Role of PLLA Plasma Surface Modification in the Interaction with Human Marrow Stromal Cells

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ABSTRACT: The effects of oxygen-based radio frequency plasma enhanced chemical vapor deposition (rf PECVD) on the surface of poly(L-lactide) (PLLA) polymers and the influence thereof on protein adsorption and on bone–cell behavior have been studied. Thin films and porous scaffolds based on PLLA polymer were developed, and the role of surface modifications were investigated extensively. PECVD surface treatments were used to alter surface functionality and modulate protein adsorption on the PLLA polymer matrix. In particular, Bovine Serum Albumine fluorescein isothiocyanate (fitc-BSA) conjugate adsorption on patterned surfaces of treated PLLA was analyzed by fluorescence microscopy. Human marrow stromal cells (MSCs) were cultured on scaffolds and cell adhesion and morphology were assessed using fluorescence microscopy. The results indicated that the PLLA surface became hydrophilic and its roughness increased with the treatment time and it had a dominant influence on the adsorption process of the protein. The outcome of the plasma treatment of various PLLA surfaces has been shown to be the up-regulator of the cell-adhesive proteins expression and consequently the improvement of cell adhesion and growth. Oxygen-treated PLLA promoted higher adhesion and proliferation of the MSCs in comparison to the untreated samples. It can be concluded that following plasma treatment, PLLA samples show enhanced affinity for osteoprogenitor cells. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 3602–3611, 2009

Key words: biodegradable; biocompatibility; biological applications of polymers; cold plasma; surfaces

INTRODUCTION

Surface modification techniques of polymer biomaterials have been used to tune surface properties and it is becoming an increasingly popular method for the improvement of device multifunctionality, i.e. biological and mechanical behavior.

Since the first interaction between the cells and the substrate is protein adsorption and then cell adhesion, surface properties, that affect the protein affinity, are a key factor in governing the success of the engineered tissue. Recent studies have highlighted the mechanisms of cell-surface recognition and have provided solid data to obtain novel materials that are able to guide and activate specific cell behavior on biomedical implant surfaces.[1–3]

The strategy of the surface modification of biomaterials has been adopted over the years to change the area of the biomaterial that first comes into contact with a biological environment, to prevent or improve the adsorption of proteins and the adhesion of cells to biomaterial surfaces.[4–7]

Due to its biocompatibility and high mechanical properties, poly(L-lactide) (PLLA) has been approved for use in vivo, as it degrades to natural metabolites.[8,9] The key premise in tissue-engineering strategies, however, is the use of biodegradable polymers to make three-dimensional (3D) porous scaffold structures which allow entrance and adhesion of regenerating cells. PLLA has been the predominant choice for materials in sutures and biodegradable implantable meshes for guided tissue regeneration.[10] PLLA macroporous or fiber-based scaffold and PLLA highly porous sponges have been fabricated to obtain synthetic extracellular matrix (ECM) employed to engineer tissues. Various cell types have been cultured on these fibrous scaffolds and successfully transplanted into animal models.[11]

However, it is well-known that the polyesters show a hydrophobic surface that is not ideal for cell attachment and growth compared to the natural

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ECM: they do not expose functional groups for the attachment of biologically active molecules.[12,13]

Therefore, many approaches to modify the surface of PLLA have been undertaken to introduce useful surface characteristics to the polymer without changing bulk properties.

Plasma enhanced chemical vapor deposition (PECVD) treatment has the advantages to modify the outer atomic layers of a substrate without changing the bulk properties.[14–17] Since the plasma treatment is conducted in a vacuum and tends to be pervasive, it is advantageous for the treatment of surfaces with complex shapes,[16] such as in treating scaffolds for tissue engineering with interconnected porous structures.[18] Using plasma processes, it is possible to change the chemical composition and properties such as wettability, surface energy, metal adhesion, refractive index, hardness, chemical inertness, and biocompatibility.[16,17] Plasma techniques can easily be used to induce the desired groups or chains on the surface of a material,[19–21] so it has a special application to improve cell interaction with the scaffolds. An interesting and widely investigated approach is to use the new functional groups on biomaterial polymer surfaces to immobilize biomolecules: using specific proteins with suitable functions, cell adhesion and tissue responses may be controlled. Proteins, recognized by cell adhesion receptors, modulate cell adhesion, morphology and functions.[22–26] Consequently, the study of protein adsorption and any associated conformational change following interaction with biomaterials is of great importance in the area of implants and tissue regeneration.[27]

In this study, a plasma method to functionalize PLLA surfaces has been proposed. The changes in the surface properties of the PLLA films and scaffolds were characterized. Protein immobilization on PLLA films, subsequent to plasma surface modifications was investigated by means of fluorescence microscopy. The osteoconduction of the scaffold before and after surface modification was evaluated using human marrow stromal cell cultures.

The goal of this study is to investigate the effects of oxygen plasma treatments on PLLA samples prepared as flat films and porous scaffolds. Since the last one is the main representative structures of the in vivo element, the possibility to modulate its surface properties by PECVD is a fundamental aim of this research.

EXPERIMENTAL

Sample preparation and characterization

A poly(L-lactide) polymer matrix with an inherent viscosity of 0.90–1.2 dL/g was purchased from Lactel-Absorbable Polymers and used as received. Samples were prepared in a thin film geometry using the solvent casting process, and porous structures using a solvent casting-particulate leaching process, with chloroform ($CHCl_3$, Sigma-Aldrich).

