Surface modification of starch based biomaterials by oxygen plasma or UV-irradiation

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Abstract Radiation is widely used in biomaterials science for surface modification and sterilization. Herein, we describe the use of plasma and UV-irradiation to improve the biocompatibility of different starch-based blends in terms of cell adhesion and proliferation. Physical and chemical changes, introduced by the used methods, were evaluated by complementary techniques for surface analysis such as scanning electron microscopy, atomic force microscopy, contact angle analysis and X-ray photoelectron spectroscopy. The effect of the changed surface properties on the adhesion of osteoblast-like cells was studied by a direct contact assay. Generally, both treatments resulted in higher number of cells adhered to the modified surfaces. The importance of the improved biocompatibility resulting from the irradiation methods is further supported by the knowledge that both UV and plasma treatments can be used as cost-effective methods for sterilization of biomedical materials and devices.

Abbreviations

SEVA-C	Blend of starch with poly[ethylene-co-(vinyl alcohol)] copolymer (50/50 wt%)
SCA	Blend of starch with cellulose acetate (50/50 wt.%)
SPCL	Blend of starch with poly(ε-caprolactone) (70/30 wt.%)
RT	Room temperature
XPS	X-ray photoelectron spectroscopy
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscope
AFM	Atomic force microscopy
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
PBS	Phosphate buffered saline solution

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1 Introduction

Biodegradable polymers have gained a remarkable place in the biomedical field as materials for fabrication of various devices and tissue engineering applications [1–4]. The versatility of their chemical composition, mechanical properties and consequently the large pool of possible processing methodologies together with the tailored biodegradability at physiological conditions have made them the obvious material choice for many biomedical applications [5]. Undoubtedly, all those bulk properties are determinant for the long performance and the proper function of a biomaterial. However, the initial acceptance or rejection of an implantable device is dictated by the crosstalk of the material surface with the bioentities present in the physiological environment. Unfortunately, it is rare that a biomaterial with good bulk properties also posses the surface characteristics suitable for clinical application and very few surfaces are truly biocompatible [6]. Therefore, a common approach is to fabricate biomaterials with adequate bulk

properties and then to modify those materials using a specific treatment which results in enhanced surface properties [7].

Starch-based biomaterials have been proposed for different biomedical applications [5, 8–10]. While there are several studies describing the optimisation of their degradation behaviour [11, 12] or mechanical properties [13, 14], only few works focused on their surface properties and the possibility to tailor them have been published [15–19]. Targeting an orthopaedic application, Oliveira et al. [15, 19] have proposed different methodologies for preparation of calcium phosphate coatings in order to promote osteoinduction and osteointegration. However, the main disadvantage of the coating methodologies is the long term instability of the produced layer and the commonly observed delamination as a result of mismatch between the mechanical properties of the polymer substrate and the formed layer [19]. Wet chemical treatments such as grafting of acrylic monomers [18] or oxidation by potassium permanganate [17] have been proposed as alternative approaches for surface modification of starch-based blends. The treatments have resulted in both apatite layer formation and cell adhesion/proliferation enhancement on the modified blends. The wet chemical treatments have advantages over the coating in several points, including covalent attachment of graft chains onto a polymer surface which avoids their delamination and well defined, controlled surface chemistry. However, two main drawbacks should be also considered [20]:

(i)   The depth of the modified layer—due to the interactions between the solvent and the material, the modification is not always confined to the material's surface;
(ii) Ongoing hydrolysis processes—in the case of degradable biopolymers these processes most probably occur.

Therefore, physical surface modification techniques are an alternative, allowing partial over passing of those side effects. Herein, we propose the use of two free-solvent methods, namely plasma and UV-irradiation, for surface modification of biodevices fabricated from starch-based blends. The characteristic feature of these two modification methods is their action only on the very top surface layer. Thus, the bulk of substrate remains unchanged and the modified material keeps the mechanical properties and the degradation profiles [21, 22].

Plasma treatment is probably the most versatile surface treatment technique [21, 23, 24]. Different types of gases can produce unique surface properties required for various applications. For example, oxygen and oxygen containing plasmas are commonly employed to modify polymer surfaces. The treatment can increase the surface energy of polymers and their hydrophilicity. Similar effect can be observed [25] when UV-irradiation including surface photo-oxidation is applied. When polymers are exposed to UV-light, depending on the level of the chosen power, chemical (photo-crosslinking, photo-oxidation in air, or photochemical reactions in reactive atmosphere) or physical (surface morphology, etc.) changes can occur. The extent of reaction also depends on the reactants and on the absorption coefficient, that is, photon absorption as a function of photon penetration depth [21].

In this study, we have applied UV-irradiation and oxygen-plasma treatment on starch-based biomaterials in order to achieve the following objectives:

(i)   To introduce surface functionality for specific interactions with functional groups and biological items (e.g., proteins);
(ii) To tailor the surface hydrophilicity;
(iii) To modify the surface morphology in terms of increasing roughness.

Changes of surface properties such as chemistry, wettability or roughness are known to control both protein adsorption and cell response to a biomaterial [6, 26–29]. Generally, surfaces with intermediate wettability are reported to be better substrates for cell adhesion [30–33]. The introduced functional groups or rather the charge they bear do also influence the behaviour of both proteins and cells. Hence, some authors [31, 34, 35] presented evidences that adsorption does occur also on hydrophilic surfaces when charge interactions or protein conformation changes provide the necessary driving force. Therefore, the hydrophilicity of the surface and its influence on the protein adsorption is a contradictory issue; this surface property can't be considered alone as a premise for protein adsorption but in combination with the other surface properties. Whitesides et al. suggested that functional groups that made the surfaces inert for protein adsorption have some common features; they are (i) hydrophilic, (ii) hydrogen bond acceptors, and (iii) overall electrically neutral [36]. Similar results have been observed for different type of cells. It has been reported that cells adhere well to surfaces with charged functional groups such as –COOH and –NH$_2$, whereas poorly to surfaces carrying –CH$_3$ groups [36, 37]. Surface topography is another important property by which the material-cell interactions can be altered. While anisotropic topographies such as ridges and grooves affect the individual cell behavior (cells align along the anisotropic direction), isotropic topographies, such as evenly or randomly distributed pits or protrusions affect collective cell behaviors [26, 28, 29].

