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Modulating bone cells response onto starch-based biomaterials by surface plasma treatment and protein adsorption

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Abstract

The effect of oxygen-based radio frequency glow discharge (rfGD) on the surface of different starch-based biomaterials (SBB) and the influence of proteins adsorption on modulating bone–cells behavior was studied. Bovine serum albumin, fibronectin and vitronectin were used in single and complex protein systems. RfGD-treated surfaces showed to increase in hydrophilicity and surface energy when compared to non-modified SBB. Biodegradable polymeric blends of cornstarch with cellulose acetate (SCA; 50/50 wt%), ethylene vinyl alcohol (SEVA-C; 50/50 wt%) and polycaprolactone (SPCL; 30/70 wt%) were studied. SCA and SCA reinforced with 10% hydroxyapatite (HA) showed the highest degree of modification as result of the rfGD treatment. Protein and control solutions were used to incubate with the characterized SBB and, following this, MG63 osteoblast-like osteosarcoma cells were seeded over the surfaces. Cell adhesion and proliferation onto SCA was found to be enhanced for non-treated surfaces and on SCA + 10%HA no alteration was brought up by the plasma modification. Onto SCA surfaces, BSA, FN and VN single solutions improved cell adhesion, and this same effect was found upscaled for ternary systems. In addition, plasma treated SEVA-C directed an increase in both adhesion and proliferation comparing to non-treated surfaces. Even though adhesion onto treated and untreated SPCL was quite similar, plasma modification clearly promoted MG63 cells proliferation. Regarding MG63 cells morphology it was shown that onto SEVA-C surfaces the variation of cell shape was primarily defined by the protein system, while onto SPCL it was mainly affected by the plasma treatment.

Keywords: Starch-based materials; Oxygen-based rfGD; Protein adsorption; Cell adhesion and proliferation

1. Introduction

The strategy of surface modification of biomaterials has been adopted over the years in order to alter the area of the biomaterial that first comes in contact with a biological environment. Surface modifications methodologies have been used in a variety of applications, with many researchers concentrating on the study of different surface stimuli to optimize the short-term and long-term performance of biomaterials. Examples are attempts to prevent or improve adsorption of proteins and adhesion of cells to biomaterial surfaces [1–3]. Plasma surface modification methodologies have been used for biomaterials in a variety of applications such as post-treatment grafting processes [4,5] for altering surfaces functionality, and modulate proteins [6,7] and cell behavior [8,9]. The ability to retain the bulk properties constant while surfaces are changed is the key to the success of such type of approaches [10,11]. Radio frequency glow discharge (rfGD), has been used for surface modification over the years because it is considered an economical technique and clearly due its reproducibility, flexibility and clean nature [7,12]. The degree of interaction of the plasma with the polymer is partly

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determined by the chemical structure and composition of the surface and is usually accompanied by roughening of the surface [13,14]. Although, plasma modification can yield instable and irregular surface chemistries [14], enables the use of a diversity of chemicals and thus the production of different special functional groups on the surface [12]. Interestingly both surface roughness [15,16] and chemical features [17] direct the response of osteoblastic cells.

Protein adsorption has been described by different authors [18,19] as the initial and key step following the contact of an artificial surface with blood. Either in in vivo or in vitro conditions, cells are known not to interact with biospecies free-surfaces [20,21]. Along with a number of other interfacial processes, the amount, type and conformation of adsorbed proteins directs the bio-integration of an implant, thus defining its final outcome: integration or rejection [22].

Protein adsorption is dependent on the chemistry, wettability, energy and topography of a polymeric surface [23,24]. For instance, studies with chitosan [25] show that an increase in hydrophobicity (water contact angle of $\sim 100^{\circ}$) lead to increased protein adsorption compared to the more hydrophilic non-modified surfaces. In this case, hydrophobic interactions govern the protein adsorption and the majority of blood proteins form proteinaceous layers over the surface [25,26]. On the other hand, very hydrophilic surfaces also favour high biocompatibility due to the preferential adsorption of albumin, which firmly binds in high concentration [26,27]. In high concentrations albumin reaches the surface and binds, leading to a thrombogenicity lowering effect [28]. In contrast, other authors [29,30] state that strongly hydrophobic or hydrophilic surfaces show a very low ability for protein adsorption.

When contacting a material cell behavior is dependent on the orientation or three-dimensional conformation and nature of the adsorbed biomolecules. Plasma treatment of different surfaces has for instance shown to up-regulate the expression of adhesion molecules and improve adhesion and growth of endothelial [8] and bone related cells [31]. The biological response of tissues to biomaterials is to a large extent cell specific and also depends on the subsequent procedures after the initial surface preparation processes [13].