PLLA polymer films were realized by casting the polymer solution onto clean glass substrates. The solvent was allowed to evaporate slowly at room temperature for 48 h and then the samples were dried under vacuum.

Porous scaffolds were fabricated with a solvent casting-particulate leaching process, using NaCl as the water soluble porogen.[28] The NaCl crystal sizes were 100–200 μm. Three different polymer/salt compositions were used in our studies: 70, 80, and 90 wt % salt.

Films and scaffold based on PLLA were characterized by means of Perkin–Elmer Pyris 1 differential scanning calorimeter (DSC), at a scanning rate of 10°C/min in a range from −25 to 250°C. Two heating scans and one cooling scan were performed.

Surface modification

The plasma treatments were carried out using a Sistec Plasma Deposition system, 13.56 MHz radiofrequency generator (Huttinger). Typically the sample was capacitively coupled to a 13.56 MHz rf-potential, so that a negative dc-bias developed on the surface. The main effect of this dc-bias is to accelerate ions in the direction of the sample.

Samples were placed on the electrode in the plasma chamber. The chamber was evacuated to 10^{-3} Torr before filling it with the desired gas. After stabilizing the chamber pressure to a proper value, a glow discharge plasma was created by controlling the electrical power for a predetermined time; oxygen (O_2) was used. The main parameters for this process were: gas pressure, power supply and treatment time; in particular, the effects of power supply and treatment time were investigated in this study. The surface of bi-dimensional (2D) and tri-dimensional (3D) specimens (films and porous scaffolds) were modified using 10 W and 20 W power supplies. A power supply of 20 W was applied for 2, 5, and 10 min on samples. The chamber pressure was maintained at a constant pressure of 10^{-1} Torr, with an oxygen flow rate of 60 sccm.

Surface properties were analysed and were connected to the PECVD process parameters. Atomic force microscopy was used to study the topography of PLLA film surface before and after oxygen plasma treatment by using an easy Nanosurf Scan AFM, Swiss. AFM images were recorded in a tapping mode at room temperature in air, using silicon cantilevers. The surface micro- and nano-topography, as well as the roughness were measured.

Surface roughness values were determined in five random areas per sample, scanning across 18 × 18 μm^2 areas. Calculation of the roughness parameters, including Ra (arithmetic average) and Rq (root mean square) were performed using the easy Scan DFM software.

The contact angle of treated and untreated PLLA films was assessed by the sessile drop method in air, using FTA1000 Analyzer. The samples were prepared by casting the polymer solution onto glass substrates, to realize thin and flat layers of polymer. Water drops of 5 μL (HPLC grade water) were placed on PLLA films and measurements were recorded 10 s after the liquid made contact with the surface. The role of treatment time and bias voltage was investigated.

Infrared spectroscopy of PLLA films before and after plasma treatments was carried out to characterize the sample surface and to confirm the composition and the treatment effects. Measurements were obtained with attenuated total reflection spectroscopy (ATR) using a JASCO FT-IR 615 spectrophotometer in the range of 400–4000 cm^{-1} and with a 4 cm^{-1} resolution.

A morphological analysis of the PLLA porous scaffold was conducted using a field emission scanning electron microscope (FESEM, Supra 25 Zeiss). Scaffolds were sputter coated to obtain a thin Au layer. The effect of the surface treatment on the scaffold morphology was investigated.

Protein immobilization

Protein adsorption was evaluated using a Bovine Serum Albumin fluorescein isothiocyanate conjugate (fitc-BSA, Sigma-Aldrich).

The PLLA films were first modified with an oxygen plasma chemical vapor deposition method, to activate the functional groups and to promote protein immobilization. A metal mask was positioned over the samples before the plasma treatment, so as to obtain a patterned surface with a functionalized surface confined in a specific region. The oxygen plasma treatment coupled with the masking technique was used to investigate the protein immobilization. Copper grids 400 and 1000 mesh (Sigma-Aldrich) with a diameter of 3.05 mm and open squares of 40 μm and 20 μm in length (l) at intervals of 20 μm and 10 μm (s) respectively, were used in the experiments.

The samples were treated by rf PECVD as described in the previous paragraph, by using a fixed bias voltage of 20 W for 5 min. A specific surface topography was created following the treatment, where the squares corresponded to the area modified with functional groups, while the grooves exposed the original PLLA without any surface modification.

Subsequently, fitc-BSA was incubated on these samples. PLLA samples were treated at room temperature for 2 h with a 100 μg/mL BSA solution in phosphate-buffered saline (PBS). To avoid the effects of salt precipitation, a magnetic stirrer was used in the solution. Static adsorption experiments were performed by immersing the modified polymer substrates in the solution.

At the end of the treatment the samples were washed three times with PBS and deionized water to remove any residual protein. Then, the films were dried in a nitrogen atmosphere, and stored overnight in a desiccator before analysis. The experiments were carried out at room temperature. Since protein-surface interaction is highly dependent on the experimental system, all assay conditions were previously optimized and kept constant throughout the performed replicates.

Fitc-BSA patterning on treated PLLA was analyzed by means of an inverted Nikon Fluorescence TE2000-E microscope (Nikon Instruments).