2 Experimental Part

2.1 Materials

The materials studied in this work were commercially available (Novamont, Italy) polymeric blends of corn starch with (i) 40/60 (mol/mol) poly[ethylene-co-(vinyl alcohol)] copolymer (SEVA-C, 50/50 wt.%); (ii) poly(ε-caprolactone) (SPCL, 30/70 wt.%); (iii) cellulose acetate (SCA 50/50 wt.%). All materials were supplied in granular form and were processed by conventional injection moulding under optimised conditions [38] in a Klockner-Ferromatik Desma FM20 machine. Produced compact discs (Ø = 1 cm) were washed prior to any characterisation in order to remove the soluble plasticizer [39].

2.2 Surface treatments

2.2.1 UV-irradiation

The UV treatment was performed by Hanovia Uvitron system with a 100 W high-pressure mercury lamp at 254 nm wavelength. The samples were fixed at a distance of 10 cm and irradiated for 24 h. Then, they were washed in order to remove the low molecular weight products produced during the treatment, dried at room temperature (RT) and characterised.

2.2.2 Plasma surface modification

The plasma treatment was performed in a home-made reactor (Fig. 1) using previously optimised conditions [40]. Briefly, a pulsed power of 70 W at 180 kHz frequency and 25°C was applied for 15 min. The pressure in the reactor (3×10^{-3} mbar) was controlled by adjusting the flow rate of a mixture O_2/Ar (20%). The samples were kept 24 h after being removed from the reactor, then washed, dried (RT), and characterised.
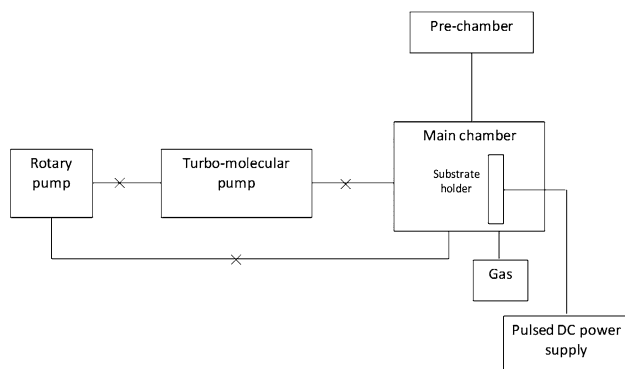

Fig. 1 Scheme of the used plasma reactor

2.3 Surface characterization

2.3.1 Surface energy/hydrophilicity

The surface wettability of modified and untreated samples were evaluated by contact angle measurements. The static contact angle measurements were obtained by sessile drop method using a contact angle meter OCA15+ with high-performance image processing system from DataPhysics Instruments, Germany. The used liquid (H_2O, 1 μl, HPLC grade) was added by a motor driven syringe at RT.

Five samples of each material were used and six measurements were carried out per sample. The normality of the data was checked by applying the Shapiro-Wilk's W-test. Student's t tests for independent samples were performed for the samples that followed a normal distribution in order to test differences among them. Throughout the following discussion, the differences were considered significant if $P < 0.05$.

2.3.2 Surface functionality

X-ray Photoelectron spectroscopy (XPS) was used to characterise the surface chemistry of treated and untreated samples. The XPS analyses were performed using an ESCALAB 200A, VG Scientific (UK) with PISCES software for data acquisition and analysis. Monochromatic Al (Kα) X-ray source operating at 15 kV (300 W) and a take-off angle of 90° relative to the sample surface were used. The measurements were carried out in a Constant Analyser Energy mode (CAE) with 100 eV pass energy for survey spectra and 20 eV pass energy for high-resolution spectra. Data acquisition was performed with a pressure lower than 1×10^{-6} Pa. Charge referencing was adjusted by setting the binding energy of the hydrocarbon C1s peak at 285.0 eV. Overlapping peaks were resolved into their individual components by XPSPEAK 4.1 software.

The surface chemical analysis was also performed by Fourier transform infrared spectroscopy (FTIR). The spectra were recorded on a Perkin Elmer System 1600 FTIR with an attenuated total reflectance device from SPECAC (MKII Golden Gate, diamond crystal, penetration depth 20 μm, active area 0.8 mm^2). Spectra were taken with a resolution of 2 cm^{-1} and were averaged over 36 scans.

2.3.3 Surface morphology

The surface morphology was observed by a scanning electron microscope (SEM) S360 from Leica (Cambridge, UK). Prior to SEM examination, a conductive thin gold film of about 10 nm was deposited (Sputter Jeol JFC 1000) on the sample surface. Quantitative analysis of the changes

of the surface roughness, introduced by the applied treatments was performed by atomic force microscopy (AFM) in air atmosphere. The analyses were carried out on at least three spots per sample using tapping mode (Veeco, USA) connected to a NanoScope III (Veeco, USA) with non-contacting silicon nanoprobes (ca. 300 kHz, setpoint 2–3 V) from Nanosensors, Switzerland. The surface roughness was calculated as Sa (average absolute distance from average flat surface).

2.4 Cell culture

The effect of the applied surface treatments on cell adhesion and proliferation was evaluated by a direct contact assay. In this assay, an immortalized cell line with an osteoblastic phenotype was used, since the materials studied in this work are to be used in bone regeneration applications. The human osteosarcoma cell line SaOs-2 was obtained from European Collection of Cell Cultures (ECACC, UK). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Life Technologies, USA), supplemented with 10% of heat-inactivated fetal bovine serum (FBS; Biochrom AG, Germany), 1% of 100,000 U/ml penicillin-G, 100 µg/ml streptomycin and 25 µg/ml amphotericin B (Sigma Chemical Co, USA) solution, and 20 mM Hepes (Sigma Chemical Co, USA) in a humidified atmosphere with 5% CO_2 at 37°C.