Starch-based biomaterials are biocompatible and non-cytotoxic materials that have been explored for several applications, including drug delivery systems [32], bone replacement and regeneration [33], and tissue engineering scaffolding [34,35]. In this study, starch-based biomaterials (SBB) modified by oxygen rfGD were analyzed in terms of surface chemical and physical changes and its potential for positively modulate bone–cells behavior was assessed. The surface modification was characterized by measurement of surface contact angles, surface energy, scanning electron microscopy (SEM), X-ray spectrophotometry (XPS) and Fourier-transformed infrared spectroscopy (FTIR). Subsequently, non-adhesive proteins such as serum albumin (BSA) [36], adhesive proteins like fibronectin (FN) [37] and vitronectin (VN) [38] and complex protein solutions such as fetal bovine serum (FBS) were incubated both with treated and original surfaces. These proteins were chosen on the basis of their importance in a variety of biomedical applications, including drug delivery and tissue engineering; and also on their characteristics: albumin represents highly concentrated, non-adhesive and globular proteins [36]; FN and VN represent adhesive sequence containing proteins also present in the extracellular matrix (ECM) and known to influence cell attachment, migration, differentiation and matrix assembly [39]. Finally, the influence of these proteins coupled to the effect of surface plasma treatment on the adhesion, growth and morphology of bone-like cells was studied.

2. Materials and methods

2.1. SBB

The materials used in this study were biodegradable polymeric blends of cornstarch with: (i) cellulose acetate (SCA), (ii) ethylene vinyl alcohol copolymer (SEVA-C) and (iii) polycaprolactone (SPCL). The amount of starch was 50% by weight (wt%) for SCA and SEVA-C and 30 wt%. for SPCL. Furthermore, a composite of SCA reinforced with 10% (wt%) of hydroxyapatite (HA) was prepared using twin-screw extrusion. Samples were processed into 10 mm circular disks using conventional injection molding technology. Samples were sterilized by ethylene oxide [40], washed, and all subsequent experimental procedures were performed under sterile conditions.

2.2. Oxygen-based plasma treatment

Surfaces were modified by means of O_2 gas plasma in a rfGD chamber (Harrick Scientific Corporation, USA). The plasma reactor chamber was stabilized at vacuum to approximately 26.7 Pa using a vacuum and O_2 was injected into chamber at a pressure of 15 psi for 30 s followed by a waiting period of a 30 s before plasma treatment. Plasma treatment was initiated for 180 s using a power of 100 W and pulsed frequency of 13.5 MHz time-related changes of treated surfaces were minimized by using the samples within the following 48 h.

2.3. Characterization of SBB surface modification

2.3.1. Water contact angle

Contact angle measurements were used to investigate the wettability of the surfaces following rfGD modification. The relative hydrophilicity of treated and untreated SBB surfaces was assessed by using the sessile drop method on a Video Contact Angle 2000 System (AST Products, Inc., USA) and ultra-pure water (Pierce, USA). Each side of water drops was recorded and averaged; 9 drops and 3 samples per condition were used. Measurements were recorded 10 s after liquid contact with the surface.

2.3.2. Surface energy and adhesion tension of water

The determination of surface energy (γ) of SBB compact samples after plasma treatment was based on the Owens and Went method [41] that discerns between a polar (γ^{p}) and a disperse or non-polar (γ^{d}) component of the surface energy. Water and diiodomethane (Sigma, USA) were used as test liquids for the determination of the surface energy. Reported surface tension values for water and diiodomethane are, respectively, 72.8 and 50.8 dyn/cm at 20 °C [42]. Furthermore, in this study polar and disperse parts of water were considered to be 51.0 and 21.8 dyn/cm and for diiodomethane 0.0 and 50.8 dyn/cm, respectively [42].

According to Janocha et al. [43], the measurement of the adhesion tension of water is an adequate alternative methodology to the calculation of the surface energy of solid surfaces due to its higher experimental or less assumption-based nature. The contact angle ϑ of water on the surfaces was measured and multiplied by the surface tension γ_1 of water (72.8 mN/m) to obtain the adhesion tension of water.

Both adhesion tension of water and surface energy were based on a sessile drop method. Drop contact angles were measured 10 s after contact with the surface 9 drops and 3 samples per condition were used.

2.3.3. XPS

XPS measurements were performed in order to characterize the surface composition of biodegradable blends of cornstarch with SCA following the rfGD treatment. The experiments were carried out using a Kratos Axis-Ultra (Kratos Analytical Inc., USA) with monochromatic Al X-ray source. X-rays energy was 1486.6 eV and base pressure approximately 2.9×10^{-11} psi. Triplicates were prepared and results collected from 5 different points of the surface of SCA samples.

2.3.4. FTIR

Spectra were obtained by attenuated total reflection (ATR) using a Nicolet Spectrometer (Nicolet Instrument Coorporation, USA). Each spectrum was recorded with a total of 32 scans and 4.0 resolution after 20 s of vacuum for chamber stabilization. Original and treated surfaces were analyzed in triplicates 400–4000 cm^{-1}.