Biocompatibility evaluation

Human marrow stromal cells (MSCs) were obtained from two patients (aged 69 and 72 yrs) during surgery for total hip joint replacement. The study was approved by the Institutional Ethical Committee and consent was obtained from the donors. During surgery, drilling of the femur was performed prior to prosthesis insertion and bone marrow with bone fragments were collected aseptically. In the laboratory, bone fragments were separated using a cell strainer and the marrow was layered onto a Ficoll gradient to obtain mononuclear cells by centrifugation and sequential washing with PBS. Following the seeding of the mononuclear cells in a 25 cm^2 flask, using α-MEM with 10% fetal bovine serum (FBS), 2 m*M* glutamine, 50 μg/mL ascorbate-2-phosphate and 1% penicillin/streptomycin solution at 37°C with 5% CO_2, the MSCs were obtained by adhesion to tissue culture plastic, expanded *in vitro* and used for experiments. MSC were seeded on porous scaffolds prepared using 70, 80, and 90% salt/polymer ratio (w/w) with or without 20 W plasma treatment for 5 min. Scaffolds were prepared for cell seeding by soaking in 70% ethanol for 1 h, then in 1% antibiotic/antimycotic in PBS for 2 h and prewetting in culture medium for 2 h.

MSCs were seeded dropwise 1.5×10^4 per sample and allowed to settle on the sample surface for 30 minutes, thereafter 2 mL of medium were added to the wells. The seeded scaffolds were cultured in the complete medium for 2 weeks, changing the media twice a week.

Cell-loaded scaffolds were assayed for cell numbers using Alamar blue, and for alkaline phosphatase

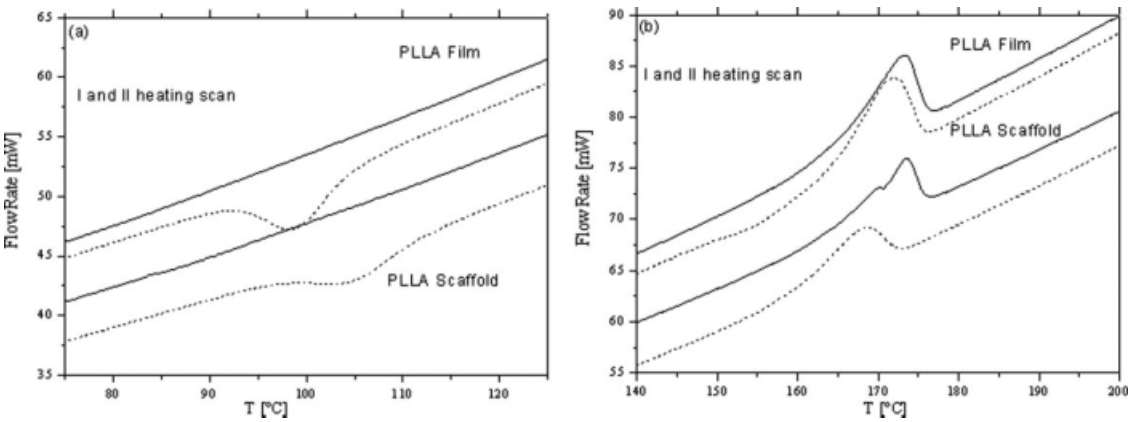

Figure 1 DSC thermograms of a PLLA film and scaffold in the first (solid) and second heating cycle (dash line), in the crystallization (a) and the melting region (b).

content using an alkaline phosphatase kit (Procedure No. ALP-10, Sigma) as detailed elsewhere[24] at 2, 6, 10, and 14 days from seeding. Human MSCs were preloaded for 5 min at 37°C with 20 m*M* CM-Dil [3,3-dioctadecyl-5,5′-di(4-sulfophenyl)-oxacarbocyanine, sodium salt, SP-DIOC18, Molecular Probes], a lypophilic fluorochrome which is retained within cells without affecting viability, seeded as described above and observed for cell attachment at 14 days by means of fluorescence microscopy.[29]

Data in the graphs are representative of two independent experiments and donors with two scaffolds per time point. The differences in cell numbers were assessed using a paired t test and the level of significance was established at $P < 0.001$.

RESULTS AND DISCUSSION

Sample characterization

PLLA films and solvent casting porous scaffolds were characterized by DSC to understand the role of the process used on the polymer properties. Figure 1 compares DSC thermograms on the PLLA film and scaffold (80 wt %) during the first and the second heating scan in the crystallization (a) and the melting region (b). During the cooling scan, both samples did not exhibit any crystallization behavior. During the second heating scan, films and scaffolds showed a cold crystallization at about 100°C [Fig. 1(a)]. Figure 1(b) shows that all the samples had a melting temperature between 168 and 175°C. It is evident that the thermal behavior of a PLLA scaffold is quite similar to a PLLA film, according to values found in literature.[30,31] In conclusion, the characteristic thermal transitions of the polymer matrix were not affected by the two different processes used.

Effects of surface modification on PLLA film

Surface topography

PLLA film surfaces before and after oxygen plasma treatments were analyzed by means of atomic force microscopy and the role of treatment time was investigated in detail. In Figure 2, AFM images of pristine PLLA surfaces (a) and oxygen treated