A cell suspension of SaOs-2 (3.3×10^4 cells/ml) was prepared by trypsinisation (0.25% trypsin/EDTA solution, Sigma, USA). The samples were placed in 24-well plates and 1.5 ml of the cell suspension was seeded onto each sample. The 24-well plates were incubated for 3 and 7 days. Culture medium was changed every 2 days. Tissue culture polystyrene (TCPS) wells were used as a control.

After each pre-determined time of culture, the samples (three samples per material per time point) were washed with a 0.1 M phosphate buffered saline solution (PBS, Sigma Chemical Co, USA), fixed with 2.5% gluteraldehyde (BDH, UK) solution in PBS for 30 min at 4°C, washed again and kept in PBS at 4°C until being stained. Methylene blue (0.4% water solution, 1 min) was used to stain the viable cells and the samples were observed under an Axioplan Imager Z1 (Zeiss, Germany).

3 Results & discussion

During the contact of material's surface with biological fluids, protein adsorption occurs almost instantaneously. This proteins' layer mediates key material-bioenvironment interactions, further directing the acceptance or rejection of the device. Physical adsorption of proteins occurs when the
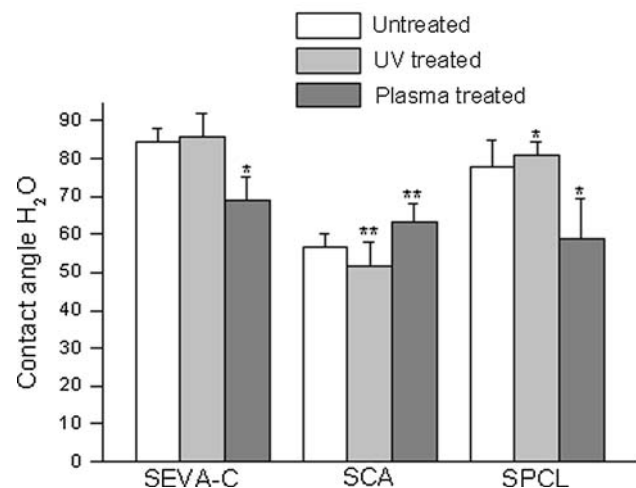

Fig. 2 Water contact angles for original and modified SEVA-C, SCA and SPCL. * $P < 0.05$ indicates a statistically significant difference between untreated materials and modified ones; ** Data do not follow normal distribution

change in Gibbs free energy of the system decreases during the adsorption process. Therefore, several surface parameters such as charge, morphology and wettability can influence this process. As mentioned before, surfaces with intermediate to moderate wettability are better substrates for protein mediated cell adhesion [31, 32, 36, 41]. On the contrary, hydrophilic surfaces are repellent for protein adsorption and therefore, they are often used as non-adhesive surfaces (e.g., PEG modified surfaces). Contact angle values of the studied materials before and after surface modification are shown in Fig. 2.

SCA was found to be the most hydrophilic material with a water contact angle of $56.4 \pm 3.9°$. Relatively hydrophobic behaviour was observed for the other two blends, SPCL and SEVA-C, with contact angles of $77.7 \pm 7°$ and $84.3 \pm 3.4°$, respectively. Previously reported results from our group [42] showed that the most hydrophilic blend (SCA) adsorbs less protein than the blend with the highest water contact angle (SEVA-C) in unitary (fibronectin or vitronectin) or complex proteins' solution system. On the other hand, a comparative in vitro study of starch-based materials [43], using L929 mouse fibroblasts, showed that the number of cells adherent to the SCA surface is higher than the number of cells on SEVA-C. Different proteins' conformations are probably the reason for the observed discrepancy between the quantity of the adsorbed proteins and the number of attached cells. Increasing of the surface oxygen content/oxygen functionalities is expected to result in more tightly binding of the proteins to the surface, hence providing a conformation, which is favorable for cell attachment and growth [44]. In fact, it has been shown [17] that chemical surface oxidation of starch-based biomaterials influences positively cell adhesion.

The performed plasma treatment resulted in more hydrophilic surfaces for SEVA-C and SPCL (Fig. 2). Unexpectedly, a relatively sharp increase in the water contact angle for SCA was observed. Ongoing crosslinking processes or cleavage of the acetate side functionalities from the cellulose chain during the plasma etching are two possible reasons for the observed result. Different wettability was also measured for the studied blends after UV-irradiation. A decrease in the water contact angle was detected for SCA, while SPCL showed an opposite behaviour. Interestingly, the UV treatment did not seem to affect SEVA-C surface. In addition to surface composition, other parameters such as surface roughness are known to affect contact angle measurements (e.g., Cassie and Wenzel effects) [16, 45]. The surface morphology of the starch-based polymers was observed by SEM before and after treatment (Fig. 3).

SEM micrographs showed different surface texture after the applied modifications. Although this effect was not pronounced for the materials treated by UV-irradiation, it is noteworthy for the samples modified by plasma. Etching processes, typically ongoing during the treatments by plasma, are the reason for the observed difference. Those processes depend on the used power, which determines the acceleration of the active species toward the material surface, on the time during which the material is exposed to this bombarding with active species, and of course on the material itself. In order to get quantitative information for the introduced morphological changes by the plasma treatment, AFM analysis (Table 1) was also performed.

The highest value of mean roughness was found for the plasma modified SCA (Fig. 4). Since SCA is the blend with the richest oxygen-containing surface and generally, these materials are more sensitive to degradation processes [46, 47], this is an expected result. On the contrary, SPCL (less oxygen) was almost unaffected.

