore increasing oxygen concentration [11]. These considerations are in agreement with Table 1 where it is possible to observe that the carbon content slightly decreases when the plasma modification occurs. Fig. 5 shows the possible chemical modifications that can lead to variations in the amount of carbon and oxygen contents in the PLLA surface of fibers and films [26]. The formation of these polar groups on the PLLA surface leads to an increase in the hydrophilicity of the treated samples.

Table 1
Surface chemical composition of untreated PLLA membranes and films modified by O_2 and Ar plasma at different treatment times.

Elemental composition (%)			
Surface	C	O	O/C
Untreated membranes	70.0 ± 0.2	30.0 ± 0.2	0.4
O_2 treatment time (s)			
10	68.0 ± 0.2	32.0 ± 0.2	0.5
970	69.0 ± 1.1	31 ± 1.1	0.4
Ar treatment time (s)			
25	64 ± 0.6	36 ± 0.6	0.6
970	66 ± 0.6	34 ± 0.6	0.5
Untreated film	68 ± 1.6	32 ± 1.6	0.5
O_2 treatment time (s)			
10	65 ± 1.7	35 ± 1.8	0.5
970	65 ± 1.2	35 ± 1.2	0.5
Ar treatment time (s)			
25	68 ± 1.2	32 ± 1.2	0.5
970	66 ± 0.3	34 ± 0.3	0.5

3.4. Samples wettability

Once the effect of the plasma treatment on the morphological and physico-chemical properties of the samples is understood, it is important to evaluate the effect on the wettability of the membranes. Plasma treatment of the samples generally causes a reduction of the contact angle and an increase in the surface energy. The contact angle measurements for PLLA samples before and after Ar and O_2 plasma treatment are presented in Fig. 6. The water surface contact angle of the untreated fibers is 141 ±4.3° being in agreement with the strong hydrophobicity associated to PLLA [3]. Fig. 6 also shows that the surface wettability of PLLA fibers and films depends on plasma treatment composition, time and power. Increasing argon plasma treatment time for a power of 100 W leads to a significant decrease in the contact angle of the PLLA fibers (Fig. 6a), indicating a more hydrophilic surface. For argon treatment times higher than 20 s, the PLLA electrospun fiber mats become

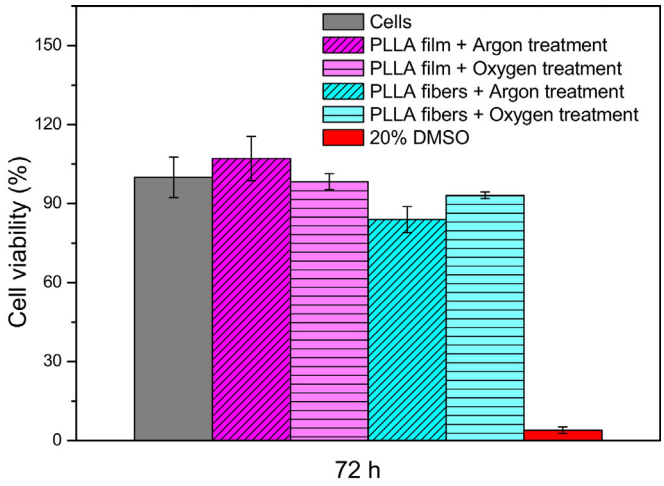


Fig. 7. Cell viability of MC3T3-E1 pre-osteoblast cells in contact with as-prepared extraction media exposed with the different PLLA samples up to 72 h.

superhydrophilic with a total water droplet absorption. Similar results are observed by varying plasma power for a given time (data not shown). Thus, for a treatment time of 20 s it was verified that 70 W is the minimum plasma power needed to achieve superhydrophilic membranes. The O_2 plasma treatment also enhances the PLLA surface hydrophilicity (Fig. 6c), and a treatment time of 10 s leads the formation of superhydrophilic membranes. In fact, a minimum power plasma of 40 W is needed for obtaining superhydrophilic membranes for a treatment time of 10 s.

The decrease of the contact angle in the PLLA films after plasma treatment with both plasma treatment gases is not as pronounced as for the electrospun membranes. Fig. 6b and d show a decrease from $78 \pm 2.6^\circ$ in untreated PLLA films to approximately 60° for both Ar and O_2 treatments. The fact that hydrophilic electrospun membranes are obtained is thus associated to the variation of the contact angle of the polymer itself, as shown by the variations in the films, and capillarity effect of the electrospun membranes, which accounts for the major part of the effect. Variations in the polymer surface roughness and polymer mat morphology leads to an associated variation of the surface tension of the membrane, leading thus to a penetration of the water drop in the membrane and the consequent hydrophilic behavior [11].

3.5. Cell viability studies

For tissue engineering applications, it is important to evaluate whether plasma treatments lead to cytotoxicity of biocompatible PLLA films and electrospun membranes due to the induced surface modifications. The effects of polymer extract medium on the metabolic activity of MC3T3-E1 pre-osteoblast cells were performed using MTT assay after 72 h (Fig. 7). According to the ISO standard 10993-5, the samples are considered cytotoxic when the cell viability reduction is larger than 30%. So, based on the results shown in Fig. 7, all samples are not cytotoxic and thereby the treatment with argon or oxygen allows modifying the wettability of the PLLA samples with no influence on the toxicity of the material.

4. Conclusions

The influence of Ar and O_2 plasma treatments on PLLA films and electrospun membranes on their morphology, physical–chemical properties and wettability was determined.

A similar fiber mesh and average size was obtained for membranes treated under Ar plasma while an increase in average fiber diameter is observed after O_2 plasma treatment, mainly due to fiber joining. Associated to this effect, there is an increase in surface roughness, as observed in PLLA films surface after Ar and O_2 plasma treatments. On the other hand, no relevant variations are observed in the degree of crystallinity and polymer bond characteristics after the plasma treatments. Both electrospun membranes and films being nearly amorphous. The contact angle of the samples is dependent on the plasma treatment conditions (used gas, time and power) and it is possible to modify hydrophobic electrospun fiber mats into superhydrophilic ones. This effect is obtained in the fibers mainly due to variations of the surface tension as plasma treatment in the films results in roughness variations. The formation of free radicals and to an increase of the polar groups in the PLLA surface, leading to lower variations of the surface water contact angle. Independently of the plasma treatment, the obtained samples are not cytotoxic. Therefore, a way is shown to obtain superhydrophilic membranes suitable for biomedical applications.

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