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Plasma-induced polymerization as a tool for surface functionalization of polymer scaffolds for bone tissue engineering: An in vitro study

Paula M. López-Pérez, Ricardo M.P. da Silva, Rui A. Sousa, Iva Pashkuleva *, Rui L. Reis

3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
IBB – Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

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ABSTRACT

A commonly applied strategy in the field of tissue engineering (TE) is the use of temporary three-dimensional scaffolds for supporting and guiding tissue formation in various in vitro strategies and in vivo regeneration approaches. The interactions of these scaffolds with highly sensitive bioentities such as living cells and tissues primarily occur through the material surface. Hence, surface chemistry and topological features have principal roles in coordinating biological events at the molecular, cellular and tissue levels on timescales ranging from seconds to weeks. However, tailoring the surface properties of scaffolds with a complex shape and architecture remains a challenge in materials science. Commonly applied wet chemical treatments often involve the use of toxic solvents whose oddments in the construct could be fatal in the subsequent application. Aiming to shorten the culture time in vitro (i.e. prior the implantation of the construct), in this work we propose a modification of previously described bone TE scaffolds made from a blend of starch with polycaprolactone (SPCL). The modification method involves surface grafting of sulfonic or phosphonic groups via plasma-induced polymerization of vinyl sulfonic and vinyl phosphonic acid, respectively. We demonstrate herein that the presence of these anionic functional groups can modulate cell adhesion mediated through the adsorbed proteins (from the culture medium). Under the conditions studied, both vitronectin adsorption and osteoblast proliferation and viability increased in the order SPCL ≪ sulfonic-grafted SPCL < phosphonic-grafted SPCL. The results revealed that plasma-induced polymerization is an excellent alternative route, when compared to the commonly used wet chemical treatments, for the surface functionalization of biodevices with complex shape and porosity.

1. Introduction

Tissue engineering (TE) emerged as an interdisciplinary field confronting the transplantation crisis caused by the shortage of donor tissues and organs. Since its inception, scaffolds composed of synthetic and natural polymers have been key elements of different TE approaches [1]. The use of an appropriate template to provide physical support and a local environment for cells and hence to enable and facilitate tissue development is an essential issue for a successful regeneration strategy. Nowadays, it is well accepted [2–4] that the ideal scaffold for bone TE must possess adequate porosity, resulting in an interconnected and permeable structure that allows the ingress of nutrients and cells. It is also believed that proper mechanical and physical properties, controlled biodegradability, biocompatibility and the ability to promote cellular interactions and tissue development are other main requirements for TE scaffolds [2,5–8]. Last but not least, cells and surrounding tissues interact with any external devise primarily through the surface and therefore properties such as surface chemistry and topography are also key determinants in material–bioentity interactions.

Starch-based polymers have been studied as valuable materials for several biomedical applications [4,9]. Their biocompatibility and non-cytotoxicity have been confirmed by both in vitro [4,10–12] and in vivo [13] assays. In this work, we have chosen fiber mesh scaffolds made from a blend of starch and ε-polycaprolactone (SPCL) which have been already proposed for bone tissue engineering [4,8,10,11]. Previous works have targeted their optimization in terms of degradability [7–9], porosity [4] and mechanical properties [9,14], but few studies have focused on their surface properties [15] and the possibility of improving them [12,16]. Herein, we propose plasma-induced polymerization as a way to render an appropriate surface for enhancing cell adhesion and speeding up cell proliferation, which will shorten the culture time

* Corresponding author at: 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal. Tel.: +351 253 510907.
E-mail address: pashkuleva@dep.uminho.pt (I. Pashkuleva).

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in vitro, i.e. prior to implantation. We have previously demonstrated [17,18] that this method is a very effective way for grafting of vinyl polymers on regular two-dimensional (2-D) structures without modifying the bulk properties of the material. In this study, we report the effectiveness of this method for the functionalization of 3-D structures with a complex shape and with the negatively charged sulfonic and phosphonic groups, and the influence of these groups on osteoblast cell behavior in vitro.

Anionic scaffolds have been investigated because of their ability to facilitate morphogenetic processes for tissue engineering substitutes [19,20]. For example, the negative charge of glucosaminoglycans (GAGs) is associated with their bioactivity. GAGs interact with the positively charged amino groups of extracellular proteins and these interactions determine cell–matrix adhesion. Recent studies with sulfate-derived materials indicate enhanced adhesion and proliferation of osteoblast-like cells as a result of the presence of the sulfate groups [17,21]. On the other hand, the introduction of phosphate groups has been also proposed as an attractive modification strategy in targeting bone tissue engineering applications [18,22–24]. The rationale for the use of this functionality stems from mimicry of bone-promoting proteins and the mineral–bone matrix. Phosphate-rich proteins are known to initiate nucleation of mineralized bone and tooth matrix. It is also reported that many bone-promoting proteins naturally interact with acidic polymers [25].