2.3.5. SEM

Samples morphology was analyzed by means of SEM. Surfaces were sputter coated (Med-010 Sputter Coater by Balzers-Union, USA) for obtaining a thin Au–Pd layer and examination was performed using a scanning electron microscope (Leica, UK). Triplicates were prepared for all original and plasma treated starch-based polymeric materials.

2.4. Protein incubation assay

Since protein–surface interactions are highly dependent on the experimental system, all assay conditions were previously optimized and kept constant throughout the performed replicates.

Single and complex protein solutions were prepared for the incubation with non-treated and rfGD-treated SBB surfaces. Proteins from bovine source were used: BSA, plasma FN, VN and FBS were obtained from Pierce (USA), Sigma (USA), Calbiochem (USA) and Atlanta Biologicals (USA), respectively. The saline solution was supplied by Baxter (USA). Proteins were incubated with characterized samples for 15 min at 37 °C as described elsewhere [44]. To simulate the blood protein environment, protein solutions were prepared at 1% of the concentration of those proteins in human blood plasma [45]: 350 μg/mL of BSA, 4 μg/mL of FN and 3 μg/mL of VN. By combining BSA, FN and VN in the same solution, a ternary protein system was prepared using the same concentrations described for the single protein solutions. Furthermore, 1% (v/v) of FBS was also used to mimic complex protein environments.

Cell seeding was carefully performed sample by sample and immediately after protein adsorption step to evade surface drying and consequent protein conformational changes or denaturation. Surface rinsing was not performed and any enrichment of the cell culture media that could result from remaining non-adsorbed proteins was considered negligible.

2.5. MG63 osteoblast-like cells culture, WST-1 assay and SEM

Cell response was studied using the MG63 osteoblast-like osteosarcoma cell line that has been well characterized in the literature and consists of a good model for the study of human bone cells. MG63 cells are known to present numerous osteoblastic traits, including increased levels of bone alkaline phosphatase and inhibition of proliferation following treatment with 1,25-$(OH)_2D_3$ [46,47]. Cells (American Type Culture Collection, USA) were seeded on the relevant surfaces at 4×10^4 cells/mL and incubation was performed for 1, 4 and 7 days in DMEM (CELLGRO, USA) containing 10% FBS (Atlanta Biomedicals, USA). Tissue culture polystyrene (TCPS) was used as maximum control.

After each incubation period, cultured samples were transferred to new wells with fresh media and analyzed for mitochondrial activity using colorimetric WST-1 tetrazolium conversion assay (TAKARA, Japan). Briefly, 10 μl of WST-1 reagent was added per well, and the cells were incubated for an additional 2 h. The absorbance of the WST-1-containing cell supernatant was determined at 450 nm (Benchmark Microplate Reader, Bio-Rad, USA). To avoid interference from both cell culture media and SBB biodegradable materials, the following controls were prepared and considered as blank samples: fresh media, and SBB samples immersed in fresh media but no cells were seeded. Cell morphology was evaluated by SEM. Briefly, preparation of cell-cultured samples for SEM observation was performed by using 4% formaldehyde and 1% gluteraldehyde as fixative solution (Electron Microscopy Sciences, USA). Samples were then washed using phosphate-buffered saline (PBS) solution (Sigma Diagnostics, USA) and gradually dehydrated by incubation in crescent ethanol concentrations. Drying was accomplished by means of hexamethyldisilazane (HMDS) solution (Polysciences Inc., USA), as recommended for SEM preparation of soft tissue.

2.6. Statistical analysis

Results of the tests were tabulated as mean ± SD. The effects of plasma treatment on both the surface parameters and cell density values were statistical analyzed by performing the bi-tail Students *t*-test. Significant differences were considered to exist when $p < 0.05$.

3. Results and discussion

3.1. Results of plasma treatment on the wettability and surface energy of SBB

Oxygen plasma treatment has been described to result in the grafting of atoms or activation of existing chemical groups in the outer surface layer. Literature sources [48,49] reveal that oxygen plasma exposure can render higher hydrophilicity on most polymer surfaces. Briefly, the active plasma species attack the polymer surface resulting in the increase or incorporation of carbonyl, carboxyl or hydroxyl functional groups [48–51]. The resulting wettability changes are generally due to oxidation effects, unsaturation, electrostatic charges and surface morphological effects [50,51].

The relative hydrophilicity of treated and untreated starch-based biomaterial surfaces was assessed by measuring the contact angle using ultra-pure water and the sessile drop method. Contact angle measurements are frequently applied in surface characterization and considered a high-sensitive technique to study surface changes within a depth of a few atomic layers [52]. In the technique used a drop of ultra pure water was placed on the surface of the material and allowed to spread for 10 s, after which images were acquired and analyzed. The results and characteristic drop profiles can be observed in Table 1. In general terms, all analyzed surfaces showed a significant increase in hydrophilicity following oxygen-based plasma treatment ($p < 0.05$) that was confirmed by the higher contact angles for untreated when compared to plasma-treated surfaces (Table 1). For SCA and SCA + 10% HA decreases in the