Previous works [26–29, 48] have shown that surface topography itself can be used as a factor, controlling cell behaviour. The size, order and shape of the surface feature are specific for each cell type. The introduced topographic features to all the materials were at the nano-scale and therefore comparable to those induced by adhesive proteins such as fibronectin, laminin or collagen fibers. Recently reported results have demonstrated that cells respond to the roughness of the surface by greatly altering their gene expression profile which in turn could affect cell signalling, proliferation, cytoskeletal organization, and production of extra cellular matrix proteins [29].

Surface chemistry is another factor that strongly influences materials biocompatibility. The effect of UV irradiation and oxygen plasma modification on the surface functionality of the studied blends was investigated by FTIR-ATR and XPS. However, no differences were observed in the FTIR-ATR spectra of treated and untreated
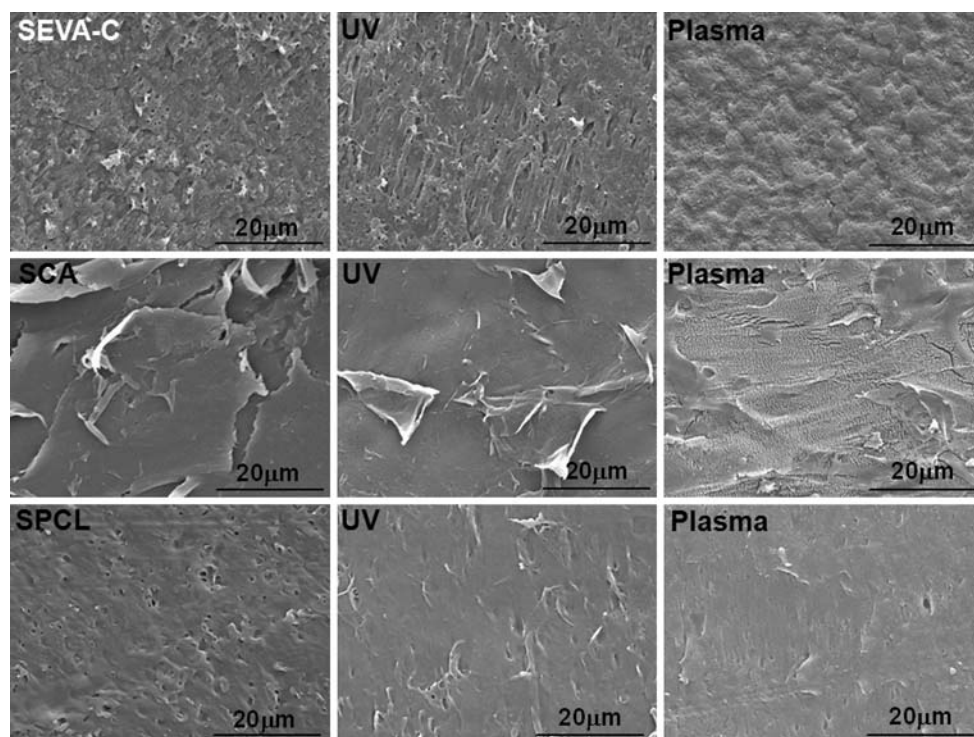

Fig. 3 SEM micrographs of the surface of untreated and modified starch-based biomaterials

Table 1 Mean roughness for untreated and plasma modified starch based polymer samples measured by AFM

Material	Mean roughness nm
SEVA-C/untreated	5.65 ± 2.05
SEVA-C/O$_2$ plasma	11.79 ± 3.29
SCA/untreated	7.43 ± 2.06
SCA/O$_2$ plasma	16.49 ± 2.47
SPCL/untreated	3.43 ± 0.74
SPCL/O$_2$ plasma	4.41 ± 1.69

materials (data not shown). Since the penetration depth of the infrared beam is in the range of 0.42–0.2 μm [49], it is quite difficult to detect structural changes on the very top surface layer. Therefore, surface analysis by XPS was used as a complementary method. The penetration depth of the X-ray beam used in this technique is about 5 nm [50]. The surface elemental compositions of starch-based blends before and after surface modification are listed in Table 2. Once again, this analysis confirmed that plasma treatment is a more powerful modification method for all studied materials.

The XPS results are in very good agreement with the measured contact angle values. An increase in oxygen surface content was observed for both SEVA-C and SPCL blends, which have also shown more hydrophilic behaviour after plasma modification (Fig. 2). The observed changes in the composition of the SCA surface after plasma treatment were quite different from those detected for SEVA-C and SPCL but also in agreement with the measured contact angle. As mentioned before, polymers containing oxygen functionalities are highly susceptible to plasma. The experimentally determined C:O ratio for SCA was 1.83 which is much lower than the C:O ratios calculated for the other two blends, 3.84 for SEVA-C and 3.06 for SPCL, respectively. This relatively high oxygen content, measured for SCA, was reduced after plasma treatment. A detailed analysis of the C1s core level spectra of this blend (Fig. 5) showed a decrease in the intensity of both C=O and C–O components compared to the C–C peak. Hence, the previous speculation for the cleavage of the acetate side functionalities was confirmed by this analysis. This same effect was also observed by other authors [51] when cellulose acetate blends were treated with CO_2 plasma.

