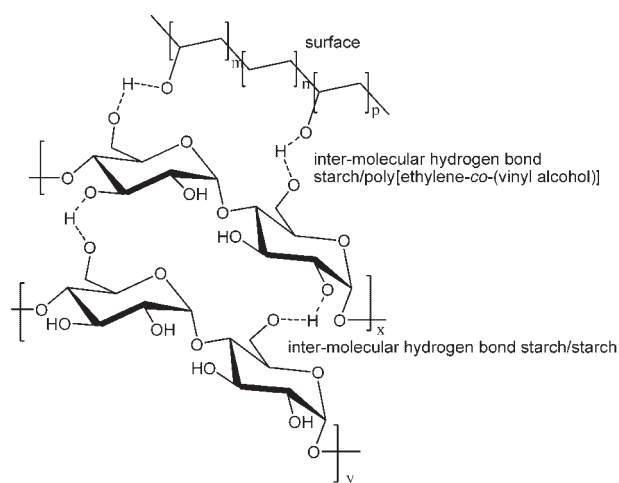


# Surface Structural Investigation of Starch-Based Biomaterials

Iva Pashkuleva,\* Helena S. Azevedo, Rui L. Reis

Surface structural characterisation of three different starch-based blends (with poly[ethylene-co-(vinyl alcohol)], cellulose acetate and polycaprolactone) was carried out. The results show that there is a difference between the bulk and the surface composition of all studied blends. Two different hypotheses were investigated – predominant presence of a synthetic component on the surface and possible inter- and/or intra-molecular bonds. The results were related to previous data for cell behaviour on those materials. It was found that both surface hydrophilicity and surface functionality are of great importance for cell adhesion and growth on starch-based biomaterials.



## Introduction

Surface is defined as the outside or top layer of the material. If the analogy with a human is used, one can say that the bulk properties of a material determine its 'character', while the surface is its 'face'. Similar to the human society, the initial acceptance or rejection of a biomaterial in the cell society is very much dependent on its face, whereas the character of a material determines its long performance and proper function.

However, it is very difficult to find a single material which possesses the desired combination of surface and

bulk properties for a certain application.<sup>[1]</sup> On the one hand, starch is a fully biodegradable material, highly available and can be easily modified, constituting, therefore, a potential biomaterial. On the other hand, starch itself has poor mechanical properties and it is difficult to process.<sup>[2]</sup> Moreover, pure starch products and even those derived from the so-called thermoplastic starch (starch with disrupted granular structure) are usually brittle and moisture sensitive, thus strongly limiting their potential fields of application.<sup>[3]</sup> Several strategies can be followed to achieve the desired material 'character', including polymer synthesis/molecular design, polymer blending, chemical modification, among others. Starch-based blends have shown<sup>[4]</sup> a great versatility, being easily processed<sup>[5–7]</sup> and proposed for applications such as drug delivery carrier systems,<sup>[8]</sup> hydrogels and partially degradable bone cements,<sup>[9]</sup> materials for bone replacement/fixation or fillers for bone defects<sup>[10,11]</sup> and porous structures to be used as scaffolds in tissue engineering of bone and cartilage.<sup>[12,13]</sup> Although the bulk properties of starch-based biomaterials have been extensively characterised

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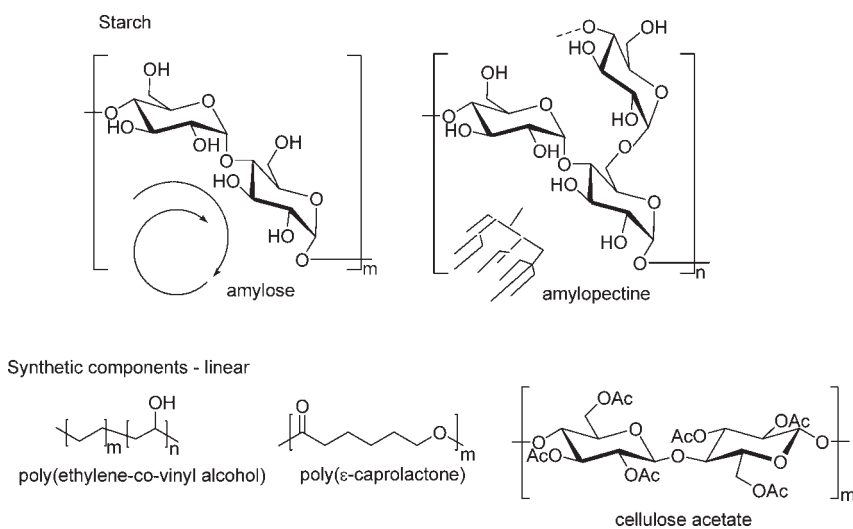
(mechanical properties, degradation behaviour),<sup>[6,11]</sup> there is no detailed study about their surface characteristics. Due to the high complexity of blend chemistry, different components may predominate at the surface, depending on the blend composition, crystallinity of the components, degree of miscibility of the system, processing conditions and also the nature of surrounding environment.<sup>[14–16]</sup>