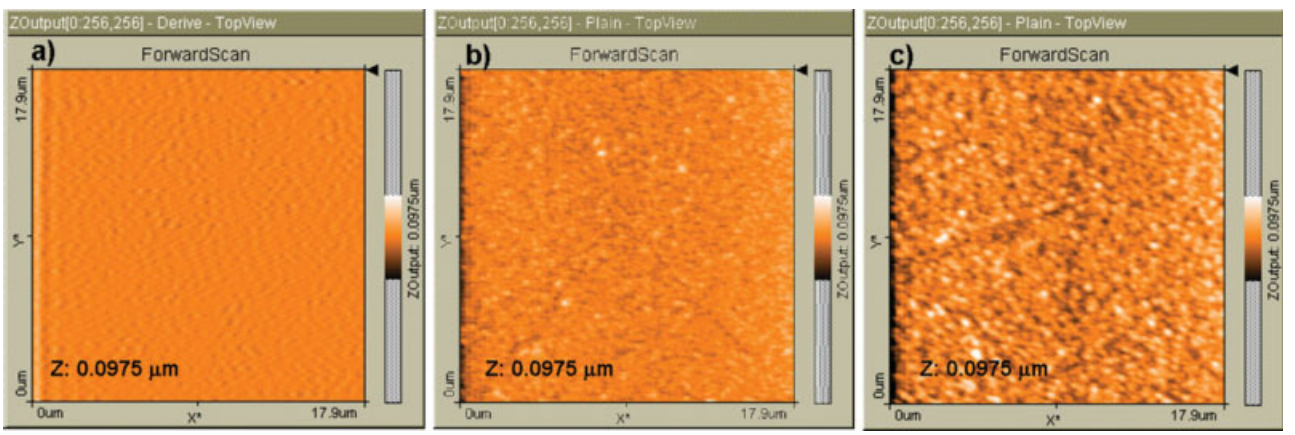

Figure 2 AFM topographies of pure PLLA film (a), after 5 min and (b) 10 min oxygen plasma treatment (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Rq and Ra Values on 18 μm Scale for Original and Surface Modified (20 W) PLLA Surfaces

Sample	Original PLLA	$PLLA_{5min}$	$PLLA_{10min}$
Ra (nm) roughness[a]	2.6 ± 0.2	4.5 ± 0.2	8.8 ± 0.5
Rq (nm) roughness[b]	3.3 ± 0.2	5.8 ± 0.3	11.2 ± 0.7

[a] Arithmetic average.
[b] Root mean square.

samples for 5 min (b) and 10 min (c) respectively, are shown. The treatment was performed by applying a power supply of 20 W to a flat PLLA film deposited with solvent casting on a clean glass substrate. Concerning physical aspect, Figure 2 shows a 18 μm window, where a variation in the roughness of the treated films is evident.

The surface of the original PLLA film is relatively smooth [Fig. 2(a)], whilst after plasma treatment it becomes rougher [Fig. 2(b,c)] and the oxygen treated PLLA films exhibit characteristic dots features of nanometric size. The surface roughness is a key factor as it has been demonstrated that the roughness of biopolymers may influence the extent to which proteins interact with the surface and the way the cells interact with the scaffold.[30,31]

Table I reports the roughness parameters: arithmetic average (Ra) and root mean square (Rq). Data are expressed as mean value and standard deviation, and show that for untreated PLLA both the Ra and Rq values were nearly two times lower than for the oxygen-treated surfaces for 5 min. However, when the treatment time was increased to 10 min, the roughness increased, revealing that the interpeak width and valley depth of the sample surface were a function of etching time.

In general, plasma treatments have an etching effect on polymer surface and produce nanostructured surface topography.

Surface wettability

Wettability is measured in terms of the changes in the contact angle of a liquid (water) on the surface: PLLA surface has a relatively high contact angle.[32] The contact angle values of the pristine and modified PLLA films was measured and reported in Table II: a correlation between the process parameters was observed. The surface wettability of the modified PLLA films was enhanced compared to the control film.

TABLE II
Contact Angle Measurements of Oxygen Treated PLLA Films

Material	Power Supply (W)	Bias (V)	Time Treatment (min)	C.A. (°)
PLLA	0	0	0	89.2 ± 0.4
PLLA oxygen treated	10	200	5	51.5 ± 0.5
PLLA oxygen treated	20	300	2	<10
PLLA oxygen treated	20	300	5	<10
PLLA oxygen treated	20	300	10	<10

Data presented are mean ± SD, $n = 5$.

In all the modified PLLA films, a decrease in the contact angle was revealed, as shown in Table II, which means that the hydrophilicity increased greatly when oxygen plasma treatment was applied. When the sample was treated with a 10 W power supply for 5 min, the contact angles decreased from 90° to 50°. Contact angles less than 10° were measured when applying a power supply of 20 W and a treatment time of 10, 5 or 2 min changing the original hydrophobic behavior of PLLA surfaces to hydrophilic. A saturation effect in the hydrophilicity was observed when a 20 W power supply was applied, even if roughness continued to increase (as shown in Fig. 2). In Figure 3(a,b,c), the behavior of the water drop on the samples treated with various power supplies is reported.

Infrared spectroscopy

The FTIR–ATR analysis retrieves up to 100 nm thickness and it is used to evaluate if the polymer chemical stability is affected by the surface treatment. The PLLA polymer film was modified by oxygen plasma with a 20 W power supply for 5 min. Spectra analysis did not suggest treatment driven-chemical changes either in the carbonyl (1756 cm^{-1}) or C—O—C (1080 cm^{-1}) stretching peaks, as can be observed in