The effect of the UV irradiation on the surface composition was not as clear as the one observed after plasma modification. Although SCA seemed to be unaffected in terms of hydrophilicity after UV treatment, the XPS analysis showed an increase in its surface oxygen content. Similar effect was observed for SPCL. These results
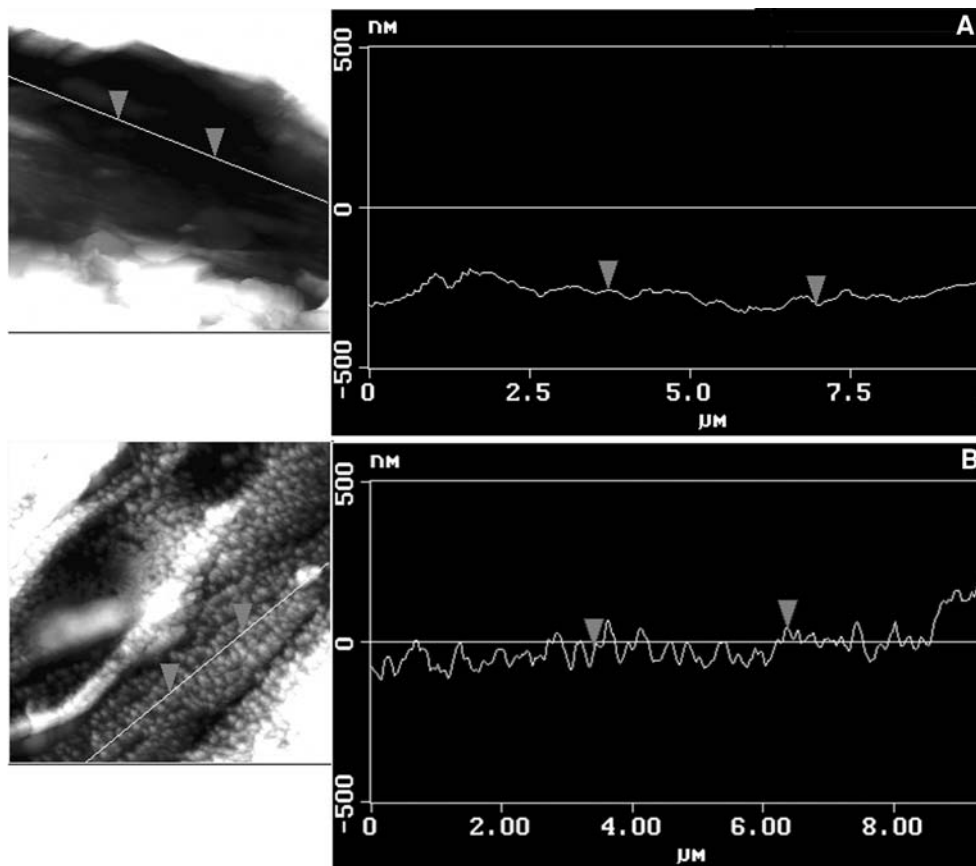
Fig. 4 AFM surface section analysis of SCA before (**a**) and after (**b**) oxygen plasma modification

Table 2 Calculated atomic concentration of the detected elements and C:O ratio on untreated, UV-irradiated and plasma treated SEVA-C, SCA and SPCL surfaces

| Material/modification	C (at.%)	O (at.%)	C:O ratio
SEVA-C/untreated	79.36	20.64	3.84
SEVA-C/UV-irradiated	80.22	19.78	4.05
SEVA-C/O_2 plasma	71.92	28.08	2.56
SCA/untreated	64.70	35.30	1.83
SCA/UV-irradiated	63.47	36.53	1.74
SCA/O_2 plasma	66.61	33.39	1.99
SPCL/untreated	75.35	24.65	3.06
SPCL/UV-irradiated	74.06	25.94	2.86
SPCL/O_2 plasma	71.39	28.61	2.50

indicate that a mild photo-oxidation of the surface of those materials occurs. During irradiation with the UV-excimer light in air atmosphere, the chemical bonds, both in oxygen and material, are split and radicals are created [52]. These radicals interact with each other resulting in simultaneous surface oxidation (new chemical groups ex. C–O or C=O) and formation of conjugated double bonds. Moreover, it is known that for polymers those processes are restricted to a very shallow superficial layer of about 50 nm thick, even after long deep-UV irradiation [21].

The opposite effect, a decrease in oxygen surface content, was measured for the SEVA-C blend. When radicals are formed on the material surface, besides surface

oxidation, a recombination to carbon double bond [52] can occur. The predominant presence of poly[ethylene-co-(vinyl alcohol)] [16] on the surface of SEVA-C might be the reason for the observed behaviour. Its linear structure together with properly positioned hydroxyl groups gives preferences to double bond formation.

How do these surface changes influence cell behaviour? Do the applied treatments enhance the biocompatibility of the studied biomaterials; are these methods suitable for the sterilization of starch-based biomaterials? Figs. 6, 7, 8 show the influence of the used irradiations on cell behaviour. As can be seen in those figures, both methods had a positive effect on the adhesion of osteoblasts-like cells.

The amount of cells adhered to the SEVA-C treated surfaces (Fig. 6c, e) clearly increased compared to the untreated ones (Fig. 6a) after 3 days of culture. This tendency of increasing cell number was kept for longer culture periods (up to 7 days) except for SEVA-C modified by oxygen plasma (Fig. 6f). The flattened and in some way oriented cells on the plasma treated SEVA-C seemed rather promising after 3 days of culture (Fig. 6e). However, at day 7 we have observed that cells do not proliferate and the majority of them detached from the surface (Fig. 6f). It should be noticed, that at this time the in vitro performance of the material is affected not only by the surface of the material but also by other parameters imposed by the presence of cells at the surface. Surface degradation of SEVA-C and consequently protein rearrangement/desorption might be another possible reason for the observed cell detachment.