The present study is an original surface structural investigation of starch-based biomaterials. It is aimed at understanding the relation between those properties and to relate them to the previous data for the behaviour of these materials in certain biological environments. The work is also aimed to propose some surface modification techniques, which could improve the biocompatibility of the studied blends in what concerns the cell-biomaterial interactions.

## Experimental Part

### Studied Materials

The materials studied in this work were commercially available (Novamont, Italy) polymeric blends of corn starch with (i) 40/60 mol-% ethylene/vinyl alcohol copolymer (SEVA-C, 50/50 wt.-%)<sup>[17]</sup>; (ii) poly( $\epsilon$ -caprolactone) (SPCL, 30/70 wt.-%)<sup>[18]</sup>; (iii) cellulose acetate (SCA 50/50 wt.-%).<sup>[19]</sup> The starch used to produce the polymer has been obtained from native maize and its typical original composition is 70 wt.-% amylopectin [ $\alpha(1 \rightarrow 4)$ - and  $\alpha(1 \rightarrow 6)$ -linked D-glucose] and 30 wt.-% amylose [ $\alpha(1 \rightarrow 4)$ -linked D-glucose] (Figure 1). All materials were supplied in granular form and were processed by conventional injection moulding under optimised conditions<sup>[20]</sup> in a Klockner-Ferromatik Desma FM20 machine. Produced compact discs ( $\varnothing = 1$  cm) were washed prior to any characterisation in order to remove the soluble plasticiser.<sup>[21]</sup>



■ Figure 1. Building components of the studied blends.

### Etching

Two different types of etching were performed: chemical with 1 M NaOH or 1 M HNO<sub>3</sub> (1 h, RT) and mechanical by polishing using the Struers RotoPol-21 machine. Chemical etching was used to remove very thin surface layer as well as to examine the reactivity/stability of this layer. Bulk properties of the materials and eventually changes in the surface chemistry introduced by the former method were analysed after mechanical etching.

### Morphological Characterisation

The surface morphology of starch-based materials before and after etching was observed by Leica Cambridge S360 scanning electron microscope (SEM). The samples were previously sputter-coated with gold in an Ion Sputter JEOL JFC 1100 equipment. Microphotographs at the surface were taken at various magnifications.

### Enzymatic Degradation Tests

The presence of starch on the surface was investigated by enzymatic degradation tests. The tests were carried out by incubating the samples (unpolished and polished) in 2 mL of phosphate buffer saline (PBS, 0.01 M, pH = 7.4) solution containing  $\alpha$ -amylase (0.03 mg enzyme  $\cdot$  mL<sup>-1</sup>) from *Bacillus amyloliquefaciens* at 37 °C for different periods of time. Control tests were also performed by incubating the samples only in PBS. After each period, the concentration of reducing sugars (RS), released into the incubation solutions, was measured by the dinitrosalicylic acid (DNS) method.<sup>[22]</sup> Duplicates were performed for each incubation time and the average was taken as the final result.

### Contact Angle Measurements

Surface wettability was evaluated by static contact angle measurements. The values were obtained by sessile drop method using a contact angle meter OCA15+ with a high-performance image processing system from DataPhysics Instruments, Germany. The used liquid (H<sub>2</sub>O, 1  $\mu$ L, HPLC grade) was added by a motor driven syringe at room temperature. Five samples of each material were used and six measurements per sample were carried out. Contact angle titration with non-buffered solutions was also performed for etched and original samples in order to determine the carboxyl groups present on the surfaces.<sup>[23]</sup>

The normality of the data was checked by applying the Shapiro-Wilk's *W*-test. Since all the samples followed a normal distribution, Student's *t*-tests for independent samples were performed to test differences among them. Throughout the following discussion, the differences were considered significant if  $p < 0.05$ , and highly significant if  $p < 0.01$ . The statistical analysis was performed with the package Statistica 6.0 (StatSoft, USA).