Table 1
Contact angle and water drop profiles, surface energy and adhesion tension of the water for plasma treated and non-treated starch based biomaterials

Surface		Contact angle		Surface energy		Adhesion tension		Water drop profiles	
		Degrees	Change (%)	Dyn/cm	Change (%)	mN/m	Change (%)	nt	t
SCA	nt	76.4±3.2	−51	43.0±2.7	46	17.0±3.9	239		
	t	37.5±2.6*		62.6±0.8*		57.7±2.0*			
SCA + 10%HA	nt	65.4±3.1	−53	46.6±2.2	43	30.3±3.6	106		
	t	30.5±5.7*		66.5±3.0*		62.4±3.7*			
SEVA-C	nt	80.0±2.6	−33	36.2±1.8	43	12.6±3.3	239		
	t	54.0±1.7*		51.9±1.1*		42.8±1.7*			
SPCL	nt	78.8±1.7	−23	46.9±1.0	19	14.1±2.1	152		
	t	60.7±3.2*		55.8±1.6*		35.6±3.4*			

nt-non-treated or original SBB surfaces; t rfGD-treated SBB.
*Statistically different from non-treated SBB surfaces (*t*-test; Bi-tail; $p<0.05$; $n>9$).

contact angle were observed, 40° and 35°, respectively. For SEVA-C and SPCL the plasma treatment resulted in moderate enhancement in wettability bringing surfaces contact angles to 54° and 61°—a result of 33% and 23% reduction, respectively.

The effect of plasma treatment was further studied by means of determining the surface energy and adhesion tension of the water. The surface energy of the SBB was determined using the Owens and Went method [41] with water and diiodomethane as test liquids. For the determination of surface energy, this method distinguishes the contribution of the polar (γ^p) and dispersion forces (γ^d), taking in consideration that γ^d are always present regardless of the chemical nature of the system. The results are shown in Table 1. After rfGD treatment, all surfaces showed a significant increase of the polar (γ^p) term compared to the original surfaces ($p<0.05$). On the other hand, the dispersion (γ^d) term was not statistically different for treated and untreated SBB samples. It was found that the increasing of the surface energy was attributed to the increasing of the polar (γ^p) term following SBB treatment. As the relative increasing ratio of the polar (γ^p) term is significantly larger than the relative increase ratio of the dispersion (γ^d) term, the values of surface energy for treated samples are higher than those untreated ones that resulted from the increasing hydrophilicity of surfaces after hydration. SCA and SCA + 10%HA composite presented the highest increase in surface energy values, of approximately 20 dyn/cm (46% change), followed by SEVA-C with 16 dyn/cm (43% change) and SPCL with 9 dyn/cm (19% change).

In parallel, adhesion tension was used as an alternative methodology to calculate surface energy. This approach permits more accurate measurements by being experimental rather than based on assumptions [43]. RfGD-treated SBB surfaces when compared to untreated ones showed higher surface energy and water adhesion tension values.

The results of water contact angle, surface energy and adhesion tension of the water showed that plasma treatment significantly affected the properties of all studied SBB. The herein-described treatment introduced higher variations of the studied surfaces when compared to other chemical-based modification methods [53]. In general, SCA surfaces were the most dramatically modified ones in opposition to SPCL that showed higher surface stability. Both the increase in surface hydrophilicity and surface energy changes are due to the density of −OH polar groups on the studied surfaces, which were highly affected by the selected surface modification technology. In the natural form both SCA and SEVA-C present the same −OH density in contrast to SPCL that is composed of less 20% of starch. On the other hand, −OH groups in the synthetic polymeric fraction of the studied SBB are 2:1:0 for SCA:SEVA-C:SPCL. The nature of the different materials, including the decrease of −OH groups from SCA to SEVA-C and finally to SPCL, gives a possible explanation for the variation of contact angle or surface energy values obtained for these surfaces after plasma treatment.

3.2. Effect of plasma treatment on total oxygen content of SBB

From the three polymeric blend studied, SCA-modified surfaces showed the lowest wettability, and highest surface energy and adhesion tension. Considering the different used surfaces, SCA blends are also characterized by a higher content of hydroxyl groups known to be directly affected by oxygen-reactive species as the environment created in the plasma reactor. As a case study, this material was further analyzed by XPS. XPS retrieves detailed chemical information [54] from the nanometer scale, more specifically with a depth up to 50 Å [50]. XPS was used in the present study to characterize the surface composition of SCA following oxygen rfGD plasma treatment. Table 2 shows the results obtained for treated and untreated SBB.

The oxygen rfGD treatment was found to add significant amounts of C–O–O bonds to the surface but on the other hand C–H and C=O functional groups decreased following plasma surface modification ($p<0.05$).