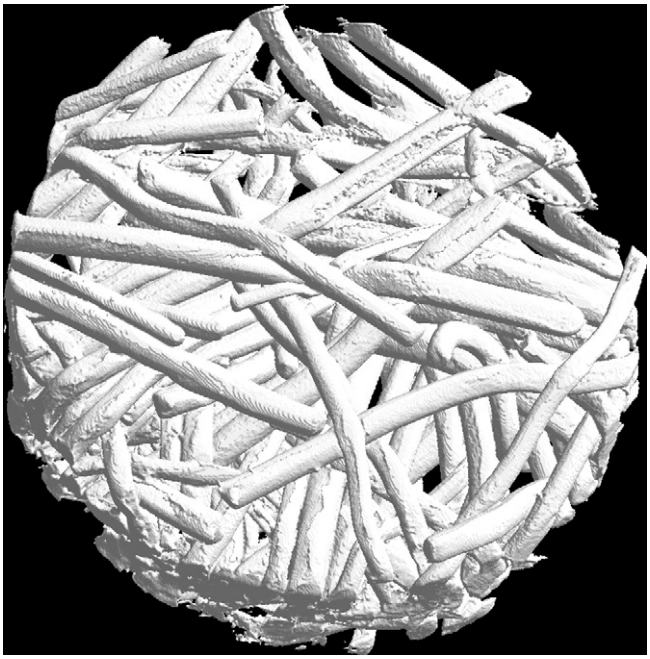

Fig. 1. Micro-computed tomography image from SPCL fiber mesh scaffold.

2. Materials and methods

2.1. Materials

In this work, we used a commercially available blend (Mater-Bi ZI01U, Novamont, Italy) of thermoplastic starch and poly(ε-polycaprolactone) (SPCL, 30/70 wt.%) [26,27]. The material was supplied in a granular form and processed by melt spun into fibers. Vinyl phosphonic acid (VPA) and vinyl sulfonic acid (VSA) were purchased from Sigma–Aldrich and used without further purification.

2.2. SPCL mesh production and modification

Fibers of SPCL were produced by melt spinning using a modular co-rotating twin screw extruder (Leistritz AG-LSM 36/25D, Germany) at a screw speed of 3 rpm and with a temperature profile in the barrel (from the feed zone to the die zone) of between 60 and 130 °C. The average output rate was 0.3 kg h^{-1}. Upon extrusion through the die, the filament was spun in two consecutive steps to a final draw ratio of approximately 1:100. The cooling of the filament was performed in air (average temperature of 17 °C). Melt-spun fibers presented a diameter in the 105–345 μm range, with a mean fiber diameter of 213 ± 50 μm. The fibers were cut into 0.5 cm lengths and used in the production of fiber mesh scaffolds by a custom-designed mould. Fiber bundles were randomly displaced into the mould cavities and subjected to thermal treatment at 60 °C for 30 min before predefined compression levels along the *Z*-axis were applied to ensure the bonding between neighboring fibers using a final compression ratio of 22%. Upon demoulding, scaffolds with dimensions 2.2 ± 0.2 mm thickness and 6 mm diameter were obtained. Their porosity was measured by micro-computed tomography and the obtained averaged value was 64.4 ± 4.4% (Fig. 1).

SPCL meshes were further modified by plasma-induced polymerization. Scaffolds were placed in a radio frequency (13.56 MHz) plasma reactor (Plasma Prep5, Gala Instrument, Germany) and exposed to O_2 plasma at 30 W of power for 15 min. During the treatment the pressure inside the reactor was maintained below 20 Pa by adjusting the gas flow. The activated meshes with free radicals formed on the surface were subsequently immersed in a degassed solution of VPA (100 mM in 2-propanol) or VSA (10 vol.% aqueous solution) at a ratio of 2 ml per scaffold. The reaction was carried out at room temperature for 2 h under stirring. The scaffolds were washed thoroughly with the solvent used for the reaction in order to remove any unreacted monomer and finally the modified samples were dried at room temperature.

2.3. Surface chemical composition

Surface elemental analysis of untreated and modified samples was performed by X-ray photoelectron spectroscopy (XPS). The spectra were obtained using an ESCALAB 200A instrument from VG Scientific (UK) with PISCES software for data acquisition and analysis. The spectrophotometer was calibrated with reference to Ag 3d5/2 (368.27 eV). Monochromatic Al Kα radiation ($h\nu$ = 1486.60 eV) operating at 15 kV (300 W) was used and the measurements were performed at a take-off angle of 90° relative to the sample's surface in constant analyzer energy mode (CAE). Survey spectra were acquire using a pass energy of 50 eV over a binding energy range of 0–1100 eV, and were used to calculate the elemental composition of the surfaces. High-resolution spectra for different regions were obtained using a pass energy of 20 eV. The peaks were fitted using the least-squares peak analysis software XPSPEAK version 4.1 and the Gaussian/Lorenzian sum function. Background counts were subtracted using a linear baseline and the sample charging was corrected assigning a binding energy of 285.0 eV to the saturated hydrocarbons C1*s* peak.

2.4. Surface topography

The topography of the samples was characterized by optical profiler analysis using a Wyko-NT 1100 interferometric profiler (Veeco) operating in vertical scanning interferometry mode. The images were processed and analyzed with the analytical software package WycoVision®32.

2.5. Protein adsorption

The effect of the surface treatments on protein adsorption was analyzed by fluorescent immunolabeling. Two adhesion proteins

were studied: fibronectin (Fn), because it is commonly used in a standard procedures applied to improve adhesion of cells; and vitronectin (Vn), because of its influence on cell spreading and migration. Unmodified and grafted SPCL scaffolds were incubated for 1 h under the same conditions as used for in vitro cell culture, i.e. in a complex protein solution composed of 10 vol.% heat-inactivated fetal bovine serum (FBS; Biochrom AG, Germany) in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, Inc., USA). Samples immersed in phosphate-buffered saline solution (PBS; Sigma–Aldrich, Inc., USA) were used as blanks. After incubation, the samples were washed with PBS and incubated at room temperature for a further for 1 h with primary antibody mouse anti-cow Vn (Santa Cruz, USA) or mouse anti-cow Fn (Santa Cruz, USA). Both primary antibodies were diluted at a ratio of 1:50 (v/v) in 1% (w/v) bovine serum albumin (Sigma–Aldrich, USA) solution in PBS. All samples were again washed and incubated for 1 h at room temperature, this time with goat anti-mouse Alexa Fluor 488 IgG (H + L) secondary antibody (Invitrogen, USA). Labeled samples were analyzed by an Olympus IX81 confocal laser scanning microscope (CLSM).