### X-Ray Photoelectron Spectroscopy (XPS)

XPS was used to determine quantitatively the surface composition of the blends. The XPS analyses were performed using an ESCALAB 200A instrument (VG Scientific, UK) with PISCES software for data acquisition and analysis. The measurements were carried out using an achromatic Al  $K_{\alpha}$  X-ray source operating at 15 kV (300 W). The spectrometer, calibrated with reference to Ag  $3d_{5/2}$  (368.27 eV), was operated in CAE mode with 20 eV pass energy. Data acquisition was performed at a pressure lower than  $10^{-6}$  Pa.

Angle-resolved XPS was used to study the difference in the chemical composition of thin (Angstroms) surface layer. Two different experiments were performed for each original blend: photoelectrons were collected from a take-off angle of  $90^{\circ}$ , which gives information about a region within  $\approx 50$  Å of the outer surface and take-off angle  $55^{\circ}$  for the very top surface layer. The XPS analyses for the etched samples were only performed at  $90^{\circ}$  take-off angle, since the material bulk (non-gradient) was the one, exposed to the beam after etching.

### Fourier-Transform Infrared-Attenuated Total Reflectance (FTIR-ATR)

The difference between the bulk and surface chemical composition, as well as eventual chemical changes introduced by chemical etching, was analysed by FTIR-ATR. The FTIR 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  $\mu\text{m}$ , active area 0.8  $\text{mm}^2$ ). Spectra were taken with a resolution of 2  $\text{cm}^{-1}$  and were averaged over 36 scans.

## Results and Discussion

The communication of an implant with the host system first takes place via the surface. This initial, direct contact between the living tissues in the body and the surface is a major determinant for the rejection or acceptance of a foreign device.<sup>[24]</sup> The surface behaviour is dependent on many parameters such as roughness, wettability, surface mobility, chemical composition, crystallinity and heterogeneity to biological reaction.<sup>[1,24]</sup> It has been shown<sup>[24–27]</sup> that wettability and surface energy of the substrate influence the adhesion of cells. High energy surfaces were generally reported<sup>[25,26]</sup> to promote cell adhesion, as opposed to low-energy surfaces. Tamada<sup>[27]</sup> and Ikada<sup>[24]</sup> have found optimal water contact angle of  $70^{\circ}$  after studying a wide variety of substrate polymers.

Starch itself is hydrophilic<sup>[28]</sup> because it is rich in hydroxyl groups. However, the wettability will be affected when it is blended with another material. Typically, the materials processed by injection moulding (blends or single polymer) present skin-core morphology. The so-called skin (the outside surface layer covering the sample bulk) can vary in chemical composition and thickness.

Melting point, viscosity and volume fraction of the components are the factors, which determine the surface composition. At the processing temperatures, the viscosity of the synthetic components (poly[ethylene-co-(vinyl alcohol)], poly( $\epsilon$ -caprolactone) and cellulose acetate) is lower than that of starch and they are present in higher volume fractions. Therefore, during the injection moulding, a preferential migration of the synthetic component towards the mould surface occurs. As a result, samples with a synthetic component rich skin and a starchy core were expected to be obtained after cooling.

Figure 2 shows water contact angles for studied starch blends before and after etching.

Surprisingly, SEVA-C, in whose composition hydrophilic ( $-\text{OH}$  groups, poly[ethylene-co-(vinyl alcohol)]) component is present, was found to be quite hydrophobic. Its water contact angle was even higher ( $p < 0.05$ ) than that measured for SPCL in which poly( $\epsilon$ -caprolactone) (PCL) is 70 wt.-%. Relatively hydrophilic properties were observed for the third studied blend, SCA. Cellulose acetate, the second component present in the blend, is quite hydrophilic and uptakes water itself.<sup>[29]</sup> Its structure is bulkier compared to the linear structure of both PCL and poly[ethylene-co-(vinyl alcohol)]. Therefore, all processes (diffusion, liquid penetration, degradation, etc.) and related kinetics for this material should be quite different from the ones for the other two blends. Hence, the presence of cellulose acetate on the SCA surface is most probably the reason for the measured lower contact angle. Moreover, its removal from the surface by polishing resulted in a less hydrophilic surface (Figure 2).