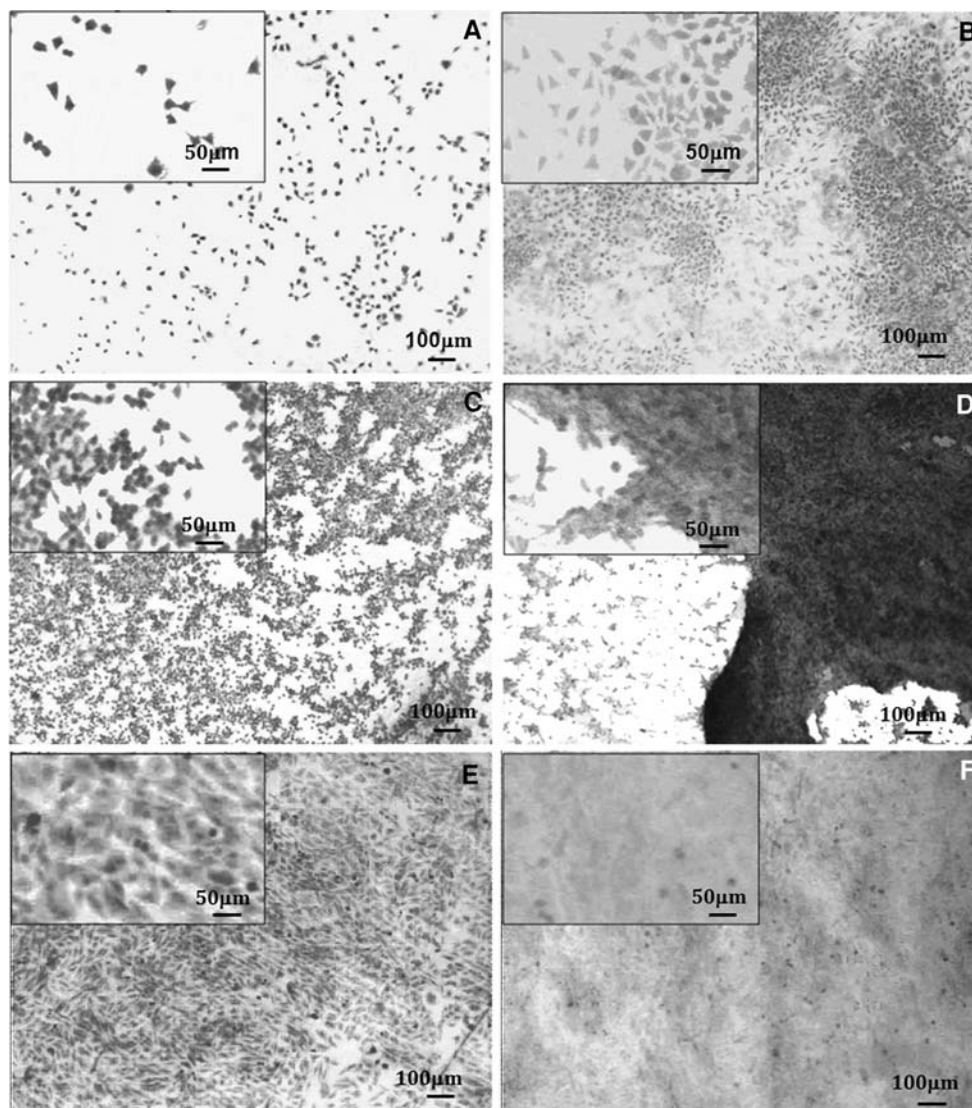**Fig. 5** C1s core level spectra for untreated and modified SCA, SEVA-C, and SPCL
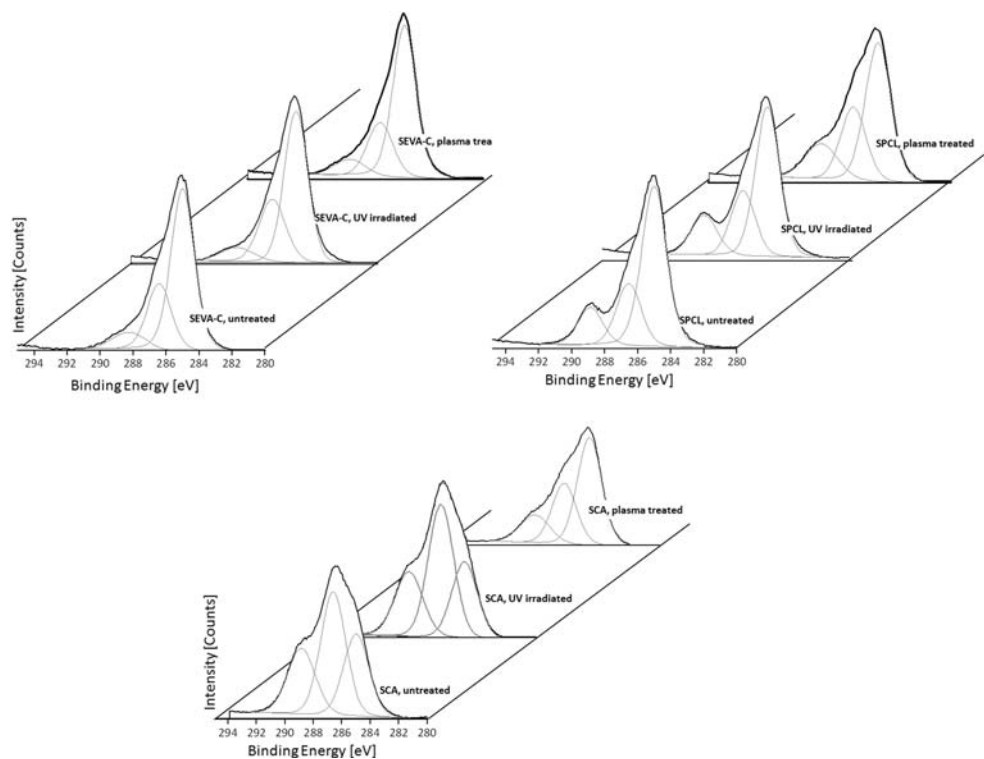
Fig. 6 Optical micrographs of osteoblast-like cells stained with methylene blue and cultured on untreated (**a**, **b**), UV-irradiated (**c**, **d**) and O₂ plasma treated (**e**, **f**) SEVA-C for 3 (**a**, **c**, **e**) and 7 (**b**, **d**, **f**) days. *Squares* on the upper corner represent an area of the micrograph at higher magnification

The analysis of the morphology of the osteoblastic-like cells cultured on the studied surfaces revealed quite interesting results. Cells cultured for 3 days on the UV irradiated SEVA-C (Fig. 6c) did not present the characteristic morphology for osteoblast-like cells and seemed to have preferential adhesion points. However, after 7 days of culture cells were forming a monolayer on the surface, confirming their viability and ability to proliferate (Fig. 6d).