2.6. Cell culture conditions and seeding

A human osteosarcoma cell line (SaOs-2), an immortalized cell line with an osteoblastic phenotype, was obtained from the European Collection of Cell Cultures (ECACC, UK) and was used in the cell culture studies. The cells were cultured in DMEM supplemented with 10,000 U ml^{-1} penicillin-G sodium, 10,000 μg ml^{-1} streptomycin sulfate and 25 μg ml^{-1} amphotericin B in 0.85% saline (Gibco, Invitrogen Corporation, UK) and 10% FBS in a humidified atmosphere with 5% CO_2. A suspension of 2×10^5 cells was added to each scaffold. The scaffolds were incubated for 3, 7 and 14 days under standard culture conditions (37 °C, 5% CO_2, humidified atmosphere).

The morphology of SaOs-2 cells was observed by scanning electron microscopy (S360, Leica Cambridge, UK). Cells were fixed using 2.5 vol.% glutaraldehyde (Sigma, USA) solution in PBS. Prior to the analysis, the samples were dehydrated by graded ethanol solutions.

Cell viability was analyzed by MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The cultured materials were incubated (3 h, 37 °C, humidified atmosphere of 5% CO_2) with 500 μl of MTS solution in DMEM culture medium without phenol red (Sigma–Aldrich, Inc., USA). Optical density was read with a microplate reader (Bio-Tek, USA) at 490 nm.

DNA quantification was used to evaluate cell proliferation. Cells were lysed by osmotic and thermal shock and the supernatant obtained was used for DNA analysis. The DNA content along the time of culture was determined using a PicoGreen dsDNA kit (MolecularProbes) and the fluorescence was read (485 nm/528 nm of excitation/emission) using a microplate reader. The amounts of DNA were calculated from a standard curve.

Triplicates were analyzed at each time point for both assays (MTS and DNA). Statistical analysis was performed and the data are reported as mean ± standard deviation. An analysis of variance (ANOVA) test for independent samples was performed and the differences were considered statistically significant if $p < 0.05$.

3. Results and discussion

The surface design of biomedical devices applied in direct contact with the body is crucial for their acceptance by or rejection from the surrounding tissues. The modification of the surface chemistry and/or topography is a way to improve the biological performance of a biomaterial without changing its bulk properties. One of the most versatile and effective tools to tailor surface chemistry and properties of solids is polymer grafting. In contrast to physical coating, grafting has several advantages, such as the covalent attachment of graft chains onto a polymer surface which avoids their delamination and assures the long-term stability of the introduced functionalities. Several approaches have been proposed for surface grafting on 2-D structures, but not all of them are viable for complex 3-D architectures. As an example, plasma surface treatment can be applied for 3-D samples whose holes/trenches are wider than the mean free path of the electrons and the Debye length because in this case the discharge which generated the active species can be sustained [28]. However, in a number of applications the dimensions of the structures can be smaller. Poll et al. [29] reported that oxygen plasma treatment of stacked textile sheets (with the distance between fibers in the range of 0.1–1 mm) for times longer than 8 min can penetrate four fabric sheets. A similar experiment was carried out by Mukhopadhyay et al. [30] for paper sheets but with flourohydrocarbon plasma. They found that up to three sheets (each with thickness of 0.15 mm) could be fluorinated by this treatment. Hollander [28] investigated the penetration of oxygen plasma in cylinders made of sintered polyethylene. He reported that samples with large pores (80 μm) are readily penetrated (5 mm in 60 s) but that there is a limit for samples with smaller pores; objects with a pore size of 7 μm can be penetrated to about 1.2 mm. Based on these previous findings, in this study we chose highly porous and interconnected SPCL fiber mesh scaffolds (Fig. 1) with a thickness of about 2.2 mm (1.1 mm from the surface to the middle of the sample) to check out the applicability of plasma-induced polymerization for the grafting of phosphonic and sulfonic groups.

3.1. Surface chemistry

The success of the applied treatment was primarily checked by surface chemical analysis. A comparison of several surface characterization techniques for the analysis of similarly treated surfaces has demonstrated [17,31] that Fourier-transformed infrared spectroscopy with attenuated total reflectance is not sensitive enough to capture the introduced changes; the penetration of the infrared beam is about 1–5 μm. On the other hand, with XPS analysis, valuable information about the elemental composition and chemistry can be obtained at the surface level of the sample within a depth of 10–250 Å. Hence, XPS survey spectra were used to assess the surface elemental composition (at.%) of untreated and modified SPCL scaffolds. Two main elements, C and O, were present on the surface of untreated samples. The ratio between them was found to be similar to the theoretically calculated one for PCL (Table 1). This result indicates that the synthetic component is predominant on the surface of the sample, which is in agreement with previously reported results for SPCL materials [12,15]. Plasma-induced polymerization resulted in a higher oxygen content. Additionally, new peaks appear in the spectrum of the VPA-grafted sample corresponding to P2*p* (128.5 eV) and P2*s* (185.5 eV), confirming the introduction of phosphonic groups. We calculated the presence of phosphorous on the surface from the P2*p* transition and a concentration of 4.6 at.% was found (Table 1). The presence of a sulfur peak (0.2 at.%) in the XPS spectrum of the sample treated with VSA confirms the success of the grafting process with this monomer, albeit with a lower efficiency than the VPA grafting. Some impurities (Si, N, Na, Sn and Cu) appear in the surface spectra of some samples, usually at very low concentration, and were excluded from the calculations of the elemental analysis (at.%) shown in Table 1.