Besides predominant presence of the synthetic component on the surface, inter/intramolecular bonds, which

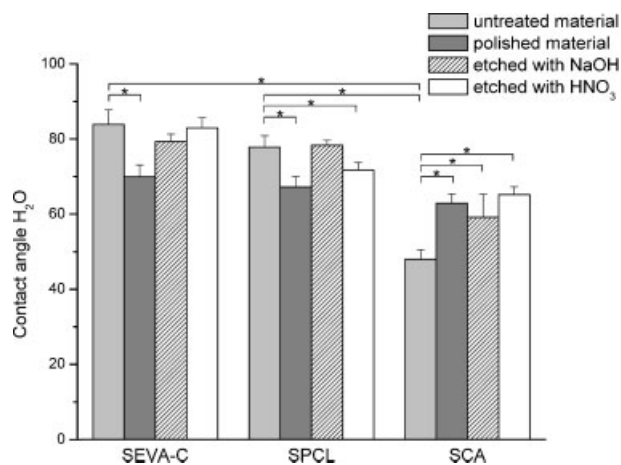


Figure 2. Water contact angle values (mean  $\pm$  SD) for SEVA-C, SPCL and SCA before and after etching. \* $p < 0.01$  indicates a statistically significant difference between untreated materials and modified ones.

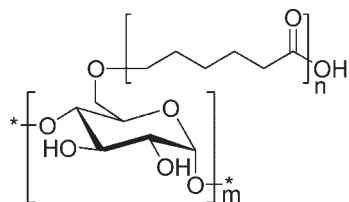


Figure 3. Ether bond which can occur between starch and PCL in SPCL.

tend to 'keep busy' the hydrophilic groups (Figure 3 and 4) could be another reason for the obtained higher water contact angle values for SEVA-C and SPCL.

A way to confirm the first hypothesis was to compare the wettability of the material before and after removing the skin by polishing. Figure 2 shows a decrease in the water contact angle for both SEVA-C and SPCL after being polished. These results could be explained by making the starch, which was entrapped during the processing of the blends, more accessible. Similar results were observed by Imam et al.<sup>[30]</sup> for injection moulded blends of starch with poly[ethylene-co-(acrylic acid)]/polyethylene. The authors have observed reduced starch hydrolysis after treatment with enzymes and detailed analyses showed that most of the starch was localised in the core of the composite.

However, the wettability could not always be directly correlated to the surface composition.<sup>[31]</sup> In fact, the wettability can vary significantly during the measurement due to the possible interactions between the two phases

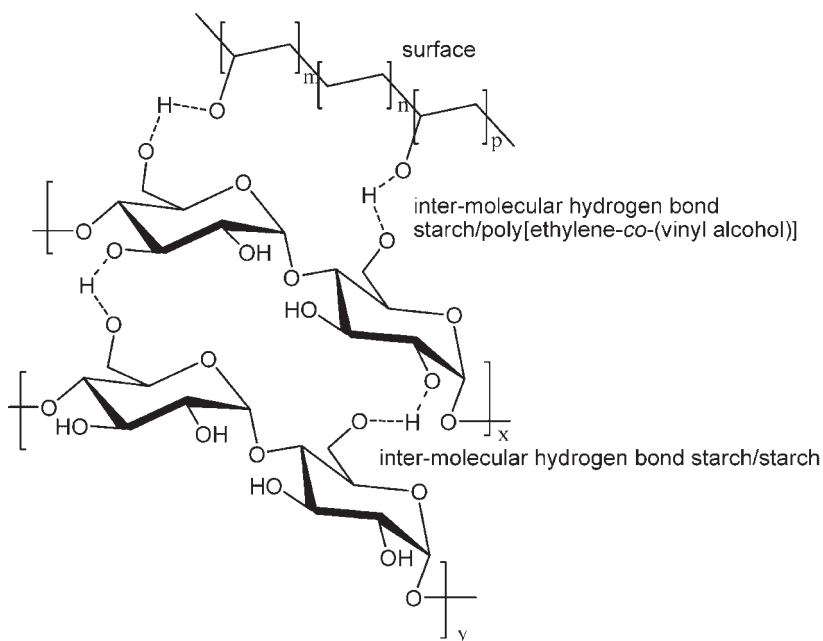


Figure 4. Intermolecular hydrogen bonds (starch/starch and starch/vinyl alcohol) in SEVA-C.