Table 2
XPS analysis of atomic percentages and functional groups for the native and oxygen rfGD modified SCA

Surface	O1 (%)	N 1s (%)	C–H (%)	C═O (%)	C–OOH (%)	Total O (%)	Total C (%)
SCA	35.19±1.07	0.07±0.16	16.83±5.14	33.68±5.14	14.23±0.85	35.19±1.07	64.74±1.12
rfGD–SCA	41.37±1.10*	1.05±0.26*	4.91±1.75*	36.57±2.31	16.09±0.86*	41.37±1.10*	57.57±0.94*

*Statistically different from non-treated SCA (*t*-test; Bi-tail; $p<0.05$; $n>9$).

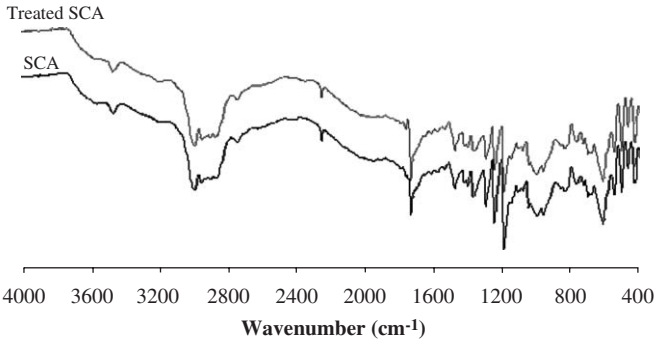

Fig. 1. FTIR spectra of original and plasma-treated SCA surfaces.

After plasma treatment, C:O ratio increases from 1:0.57 to 1:0.77, indicating an increase of oxygen on the surface of the SCA blends. Comparing the contact angle and XPS results it is seen that after the plasma treatment the increase in the total oxygen is due to an increase of OH or COOH groups as an increase in C═O group would lead to an increase in oxygen but to a more hydrophobic surface.

FTIR spectroscopy with ATR (FTIR–ATR) was also performed to characterize all SBB materials before and after plasma treatment. In contrast to surface methodologies as the wettability analysis described above, the FTIR–ATR spectra results from signals retrieved from up to 100 nm thickness, which in practice could mask true surface signals [52]. Spectra analysis did not suggest treatment driven-chemical changes either in the aliphatic, carbonyl or asymmetric stretching regions [55] as can be observed in the example presented in the Fig. 1. This indicates that the oxygen plasma surface modification did not affect the properties of the bulk of the material.

3.3. Effects of plasma treatments on the surface morphology of SBB

SEM was used to perform a qualitative surface analysis of the morphology changes introduced by the plasma treatment (Fig. 2). In opposition to SCA + 10%HA, non-modified SCA, SEVA-C and SPCL polymeric blends (Fig. 2a, e and g, respectively) presented a smoother surface when compared to treated surfaces. These preliminary results could indicate the ability of oxygen plasma treatment to modify SBB microtopography. The increase in surface heterogeneity may be another factor responsible for changing the hydrophilicity of SBB surfaces since surface micro-features affect wettability [56].

3.4. Effect of plasma treatments and adsorbed proteins on the density and morphology of osteoblast-like cells seeded on SBB

Single and complex protein solutions were prepared for the incubation with non-treated and rfGD-treated SBB surfaces. Proteins were incubated as described elsewhere [44]; to simulate the physiological environment protein solutions were prepared at 1% of the concentration of those proteins in human blood plasma [45]: 350 μg/mL of BSA, 4 μg/mL of FN and 3 μg/mL of VN. By combining BSA, FN and VN in the same solution, a ternary protein system was prepared. Furthermore, in order to mimic complex protein environments, 1% (v/v) of FBS was used. Cell response was studied using MG63 osteoblast-like osteosarcoma cell line. Cells were seeded on the surfaces for 1, 4 and 7 days; cell adhesion and proliferation was assessed in terms of absorbance and cell morphology observed by SEM. WST mitochondria assays measure viability taking in account intact mitochondrial mechanisms, consistent cellular activation and similar ECM interactions. The PreMix WST-1 enables to analyze cell proliferation and cell viability with a colorimetric assay, and is based on the cleavage of tetrazolium salts by mitochondrial dehydrogenase in viable cells [57].

Cell adhesion and proliferation on SCA (Fig. 3a) were found to be enhanced for non-treated surfaces. As for SCA surfaces, proliferation of MG63 cells was promoted on untreated SCA + 10%HA (Fig. 3b) but no alteration on cell adhesion was introduced by the plasma modification. In addition, plasma-treated polymeric blends of cornstarch and ethylene vinyl alcohol showed an increase in both MG63 cells adhesion and proliferation compared to non-treated surfaces (Fig. 3c). Even though adhesion on treated and untreated SPCL (Fig. 3d) was rather similar, plasma modification promoted MG63 cells proliferation. Between the cells and the surface, proteins are present. In the case of SCA surfaces, gas plasma treatment and subsequent protein incubation revealed to affect MG63 cells adhesion. On SCA surfaces, BSA, FN and VN single solutions improved cell adhesion levels, and this same effect was found for ternary systems.