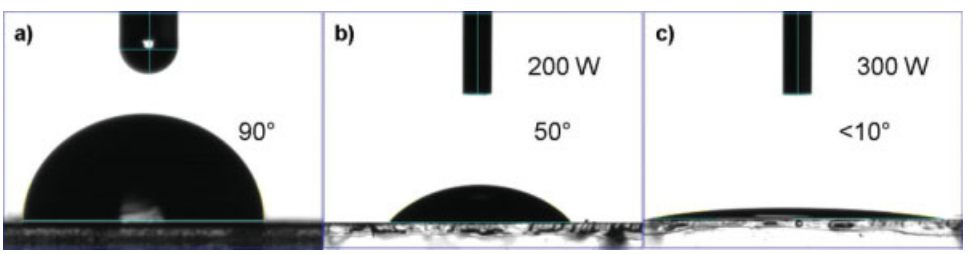

Figure 3 Contact angle images of pure PLLA (a), oxygen treated PLLA, 10 W 5 min (b) and oxygen treated PLLA, 20 W 5 min (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

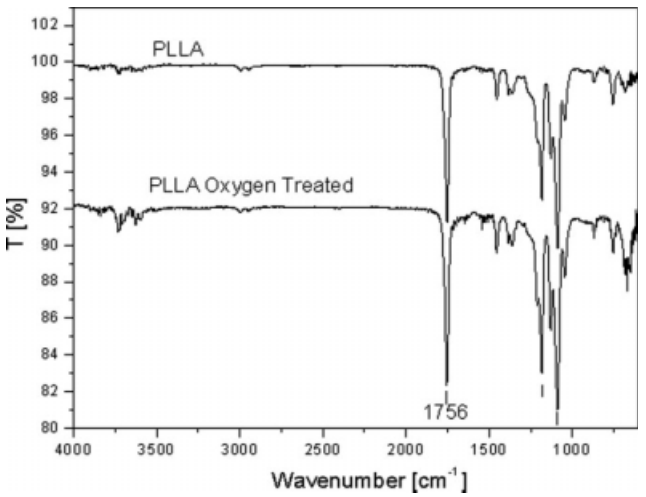

Figure 4 FTIR spectra of the PLLA film before and after oxygen plasma treatment.

Figure 4. This indicates that the rf oxygen-plasma surface modification does not affect the bulk properties of the material, as the obtained spectra maintain all the characteristic peaks of the polymer matrix.[24,33] However in the oxygen-plasma treated PLLA, new band are still visible, with a very low intensity, above 3300 cm^{-1} that is attributed to the -OH stretching. This result is related to the wettability increase, measured in the PLLA film, due to oxygen treatment.

Protein Immobilization

Protein adsorption was evaluated using a fitc-BSA, that was used as a model protein to evaluate the adsorption capacity of plasma-treated PLLA films.

It is well recognized that material biocompatibility is influenced by the structural properties of adsorbed proteins. The rearrangements in the protein structure are an important driving force for adsorption to take place.

For a detailed evaluation of the morphological variation induced by plasma treatment on the PLLA surface, AFM images were performed before protein immobilization. A PLLA film was treated in oxygen after positioning a copper grid on the surface. The grid used in this part of the experiment was a 1000 mesh, since the resulting treated squares are smaller in length and it is possible to observe more detailed AFM height and phase images and hence the homogeneity of the treatment. Figure 5 shows AFM images of the patterned surface after plasma treatment, the cross section line view [Fig. 5(a)], the 2D topography [Fig. 5(b)] and the contrast phase [Fig. 5(c)] of the surface, where squares of 400 nm in depth present the oxygen plasma treated area.

2D protein micropatterning with fitc-BSA immobilized on a PLLA film was analysed by means of fluorescence microscopy. The fluorescence images acquired at various magnifications are shown in Figure 6(a,b). The fitc-BSA molecules were distributed on the oxygen plasma treated regions of the surface, i.e. the squares, and almost no proteins were observed in the no-surface treated sector, i.e. the grooves. In the fluorescence images, the dark regions did not show any specific absorption of fitc-BSA. Results show a high degree of fluorescence on the PECVD-treated area of PLLA, and a dark region on the untreated area, indicating a complete absence of protein adsorption (Fig. 6). This means that BSA was immobilized only on the oxygen plasma modified regions.

From the microscopic studies, the modified PLLA surfaces showed great affinity with the BSA. This effect is related to the introduction of hydroxyl reactive groups that are able to immobilize protein molecules. The final result shows how the surface modification technique used in this study and the oxygen

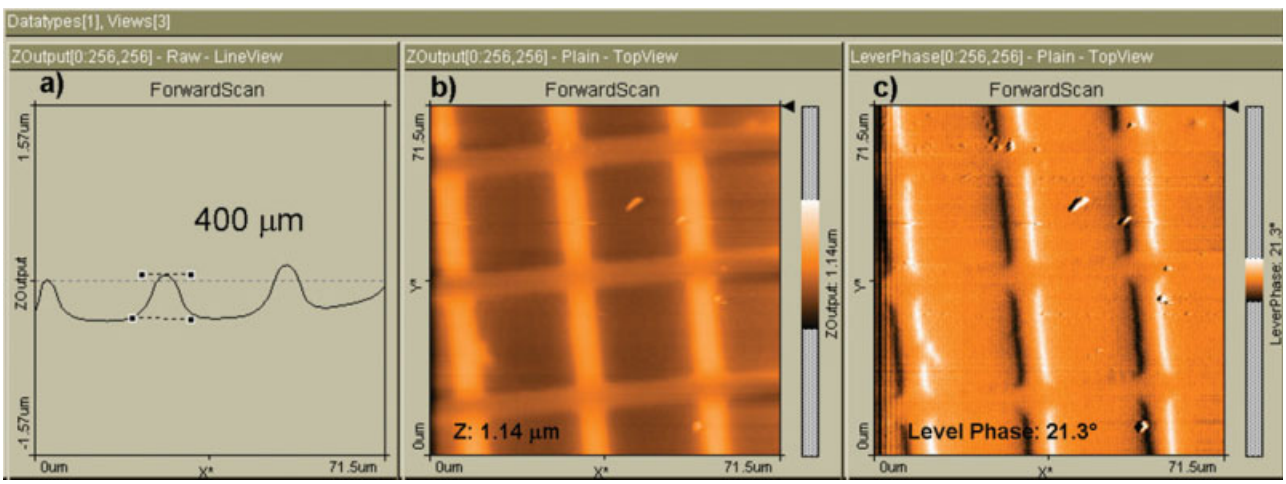

Figure 5 AFM images of the patterned surface after oxygen treatment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