(water or air and material). When subjected to a change in environmental conditions, such as temperature or incubation medium, the surface composition can be altered by the migration of certain components or groups to the surface.<sup>[32]</sup> These interactions can depend on both the concentration of  $-OH$  end groups and the mobility of the block to which the  $-OH$  belongs (Figure 5).

On the other hand, various surface parameters such as roughness can also influence the contact angle values. Surface morphology analysis by scanning electron microscopy (SEM) showed (Figure 6–8) that SEVA-C and SPCL have relatively plane surface, while SCA is the blend, which presents more irregular surface morphology. The applied chemical etchings did not alter significantly the surface roughness. However, different surface morphology was observed for all the blends after polishing. Therefore, the XPS analysis (in vacuum) was performed in order to obtain more detailed information for the chemical structures and groups present on the surface and in the bulk of the studied materials. The results are summarised in Table 1.

Generally, the results confirmed those from contact angle measurements, namely, that the synthetic components in all studied blends dominate on the surface. For all studied blends, C:O ratio was found to be in a good agreement with the theoretical one calculated for the synthetic component in the blends.

Detailed analysis of C1s core level spectra of SEVA-C samples (Figure 6) showed that the signals are composed of three main peaks – at 285 eV corresponding to the main carbon backbone, at 286 eV for the hydroxyl bonded carbon atoms and a broad component with lowest intensity at about 288 eV for the carbonyl/carboxyl-bonded carbon atoms.

The impurities are the reason for the appearance of the last peak, since none of the components present in the blend contain carbonyl or carboxyl groups. Poly(vinyl alcohol) (PVA) is a commercial material usually derived from the parent vinylacetate polymer (PVAc). Some traces of PVAc can be the reason for this peak appearance.<sup>[33]</sup> The oxygen content increases with the depth and it is highest for the polished samples. C1s core level spectrum of the material bulk (Figure 6) is different from the spectra ( $T=90$  and  $55^\circ$ ) of the sample surface. The intensity of the peak for hydroxyl-bonded carbons is much higher, which means that those are the groups responsible for both higher oxygen concentration in the bulk and the decrease in water contact angle.



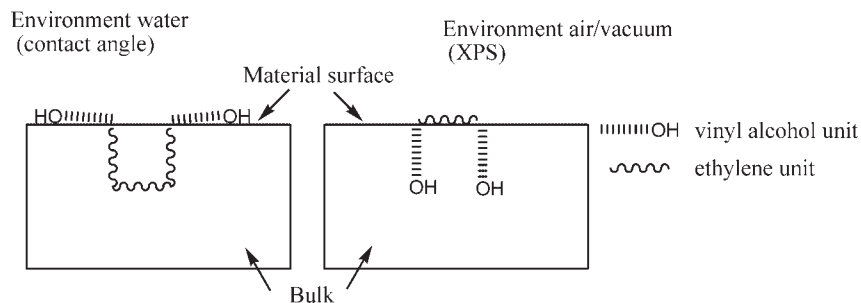


Figure 5. Schematic representation of surface variations, which could occur in SEVA-C depending on the environment.

Thermal and mechanical degradations, that are unavoidable for such type of blend during the injection moulding process, could be another reason for the lower oxygen content. Those processes most probably have

resulted in low molecular weight products, which are soluble in water and have been removed during the washing process. The results from XPS analysis after chemical etching do not show any apparent differences when compared to the untreated samples, i.e. the top surface layer is quite resistant to both acid and base action at the used conditions.

C1s core level spectra of SCA (Figure 7) contain the same components as the SEVA-C ones – at 285 eV for the main carbon backbone, at about 287 eV for the hydroxyl bonded carbons and at about 289 eV for the carboxyl bonded carbons. The intensity of the last peak is much higher than the one observed for SEVA-C and the presence of  $-\text{COOCH}_3$  groups in cellulose acetate is the reason for this. The detected oxygen content on the surface is near (but lower) the theoretical one found for cellulose acetate (Table 1) and much lower than that for pure starch. This is not surprising since it is well known<sup>[34]</sup> that there is a strong adsorption of hydrocarbon-like impurities on cellulose surfaces and this feature cannot be avoided by the washing of the samples.