Regarding MG63 cells morphology it was shown that on SEVA-C surfaces the variation of cell shape was primarily

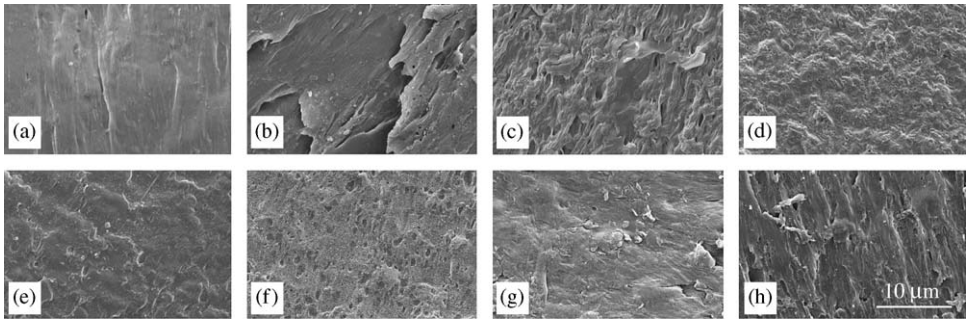

Fig. 2. Morphology of original and plasma-treated starch-based biomaterials: non-treated (a) and treated SCA (b), non-treated (c) and treated SCA + 10% HA (d), non-treated (e) and treated SEVA-C (f) and non-treated (g) and treated SPCL (h) surfaces.

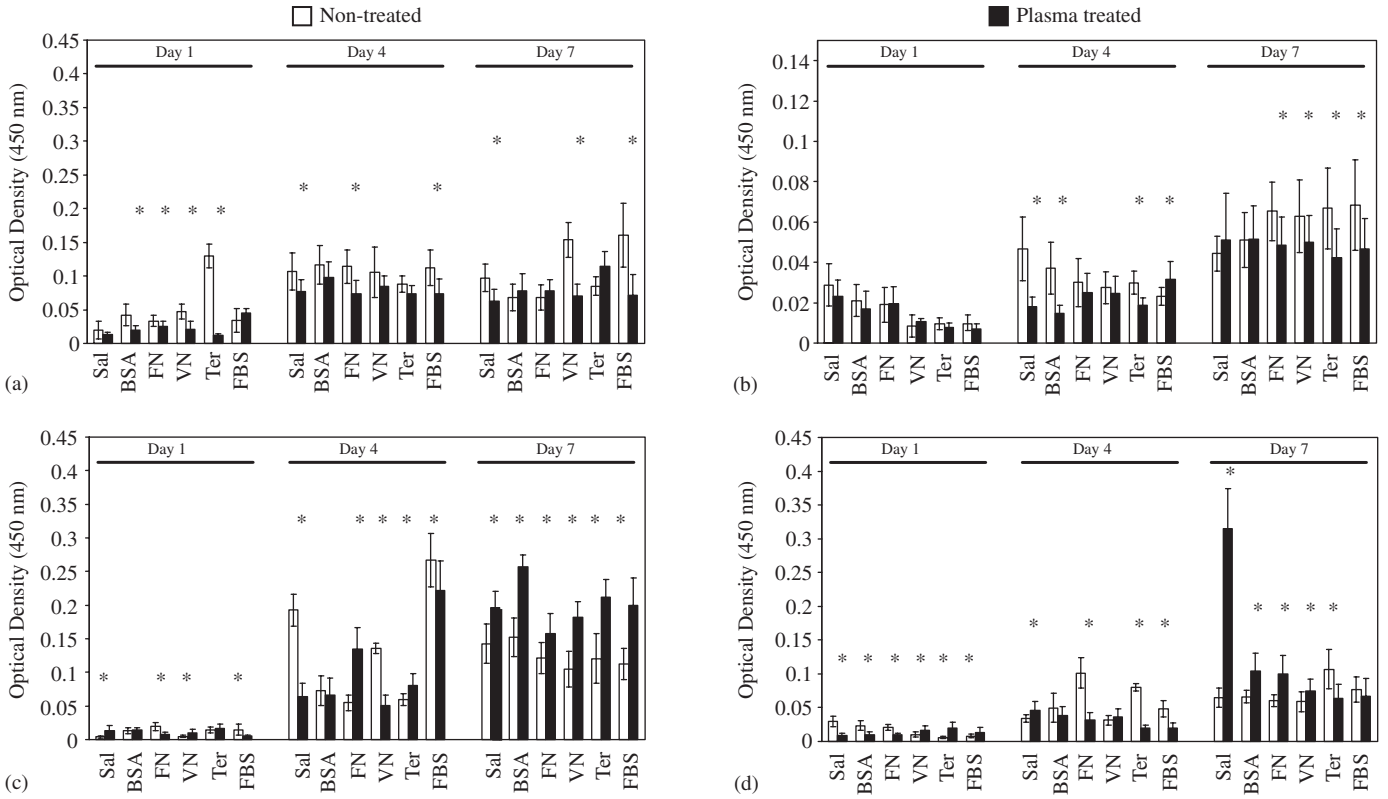

Fig. 3. Adhesion and proliferation of MG63 osteoblast-like cells onto the different non-treated and treated starch-based biomaterial surfaces: (a) SCA, (b) SCA + 10% HA, (c) SEVA-C and (d) SPCL. Leg.: *Sal*-saline solution and *Ter*-ternary solution. * Statistically different from non-treated sample (*t*-test; Bi-tail; $P<0.05$; $n>9$).