C1*s*, O1*s*, P2*p* and S2*p* core level spectra of untreated and grafted materials were analyzed in order to obtain additional details about the surface chemical composition. Fig. 2a shows the binding en-

Table 1
Elemental composition of untreated and modified SPCL fiber meshes determined by XPS.

Material	C1*s* (at.%)	O1*s* (at.%)	P2*p* (at.%)	S2*p* (at.%)	C:O ratio
Starch (theoretical)	50.0	50.0	–	–	1.0
PCL (theoretical)	75.0	25.0	–	–	3.0
SPCL (theoretical)	67.4	32.6	–	–	2.1
SPCL meshes	75.1	24.9	–	–	3.0
VSA-grafted SPCL	67.8	32.0	–	0.2	2.1
VPA-grafted SPCL	51.1	44.3	4.6	–	1.1

ergy region corresponding to the C1*s* peak (279.8–291.9 eV) for the SPCL sample. The peak-fitting was performed taking into consideration the chemical structures of both PCL and starch (Fig. 3). The peak at 285.0 eV was assigned to the C–H/C–C chemical bonds of the starch backbone and the C–C chain from PCL. The signal centered at 286.3 eV corresponds to the hydroxyl-bonded carbons (C–OH) from starch and the ester-bonded carbons (C–O) from PCL. The peak observed at 288.9 eV was assigned to the O–C–O bonds from starch and the C=O bond from the synthetic component [32]. The C1*s* spectrum of VPA-grafted samples (Fig. 2b) did

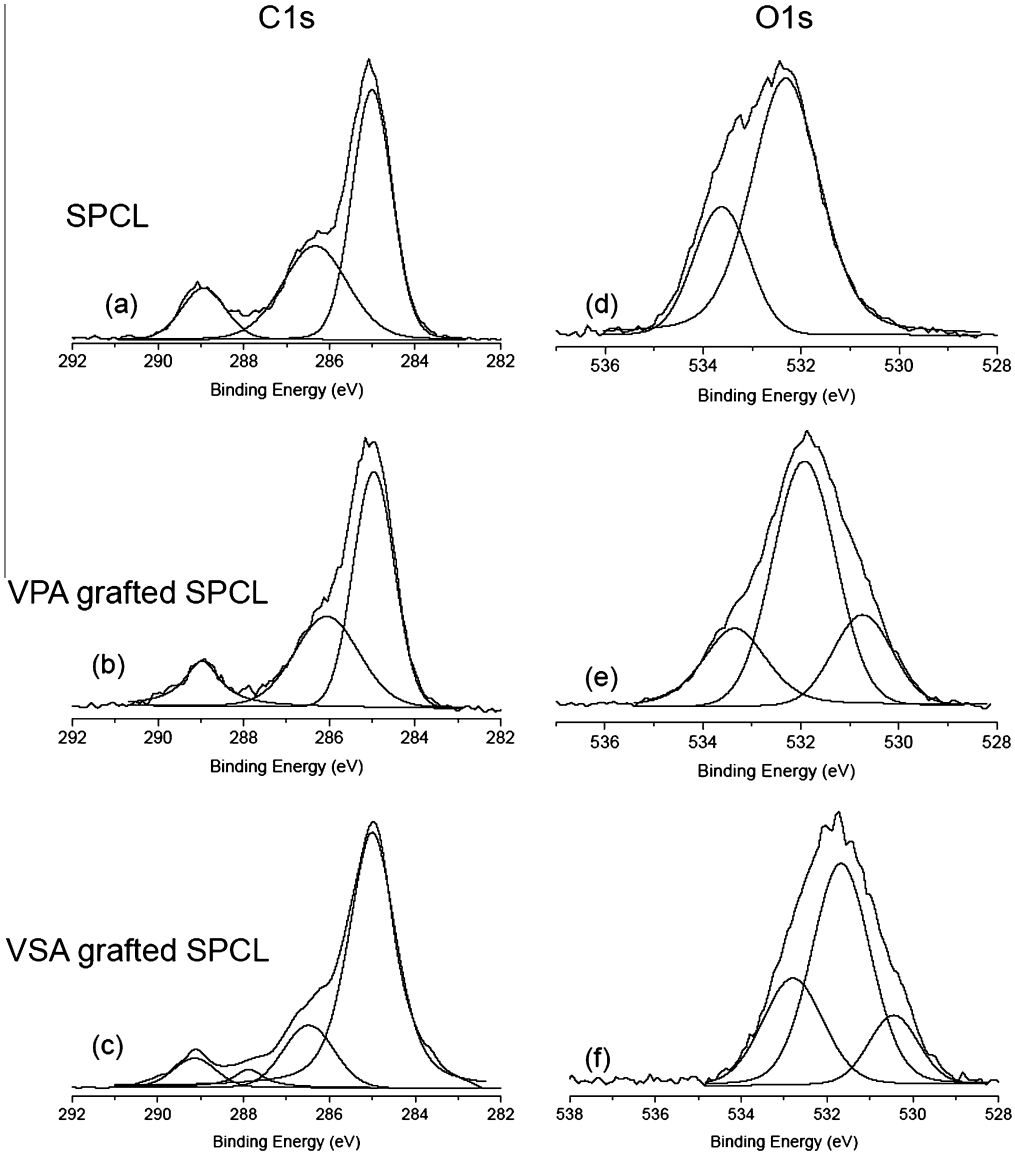

Fig. 2. XPS core level spectra of untreated and grafted samples in the C1*s* (left) and O1*s* (right) regions.