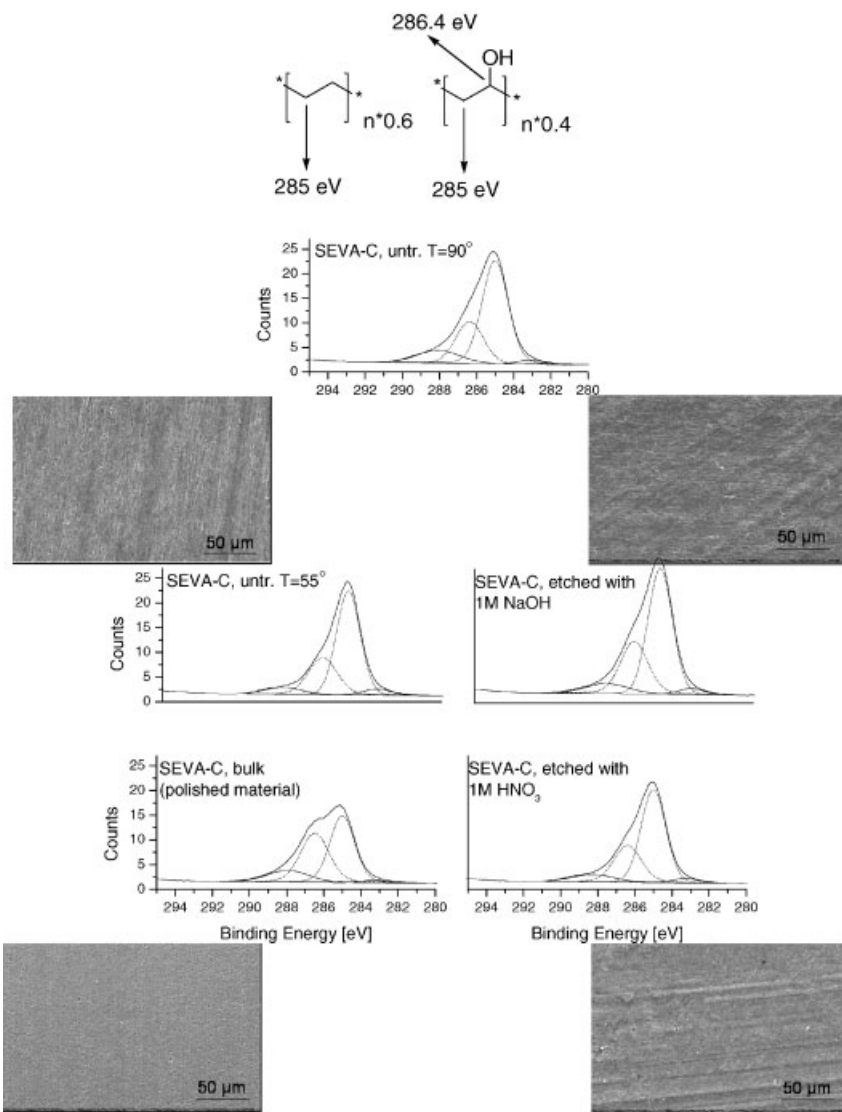
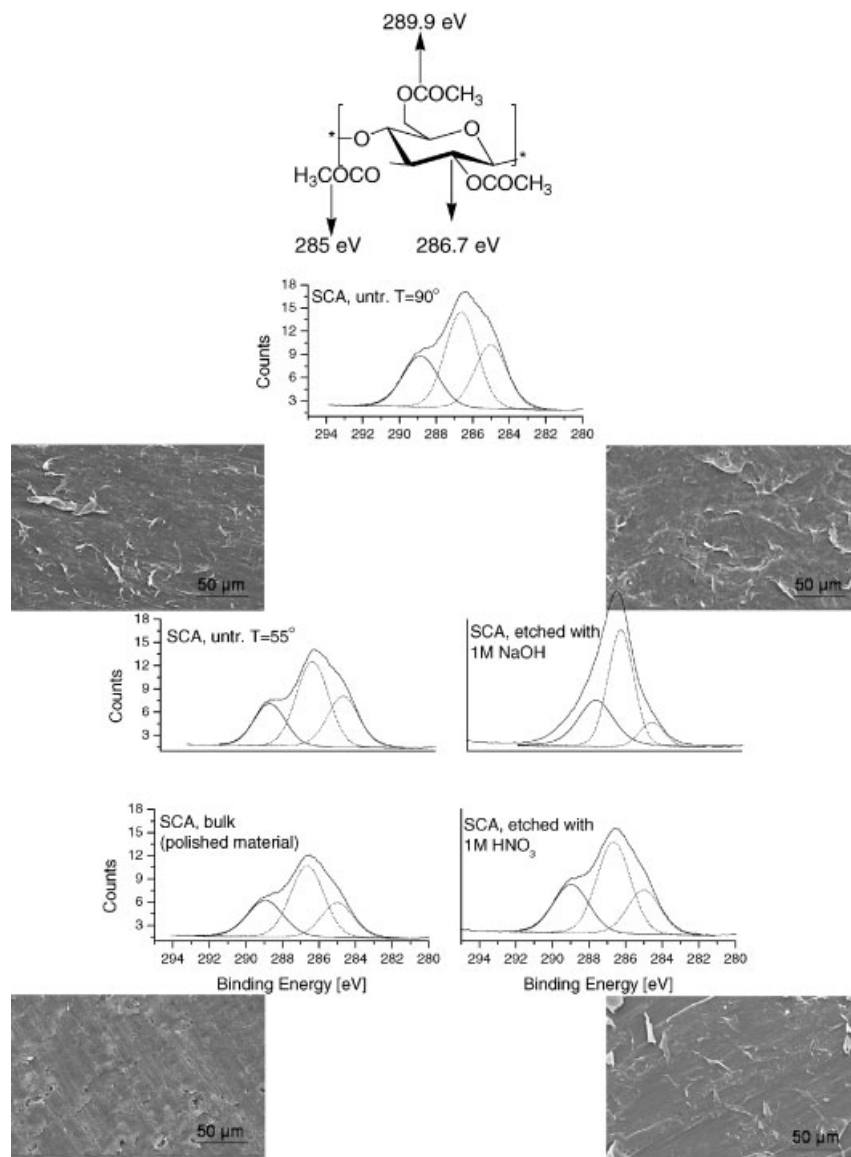