defined by the protein system used (Fig. 4). In general, cells presented a similar morphology either for treated or non-treated SEVA-C surfaces. Specific morphological characteristics were observed for the surfaces pre-adsorbed with FN (Fig. 4c and i) and VN (Fig. 4d and j), where cell spreading is increased when compared with the surfaces incubated with other protein systems. For SPCL surfaces, cell shape was affected by the plasma treatment (Fig. 5).

No cell morphological variation was observed when comparing the different protein systems within the treated or non-treated surfaces. Comparing Figs. 5a–f with 5g–l reveals that on non-treated SPCL surfaces cells present lamelipodia structures and on treated surfaces they present preferentially filapodia formation.

4. Conclusion

The surface modification technique used in this study, oxygen-based radio frequency glow discharge (rfGD) treatment was successful in changing starch-based biomaterials surface properties. Oxygen rfGD was shown to uniformly functionalize/activate the surface of SBB without affecting the bulk properties. The effect of oxygen-based rfGD on the surface of starch-based biomaterials

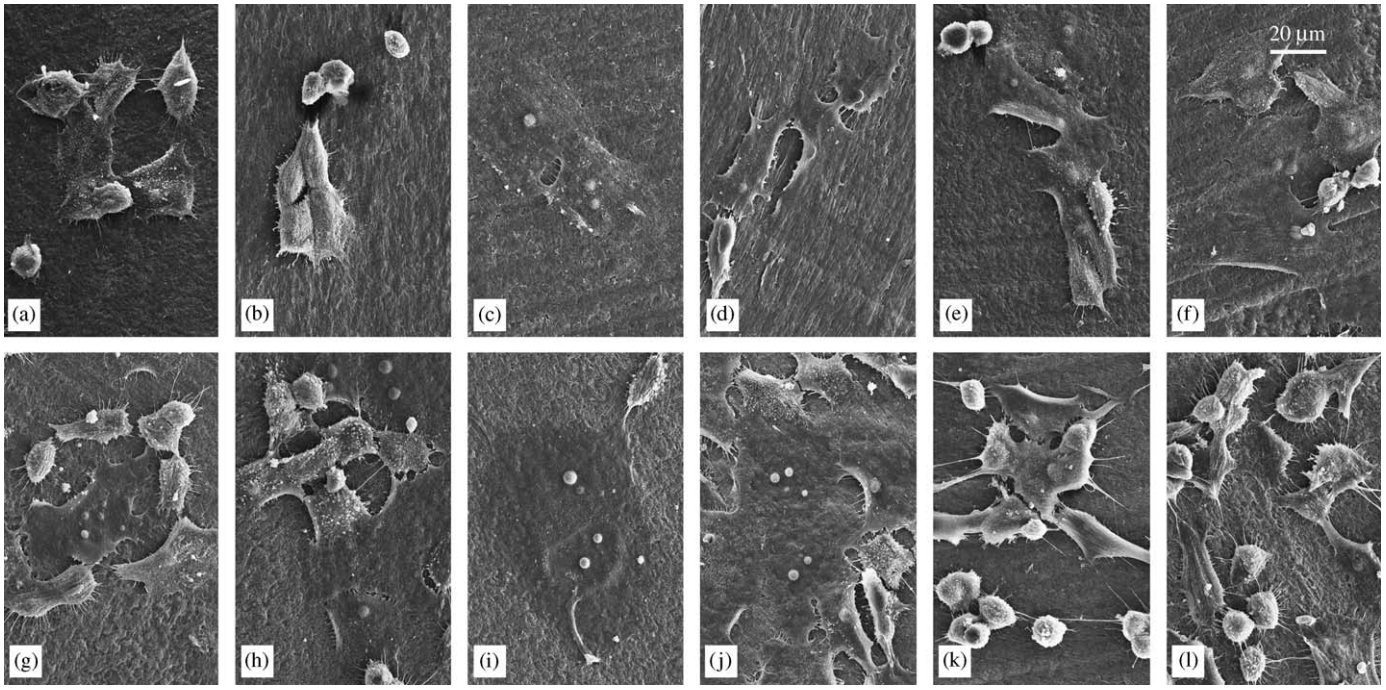

Fig. 4. Representative MG63 cells morphology over SEVA-C (a–f) and SEVA-C-treated surfaces (g–l), previously incubated with: saline (a and g), BSA (b and h), FN, (c and i), VN (d and j), ternary (e and k) and FBS (f and l) solutions.