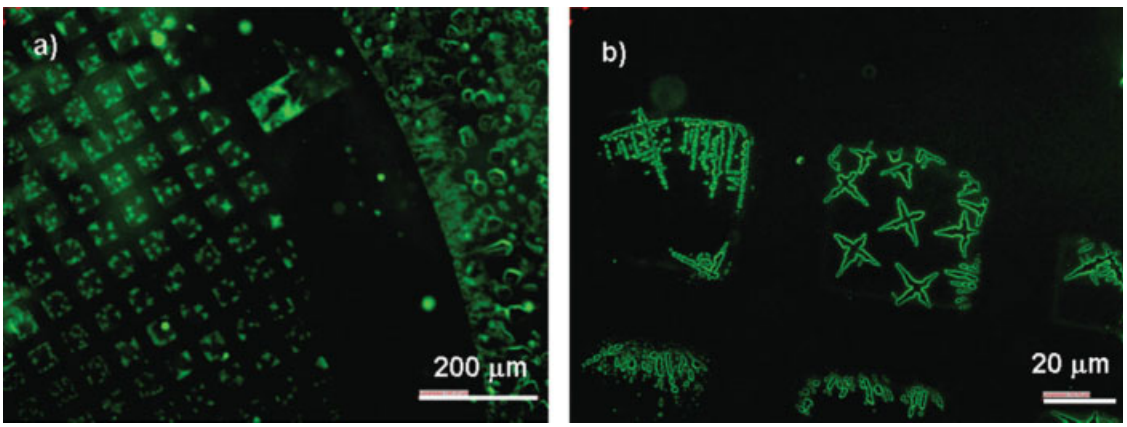

Figure 6 TE2000-Nikon fluorescent microscopy (a) fluorescein protein grid (b) particular view of the square with fitc-BSA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

plasma treatment were successful in changing PLLA-based biomaterial surface properties.

Effects of surface modification on the morphology of PLLA scaffold

According to tissue engineering principles, porous PLLA scaffolds were also fabricated and modified by oxygen plasma treatment methods as above. Porous scaffolds provide more surface area for cell attachment and growth respect to flat film. A qualitative analysis of the morphological changes introduced by plasma on the PLLA scaffold surface was obtained using FESEM.

The oxygen treatment was performed on a porous scaffold prepared with a solvent casting-particulate leaching process of the PLLA matrix, using 80 wt % porogen.

The FESEM observations of porous scaffolds in Figure 7, before (a,c) and after oxygen plasma