In the case of SCA and contrarily to the effect observed for SEVA-C, the UV irradiation did not have a significant influence on cell adhesion and cell morphology at day 3 (Fig. 7c). This result was expected since the surface characterization showed that the water contact angle, the oxygen content and the morphology were almost unaffected by the applied treatment.

The treatment by plasma resulted in a considerable increase in the number of cells adhered to the SCA surface after 3 and 7 days of culture (Fig. 7e, f). In the natural in vivo environment, cells contact with textured not smooth interfaces (extracellular matrix). Hence, the introduced nano-roughness by this treatment could be the reason for the observed result. On the other hand, the higher surface area could also affect positively the cell adhesion by increasing the quantity of the adsorbed proteins [53].

A direct relation between surface chemistry and the adhesion behaviour was demonstrated for SPCL materials (Fig. 8). The UV irradiation had an effect similar to the one observed for SEVA-C. A significant number of cells adherent to the modified surface was detected after 3 days of culture (Fig. 8c). Similarly to SEVA-C, the adhered
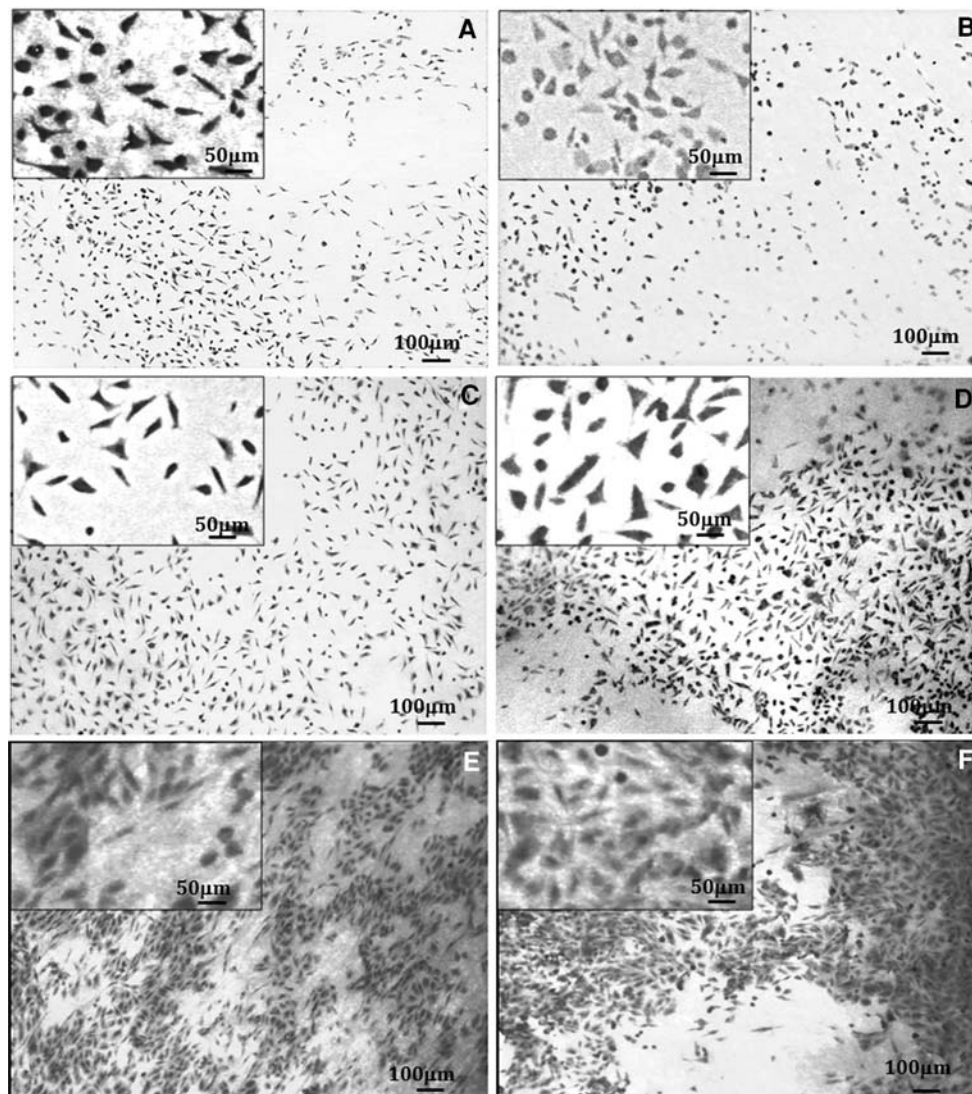
Fig. 7 Optical micrographs of osteoblast-like cells stained with methylene blue and cultured on untreated (**a**, **b**), UV-irradiated (**c**, **d**) and O$_2$ plasma treated (**e**, **f**) SCA for 3 (**a**, **c**, **e**) and 7 (**b**, **d**, **f**) days. *Squares* on the upper corner represent an area of the micrograph at higher magnification

cells did not present the typical morphology of osteoblasts and seemed to have preferential adhesion points. However, after 7 days of culture, cells were elongated and their proliferation resulted in a formation of a cell layer covering the entire irradiated surface (Fig. 8d).

Since the surface morphology of the treated and untreated SPCL samples is comparable (Fig. 3), the observed difference can be related to the change of the surface chemistry. An increase in the surface oxygen content was measured (Table 2) after UV-irradiation of the samples, thus it seems this is the main factor influencing cell adhesion to this starch blend.