Figure 6. C1s core level spectra and surface morphology (SEM) of the original and etched SEVA-C samples.

The assumption for surface contaminations appears quite reasonable, if one compares the carbon/oxygen ratios for spectra, taken at 55 and 90°. At 55°, the spectrum shows a topmost layer composition with the highest concentration of impurities, i.e. the layer, containing oxygen, is covered with hydrocarbon-like compounds. At 90° (deeper in the surface), the spectrum presents the 'real' surface. As can be seen from Table 1, the carbon/oxygen ratio is not equal to any of the theoretical ones but it is nearer to the ratio calculated for cellulose acetate. This ratio is even lower in the bulk, after polishing of the sample. Once again, the degradation processes could be the explanation for the observed results. In SCA, both components can be degraded to soluble lower molecular weight products.

The results from SCA C1s core level spectrum after chemical etching (Table 1) are in good agreement with



■ Figure 7. C1s core level spectra and SEM micrographs of SCA before and after etching.

the contact angle measurements (Figure 2). These results show that the theoretical carbon/oxygen ratio is much more similar to the one measured for the bulk than that for the surface. Figure 7 shows that the carbon signal shape, after base etching, is quite different compared to the carbon signals for all the rest of the SCA samples. The intensities of both  $-C-O-$  and  $-COO-$  signals increase dramatically while the intensity of the carbon backbone peak decreases. This means that chain scission processes and hydrolysis are taking place at these conditions. No significant change in the signal shape was observed after etching with 1 M  $HNO_3$ . The oxygen content is near to the one calculated for cellulose acetate, which shows that, by