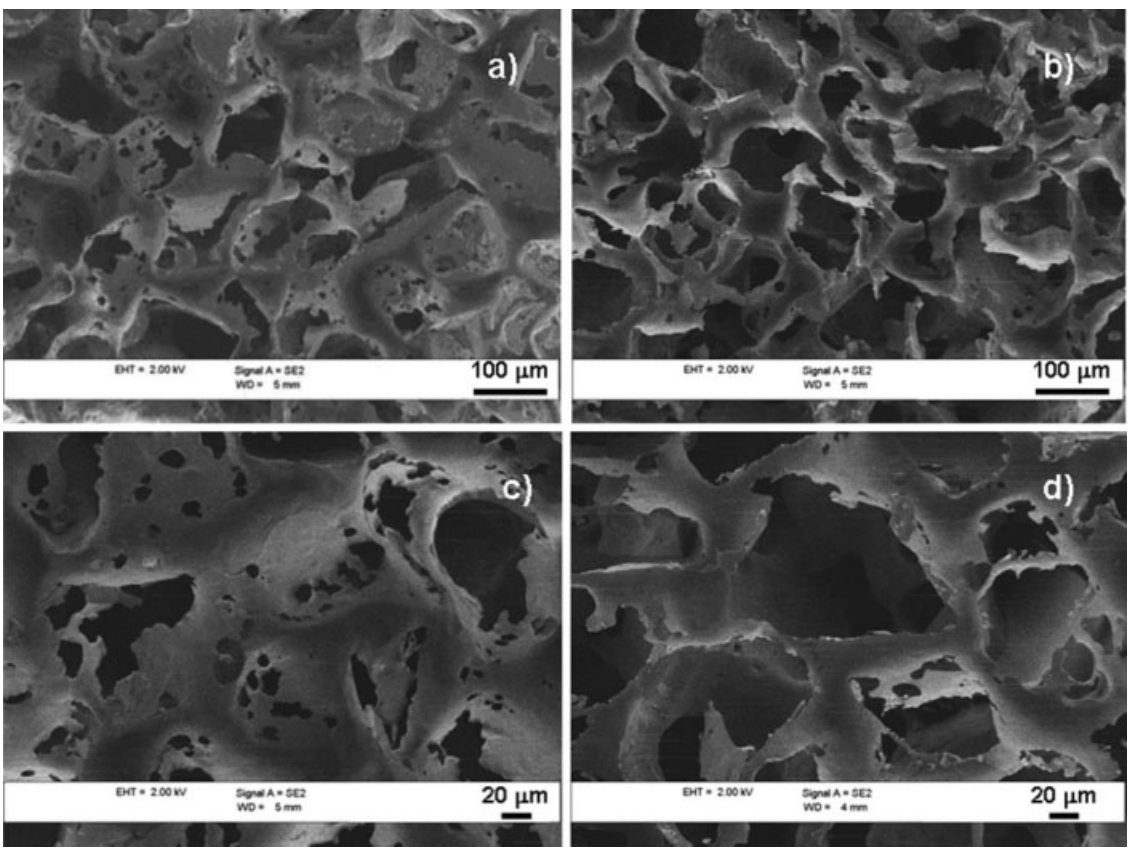

Figure 7 PLLA scaffolds before (a,c) and after oxygen treatment (b,d) at different magnifications.

treatment (b,d) showed that the PLLA sponge was highly porous with an open pore structure. Its pore size and morphology were the same as those of the sodium chloride leached particles.

It can be observed that the integrity of the scaffold is maintained under the selected conditions (20 W, 5 min): the pore walls remained well defined after the treatment, but the changes in morphology are evident. In the case of untreated porous PLLA scaffolds, two different porosities, i.e. macro (100 μm) and micro (20 μm) were marked, while after treatment, the smallest pores are not observed and the interconnection grade of pores increased. In contrast, the untreated PLLA scaffolds [Fig. 7(a,c)] presented a surface smoother than the treated ones. These results indicate that oxygen plasma treatment is able to modify the PLLA microtopography of porous scaffold and suggests that the integrity of structural properties of the scaffolds was preserved.

Effects of surface modification on the cell response of PLLA scaffold

Throughout the testing period, all the surfaces maintained viable cell adhesion at each time point, and the general pattern was that human MSC proliferated on all the surfaces.

The cell density on porous scaffolds during the culture period is shown in Figure 8. For the sake of clarity, the results have been represented in two graphs: by separate underlining of the significant differences it is highlighted how cell growth is affected by the scaffold characteristics.

In Figure 8(a), untreated scaffold and the same scaffold treated with oxygen are compared: the differences are likely to be due to the effect of the surface on cell adhesion. In Figure 8(b), the 70, 80, and 90 wt % salt/polymer ratio generated different porosities which were compared within both the untreated and the oxygen-treated groups of scaffolds: the difference in cell density may be ascribed to their porosity. In both graphs scaffolds cultured for the same time-period were compared.

As shown in Figure 8(a), the cell density on the oxygen treated surfaces was significantly higher than on the corresponding untreated surface with the same porosity at most of the observation time points.

At fourteen days, the greatest density of human MSC was observed on the oxygen-treated 90% porous PLLA scaffold.

When comparing the different porosities, the 90% porous PLLA scaffold had levels of cell density higher than those observed in the 70 and 80% porous scaffolds at each time point, with the exception of untreated 80% porous PLLA scaffolds at 14 days.

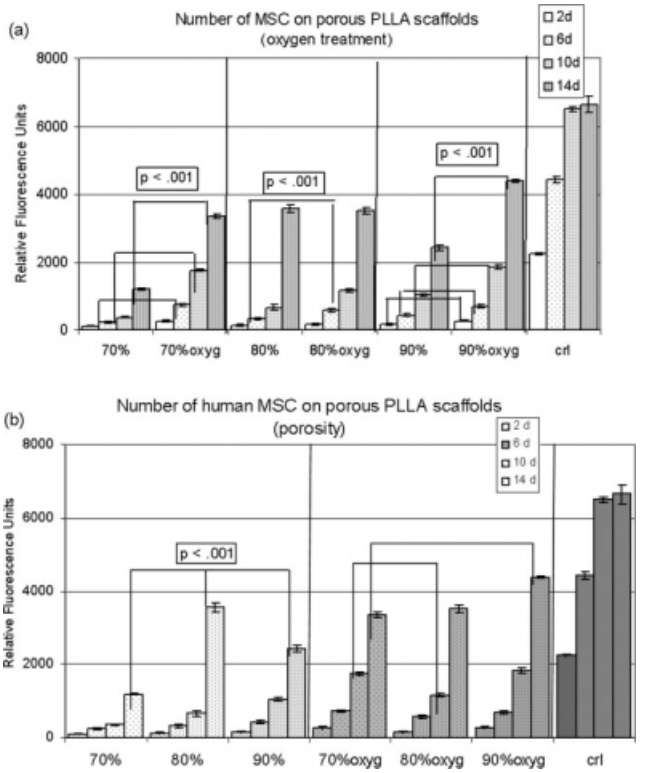

Figure 8 (a) Density of human MSCs on PLLA scaffolds with various porosities and surface treatments: the surface treatment with oxygen affects cells cultured for the same period. (b) Density of human MSCs on PLLA scaffolds with various porosities and surface treatments: porosity affects the cells cultured for the same period.

In agreement with the biochemical results, fluorescent cells were observed on all the porous surfaces. Examples of images taken on day 10 are provided in Figure 9(a–f). In a qualitative evaluation, the 90 wt % porous surfaces apparently support the highest number of human MSC, and the cell density is further increased upon oxygen treatment.