These results are consistent with other works [54, 55], correlating the adhesion process to the surface chemical composition rather than to the changes in surface wetta- bility. Moreover, this assumption was confirmed by the

results for plasma modified SPCL. According to the XPS analysis, surface oxygen content was higher after applying this modification method (C:O ratio of 2.5 compared to 2.86 for UV-irradiated SPCL). On the other hand, cells completely covered modified SPCL samples only after 3 days of culture (Fig. 8e). These cells continued to pro- liferate and after 7 days of culture, a dense monolayer of cells was formed (Fig. 8f).

4 Conclusions

The effect of UV-irradiation and oxygen plasma modifi- cations on the physico-chemical surface properties of dif- ferent starch-based biomaterials was studied. It was found that plasma surface modification is more powerful method
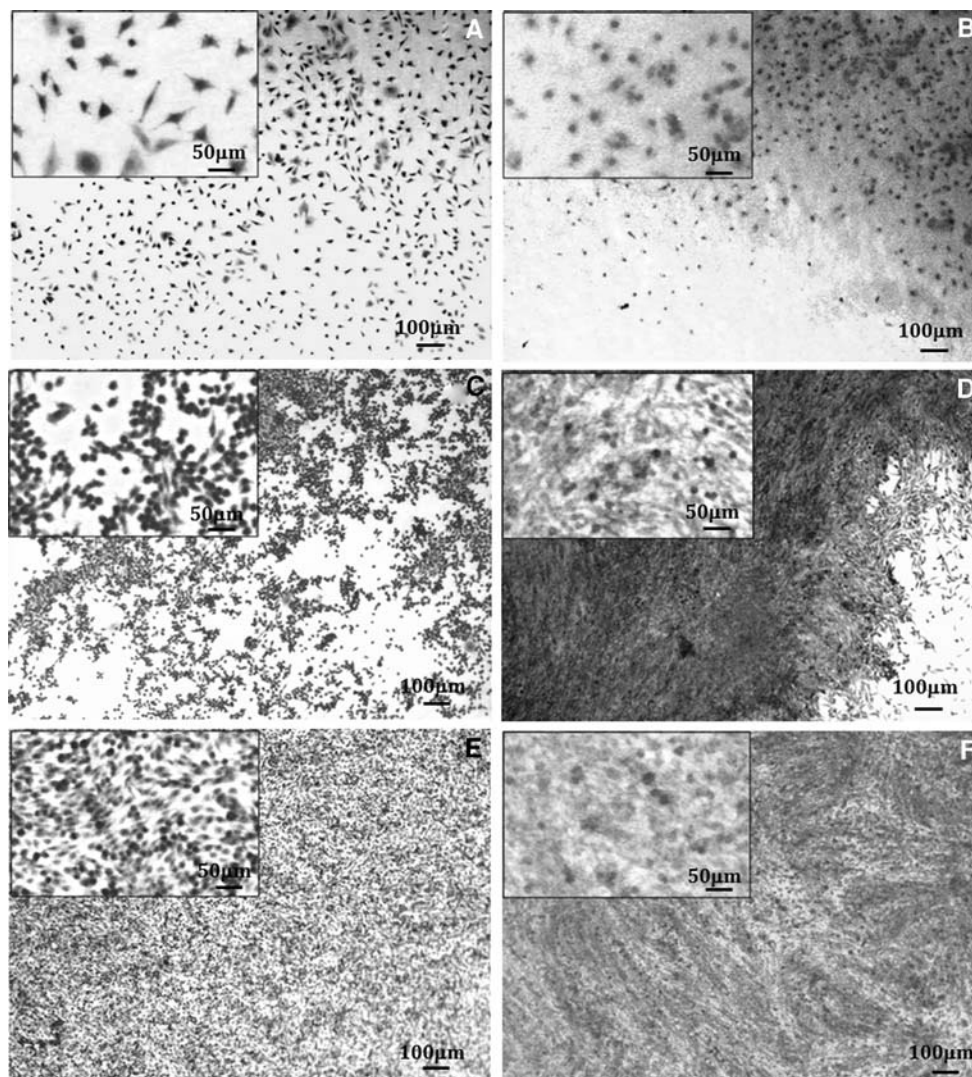
Fig. 8 Optical micrographs of osteoblast-like cells stained with methylene blue and cultured on untreated (**a**, **b**), UV-irradiated (**c**, **d**) and O$_2$ plasma treated (**e**, **f**) SPCL for 3 (**a**, **c**, **e**) and 7 (**b**, **d**, **f**) days.

Squares on the upper corner represent an area of the micrograph at higher magnification

resulting in different surface morphology, wettability and chemical composition. However, the effect of both used methods depends of the initial surface oxygen content. SCA, which is the richest in oxygen blend, was the most affected material in terms of both surface morphology and surface chemistry.

The effect of the modified surface properties on the adhesion and proliferation of osteoblastic cells was further investigated. Higher number of cells adherent to the modified surfaces was observed for short culture periods. A direct relationship between the amplitude of each surface property and cells behaviour is not possible to withdraw. Considering each studied surface parameter: the roughness induced by the surface treatments is at the biomimicking nano-scale and therefore stimulate the cell behaviour. It was found that neither very hydrophilic nor very

hydrophobic surfaces are desirable. In fact, changing the wettability of SCA from a highly to a moderate hydrophilic character favoured cell adhesion.

Sterilization of biomedical materials and devices is the final, crucial step to the application of these materials. Failing at this stage means unsuccessful end of a long process (very often years!!) of a biomaterial's creation. Oxygen plasma treatment and UV irradiation, applied on different starch-based blends, altered their surface properties in a way that do significantly improve their biocompatibility in terms of cell adhesion. The possibility to apply these techniques to three-dimensional devices with complex shape combined with their cost-effectiveness make the reported methods indispensable in further sterilization procedures of biodegradable biodevices.

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