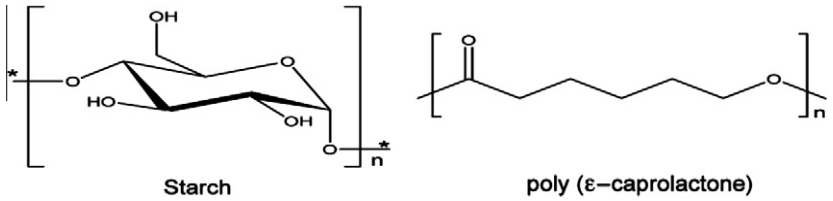

Fig. 3. Chemical structures of the components of the blend.

not reveal significant changes when compared with untreated SPCL scaffolds. The expected binding energy of C–P is around 286–286.4 eV [33,34] and therefore the C1*s* peak corresponding to C–PO_3 is probably overlapped by the C–OH/C–O peak. For VSA-grafted samples (Fig. 2c), a new peak was detected at 287.9 eV and was assigned to C–S bonds from sulfonic acid. To our knowledge only a few works have focused on the chemical shift of C–S and there is not agreement in the reported values [35–37]. Two

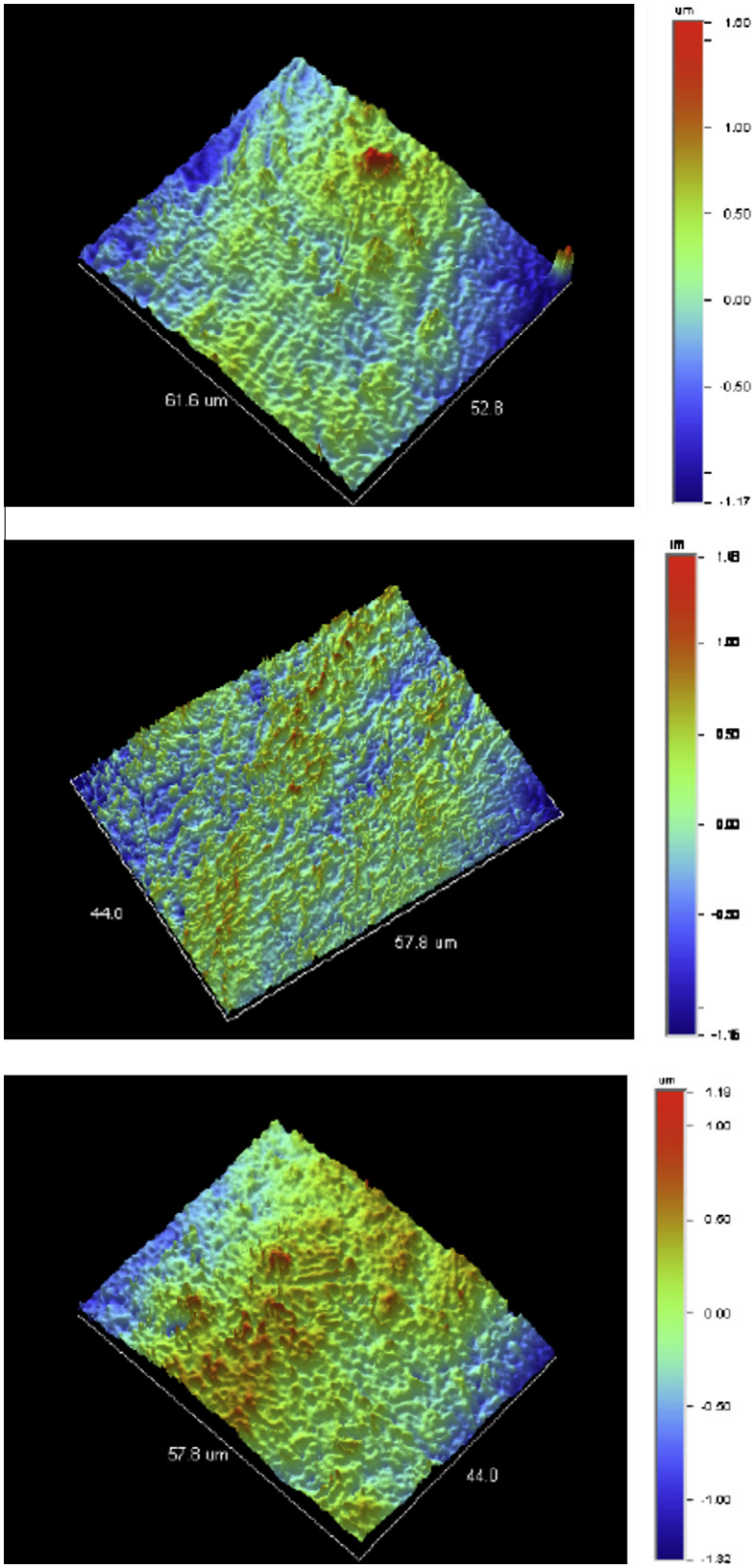

Fig. 4. Optical profile images. From top to bottom: untreated SPCL, VSA-grafted and VPA-grafted scaffolds.