CONCLUSIONS

PLLA films and porous scaffolds were successfully constructed by solvent casting and solvent casting-particulate leaching processes, respectively. The possibility, to produce films and scaffolds, with the same thermal properties, permitted to correlate the material properties changes as a consequence only of the surface treatments.

Oxygen rf-PECVD was effective in changing the surface properties of polymeric films and porous scaffolds. The plasma treatment was found to be successful in achieving three-dimensional functionalization without any adverse effect on the chemical composition and structure of the scaffolds, thus preserving their properties for tissue engineering applications.

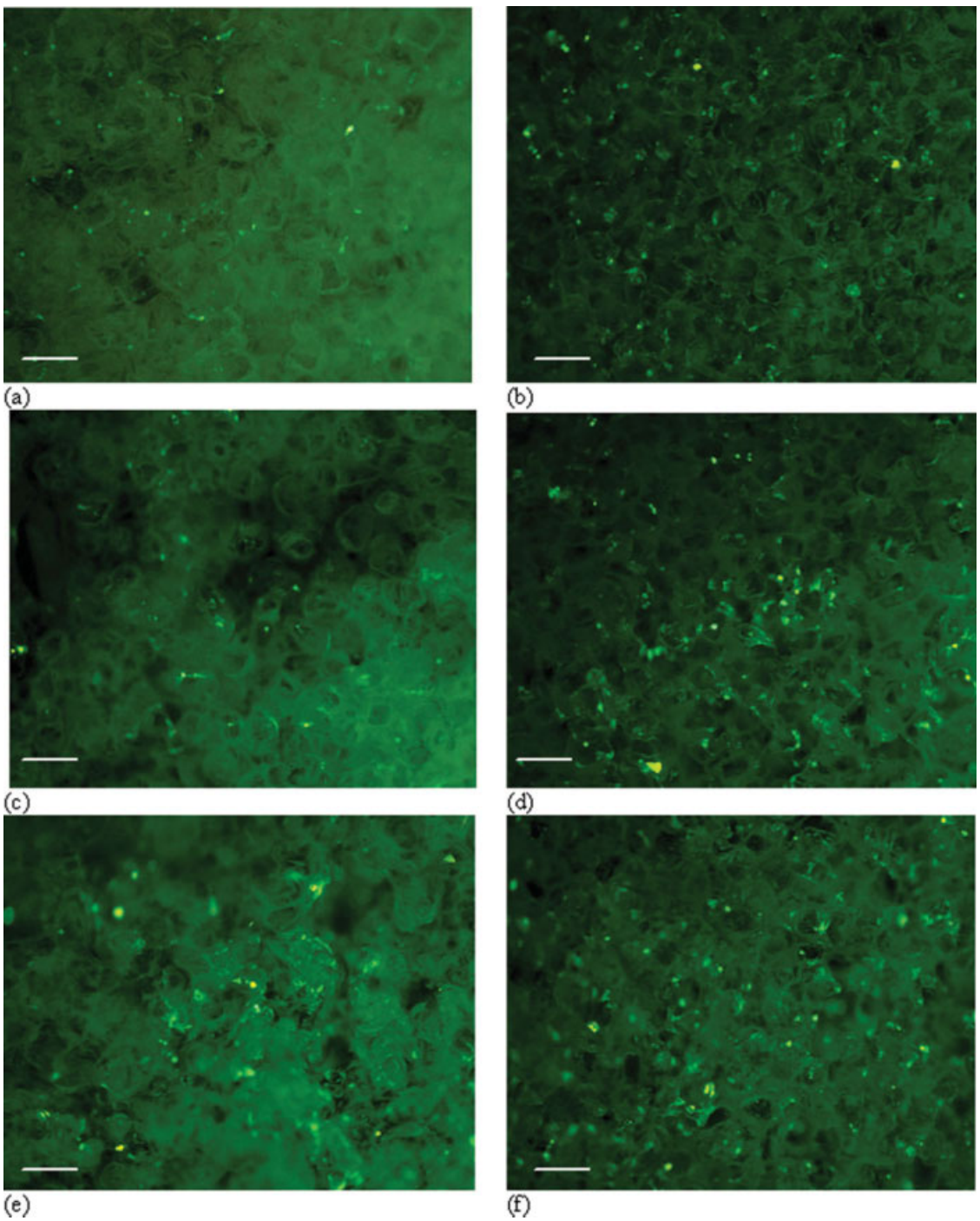

Figure 9 Fluorescent images of human MSCs cultured for 8 days on 70% (a), 80% (b), 90% (c), 70% oxygen-treated (d), 80% oxygen-treated (e), and 90% oxygen treated PLLA scaffolds (CM-dil, bar = 100 μm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The effects of the oxygen plasma treatments on the surface of the material have been shown to change wettability, roughness and to enable the interaction between the PLLA polymer surface and the protein. The present work has provided an important contribute to the understanding of the adsorption of BSA on oxygen-modified PLLA surfaces.

The cell assays of this study show that the porous PLLA scaffolds, prepared by means of a solvent casting particulate leaching technique, provide a suitable substrate for the adhesion and proliferation of MSCs. Moreover, changes of the surface properties induced by the oxygen treatment, including charge, wettability, topography, and morphology, further improved cell attachment.

In fact MSCs are profoundly affected by surface topography and surface chemistry, as signals from the substrate to the nucleus are transmitted through the cytoskeleton.

It is suggested that this approach can be used with various types of proteins and specific growth factors to modulate subsequent cell functions, such as proliferation, differentiation and migration on biomaterial surfaces.

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