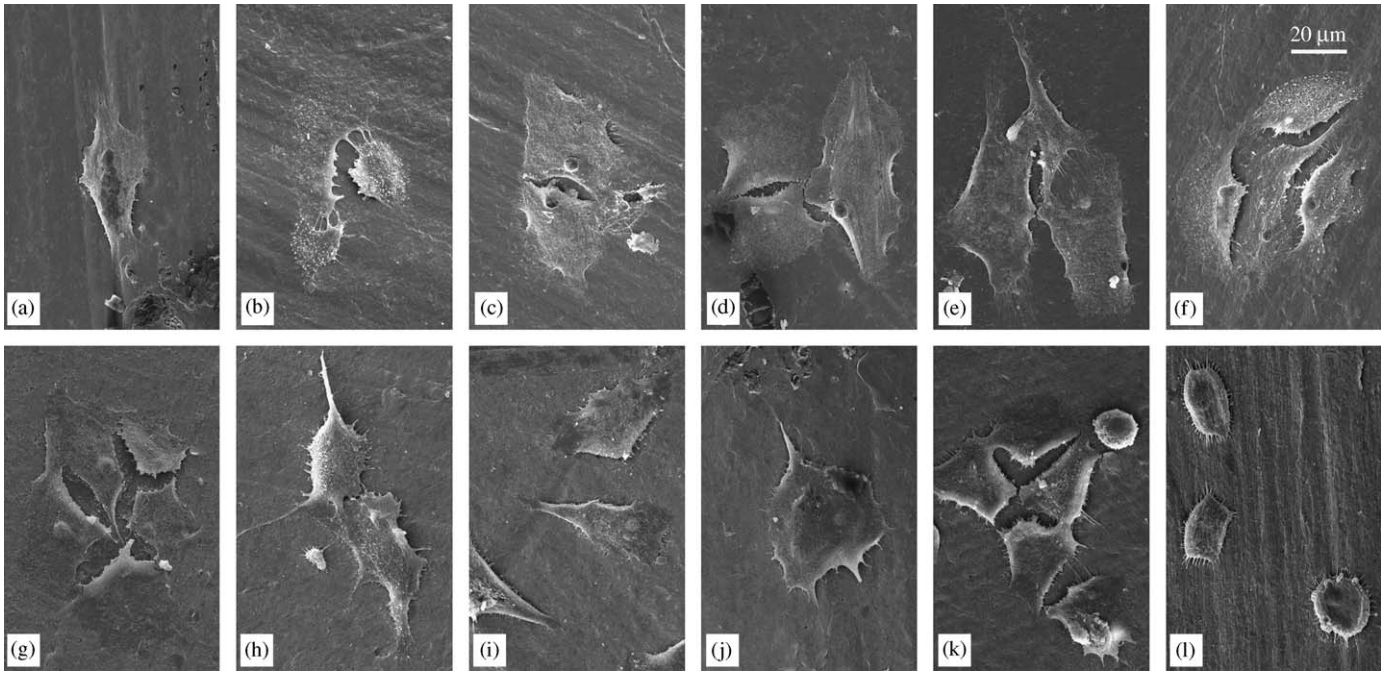

Fig. 5. Representative MG63 cells morphology over SPCL (a–f) and SPCL-treated surfaces (g–l), previously incubated with: saline (a and g), BSA (b and h), FN, (c and i), VN (d and j), ternary (e and k) and FBS (f and l) solutions.

(SBB) was investigated by means of: contact angle, surface energy, adhesion tension of water, scanning electronic microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and Fourier-transformed infrared spectroscopy with (FTIR–ATR). Both, the effects of plasma modification and the presence of different protein systems on the viability and morphology of MG63 osteoblast-like cells were also studied. RfGD-treated surfaces showed an increase in the hydrophilicity as well as the adhesion tension and surface energy when compared to non-modified SBB. Biodegradable polymeric blends of cornstarch with cellulose acetate (SCA) and SCA with 10% hydroxyapatite (HA) showed the highest change in wettability and surface energy as a result of the rfGD treatment. Surface morphological

changes were also observed by SEM. XPS analysis of SCA indicated significant differences in the C:O ratio, which increased after treating surfaces by plasma treatment, and may explain the biological response of the different polymeric blends.

In the absence of pre-incubated proteins, the plasma-treated SPCL surfaces showed to highly improve osteoblast-like cells proliferation. Protein types and the presence of other proteins were shown to be the key for cell adhesion and proliferation. In several cases, cell morphology was shown to be related to surface properties created by the plasma treatment. In contrast to SEVA-C surfaces, cell adhesion and proliferation on SCA were found to be enhanced for non-treated surfaces and on SCA + 10%HA no significant changes in cell adhesion were introduced by the plasma modification. Even though adhesion on treated and untreated SPCL was very similar, plasma modification clearly promoted MG63 cells proliferation. MG63 cells morphology on SEVA-C surfaces was primarily defined by the protein system used, while on SPCL it was mainly affected by the plasma treatment.

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