Table 2
Values for the roughness, calculated from the optical profiler images (107× magnification).

	SPCL	VSA-grafted SPCL	VPA-grafted SPCL
R_a (nm)	219.0 ± 22.7	245.1 ± 16.0	255.1 ± 14.0
R_q (nm)	283.7 ± 34.6	308.3 ± 11.7	335.6 ± 20.5

peaks were identified in the high-resolution O1*s* spectrum of SPCL scaffolds (Fig. 2d). The peak at 532.3 eV was assigned to C═O bonds from PCL [33,37] and the peak at 533.6 eV to C–OH/O–C–O from starch and C–O–C from both synthetic and natural components of the blend [37]. After VPA grafting (Fig. 2e), additional peak at 531.0 eV appears in the O1*s* spectrum which was attributed to the P═O group. The second peak at 532.0 eV was assigned to the C═O and the last peak at 533.4 eV corresponds to the C–O/C–OH/C–O–C and P–OH groups, for which binding energies between 533.0 and 533.6 eV have been reported [33,34,38]. The O1*s* core level spectra of the VSA-grafted samples (Fig. 2f) showed a new additional peak compared to the untreated SPCL scaffolds. This peak is at 531.1 eV and was assigned to O═S bonds [35,36]. The P2*p* signal that appears in the spectrum of VPA-grafted fiber meshes is a non-resolved doublet with 2*p*1/2 and 2*p*3/2 core levels. The peak is at 133.6 eV and presents a full width at half-maximum of 2.1 eV [33,38] (spectrum not shown).

3.2. Surface topography

When a polymer surface is treated by plasma, together with surface functionalization, surface degradation or etching may also occur [39]. Hence, chemical surface modification is usually associated with changes in the surface topography/morphology. Consequently, the protein and cell interactions with the material may also be modified. An optical profiler analysis was performed to evaluate the eventual topographical changes on the surface of the fibers as a result of the applied treatment. As can be seen in Fig. 4, no significant changes were detected on the fiber surfaces after the performed modification. Although the roughness calculated from 107× magnification images seems to increase in absolute value after the plasma treatment (Table 2), it should be noted that this tendency is not fully clear because of the difference in the roughness between fibers in the same scaffold.

3.3. In vitro biological evaluation: protein adsorption and cell response

When a biomaterial is brought into contact with a physiological milieu, it is very unlikely that cells will make direct contact with its surface. The very first event either in vivo or in vitro is the adsorption of protein from blood or serum on the material's surface [40–42]. The surface properties set the characteristics of the adsorbed protein layer, and the nature of the established protein–surface interactions will modulate cell adhesion and consequently cell biochemical mechanisms via interactions with cell-surface molecules, such as integrins [43,44]. Fig. 5 demonstrates that Vn adsorbed on all (untreated and modified) SPCL scaffolds, whereas Fn was not detected on any of the materials studied. This result is not surprising as it agrees with previously reported data for SPCL materials showing predominant adsorption of Vn vs Fn from complex protein solutions [12,41]. In fact, Vn is present at a higher concentration than Fn in FBS [45]. FBS for cell culture is prepared by clotting at 4 °C, which can lead to a considerable depletion of Fn but not of Vn [45]. Another process which is associated with the lower protein adsorption of Fn vs Vn is the so-called Vroman effect [46], which involves inhibition of Fn adsorption from serum by other proteins that have the ability of displacing it from the surface. This effect has been observed for Fn when serum with a concentration above 3% is used [47]. Because we were working with 10% FBS, the lack of Fn adsorption was thus an expectable result. On the other hand, under these conditions, Vn can be adsorbed from the medium (Fig. 5), and can therefore participate in the mediation of sub-

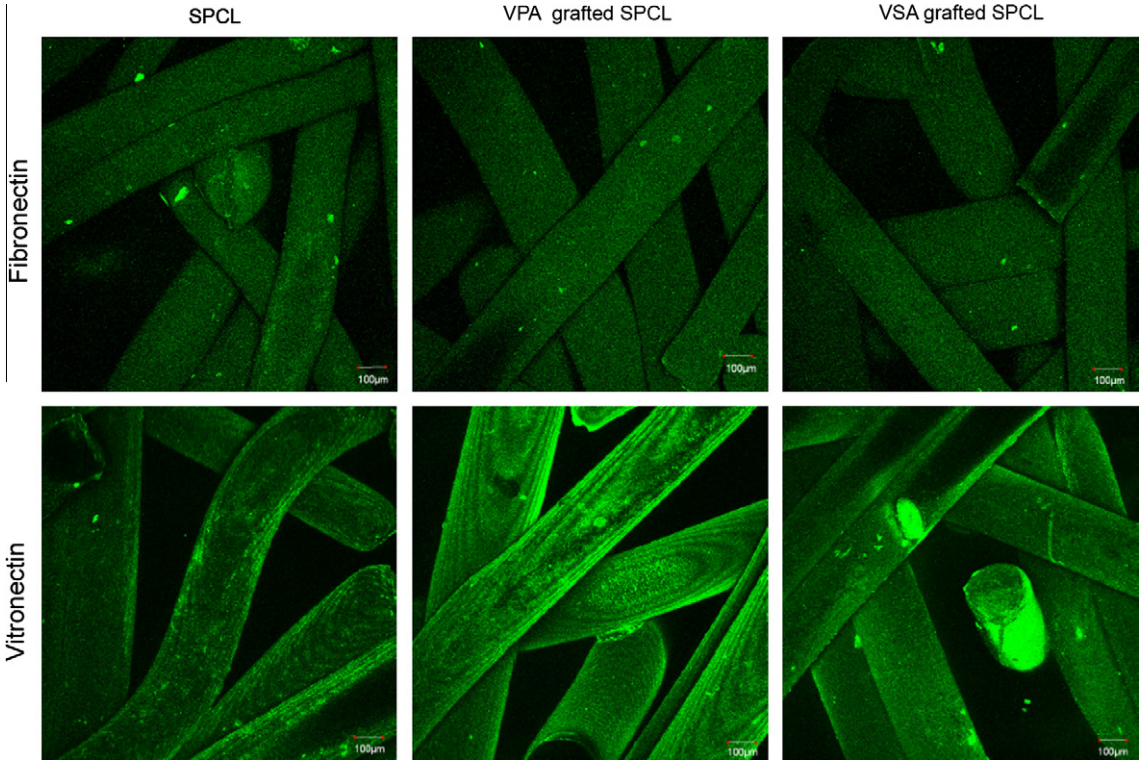

Fig. 5. Confocal laser scanning micrographs of non-modified and grafted scaffolds stained for Fn and Vn.

sequent events, such as cell adhesion, spreading and growth [48]. The Vn adsorption profiles on untreated and modified materials showed that the fluorescence intensity for SPCL scaffolds increases after the modification with VSA and even more so after VPA grafting. These results indicate that the surface chemical composition significantly influences both the content of the adsorbed protein layer and the interactions between the material surface and the adsorbed proteins [40]. Vn plays an essential role in the attachment and spatial distribution of bone-derived cells [49,50]. Hence, it is expected that the observed difference in Vn adsorption on untreated and modified scaffolds will change the behavior of those cells. Therefore, we tested osteoblast-like cells cultured in direct contact with the studied materials. Osteoblast cells in vitro have been shown to depend primarily on the adsorbed Vn or Fn for initial adhesion and spreading on materials [51]. Therefore, the ability of materials to support cell adhesion and spreading is determined mainly by their ability to adsorb these proteins from serum in an active state.

Fig. 6 shows scanning electron micrographs of SaOs-2 seeded on the surfaces of modified and untreated samples after 3, 7 and 14 days of culture. After 3 days of culture cells were able to attach and spread on all the surfaces, showing the typical morphology of osteoblast cells with a polygonal shape. However, some differences were observed between the untreated SPCL and the grafted samples. While on modified scaffolds the cells were able to extend and bridge between fibers, on untreated samples these bridges were not observed. Prolongation of the culture periods to 1 and 2 weeks resulted in higher cell density, indicating the ability of SaOs-2 to proliferate on all of the materials. At 14 days of culture, SaOs-2 formed a complete monolayer covering all of the fibers, as well as some of the contact junctions between fibers. Not only can surface properties induce morphological changes, they can also influence the metabolic activity of the cells. MTS was used to evaluate the effect of the grafted functional groups on the cell viability

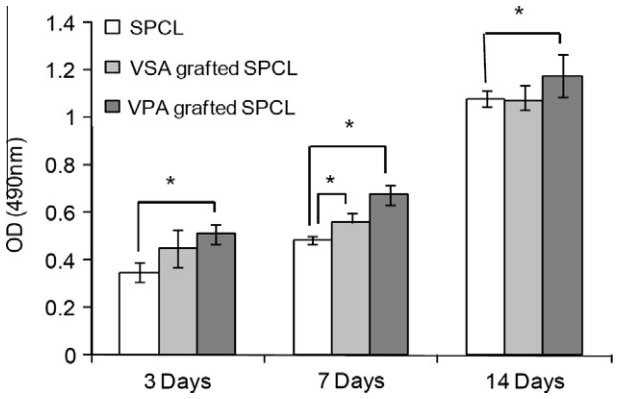

Fig. 7. Viability of SaOs-2, evaluated by MTS assay, cultured on untreated and grafted SPCL fiber meshes. *Significantly different (ANOVA, $p < 0.05$).

at different time points. The MTS test is an indirect test that determines cell mitochondrial activity, which in turn can be related to the number of viable cells. For all the materials studied, an increasing number of viable cells were detected with prolonged culture time. These results show the ability of both unmodified and treated scaffolds to support cell proliferation. However, Fig. 7 demonstrates that the VPA grafting significantly increases the number of viable cells when compared with untreated SPCL scaffolds, and this tendency remained for the whole period studied. The presence of phosphate functional groups also increases the cell viability compared with sulfonic groups grafted under the same conditions. DNA quantification was used to obtain more accurate quantitative results and to determine the cell proliferation profiles (Fig. 8). DNA quantification confirmed the positive effect of the VSA and VPA grafting on the proliferation of SaOs-2. Once again, the same order was kept: SPCL < VSA-grafted SPCL < VPA-grafted SPCL.

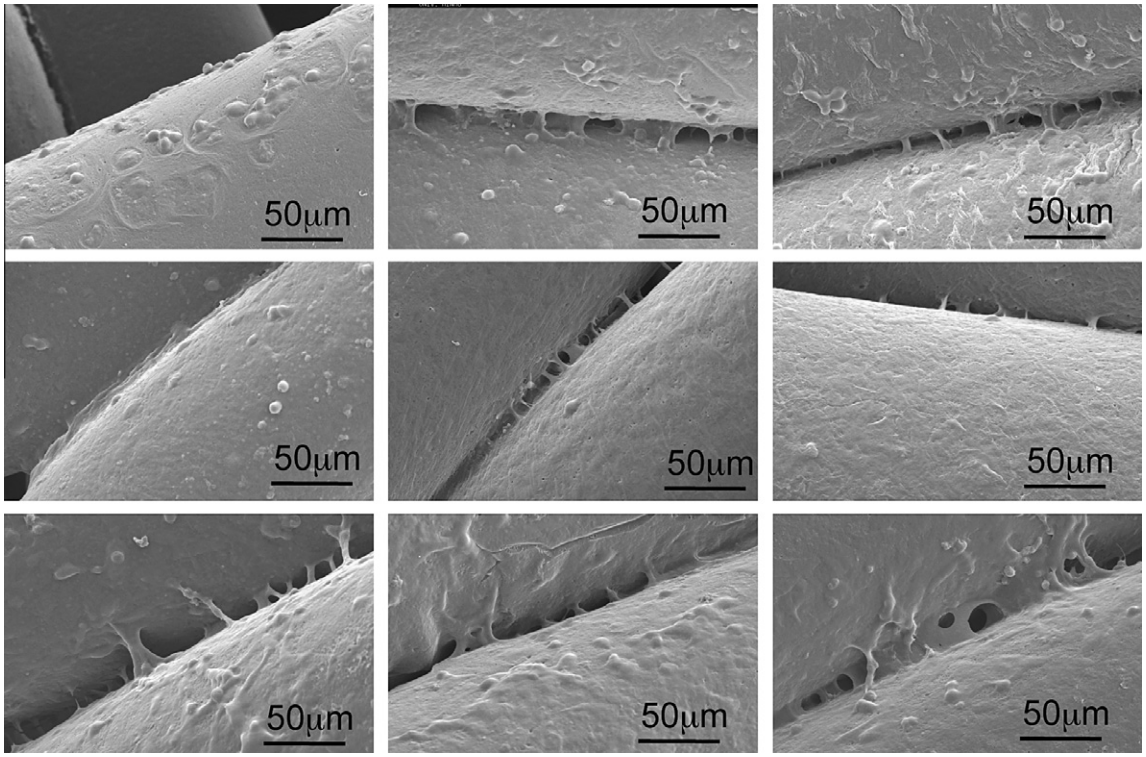

Fig. 6. Scanning electron micrographs of SaOs-2 cultures on SPCL, VSA-grafted and VPA-grafted scaffolds (from left to right) after 3, 7 and 14 days of culture (from top to bottom).

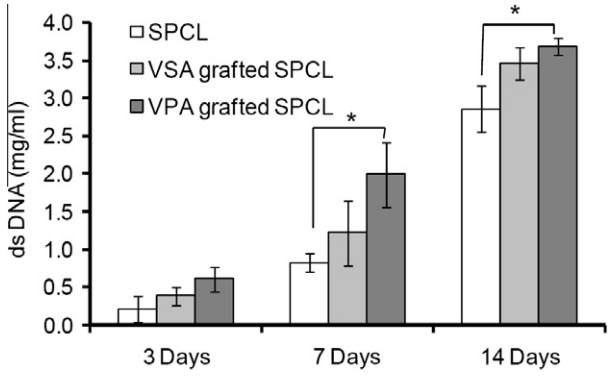

Fig. 8. DNA concentration corresponding to SaOs-2 cultured on non-modified and functionalized scaffolds. *Significantly different (ANOVA, $p < 0.05$).

4. Conclusions

Plasma-induced polymerization is proposed herein as an effective method for 3-D porous scaffold functionalization. The results from the surface analyses confirmed that, using this method, we were successful in grafting sulfonic or phosphonic groups onto materials with a complex shape without changing the surface morphology and topography. Although this method can be extended to different materials and monomers, it should be noted that its efficiency is dependent on the monomer used: we found that the reaction was more efficient for VPA, with 4.6 at.% *P* measured on the surface, whereas only 0.2 at.% *S* was registered in the surface composition of VSA-grafted surfaces. The introduced functional groups modulate the protein adsorption from serum, as indicated by confocal microscopy analysis. In this study, we observed that Vn adsorption is favored on both modified samples compared with untreated SPCL scaffolds. Comparing the treated samples, the VPA-grafted samples presented greater quantities of adsorbed Vn compared with the VSA-grafted ones. However, this result could be related with the different efficiencies of the grafting process for the two monomers studied. Vn adsorption profiles were correlated with cell adhesion and proliferation studies; materials with greater Vn adsorption presented greater cell adhesion and proliferation. We further found that grafting of negatively charged units, such as sulfonic and phosphonic groups, induced remarkably different osteoblast-like cell (SaOs-2) responses in terms of adhesion and proliferation: under the conditions studied, VPA-grafted samples showed the greatest cell adhesion and proliferation.

Overall, the results from this study further testify to the potential of surface grafting of functional groups by plasma-induced polymerization in the context of bone tissue engineering.

Acknowledgements

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 4 and 5 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.03.008).

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