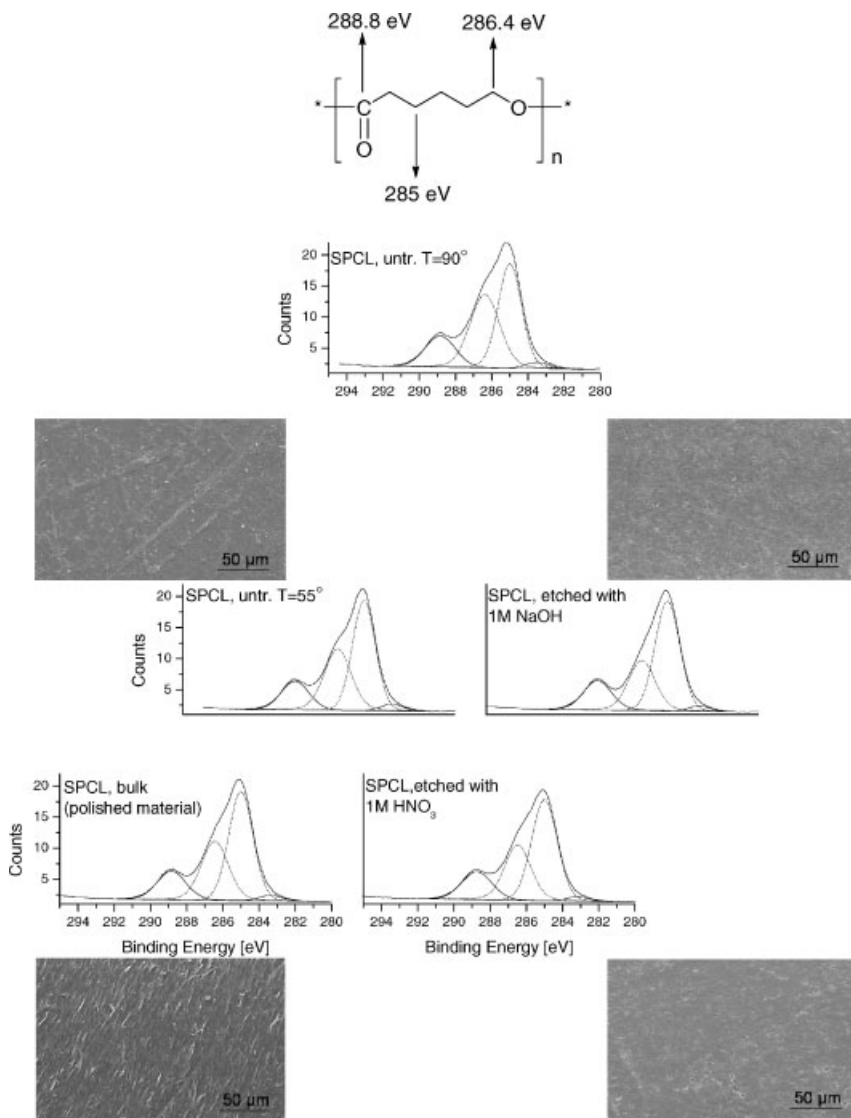
this treatment, the surface was cleaned from the impurities mentioned above.

The components of C1s XPS spectrum of SPCL (Figure 8) are at 285 eV, at about 286 eV for  $-C-O-$  and at about 289 eV for  $-C(=O)O-$  bonded carbons. The main component of the blend, PCL has all those bonds present in its structure. Surprisingly, the carbon/oxygen ratio was higher than the theoretical one. Also no significant difference was observed between the surface and the bulk composition of the material.

There could be two reasons for this result: (i) during the polishing process, the viscoelastic PCL forms a film, which covers the newly produced surface and in this way rehides the starch or (ii) in this blend starch is also present on the surface. Since the surface content of oxygen is higher than the one calculated for PCL and this value was observed to be kept in the bulk, the second reason is more probable. Contact angle values (Figure 1) also support this assumption. PCL is quite hydrophobic material – it does not present polar groups and it is not possible to have a lower water contact angle, as it was measured – than SEVA-C in which both components have hydroxyl groups. These results (from contact angle measurements and XPS) coincide with models on which starch is not present on the SEVA-C surface but it is clearly on the SPCL surface. The carbon/oxygen ratio shows again some degradation processes – although higher than the one for PCL, it is still much lower than theoretically found for SPCL.

Three-dimensional structure of starch (Figure 1) and the interactions between the blend components (Figure 3 and 4) could be another reason for the observed results. Starch polar groups may not be able to orientate at the surface and may, in contrast, exhibit strong intermolecular hydrogen bonding beneath the surface.<sup>[35]</sup> Additionally, in SEVA-C, the intermolecular hydrogen bonding between starch and vinyl alcohol hydroxyl groups could be formed keeping these groups under the surface (Figure 3). Similarly, an etherification reaction (intermolecular bond) between starch and PCL has been proposed<sup>[2]</sup> for SPCL (Figure 4).

Enzyme degradation was also performed for the studied blends (Figure 9) in order to check out this hypothesis. In



**Figure 8.** Surface morphology (SEM) and C1s core level spectra for SPCL before and after etching.

fact, enzymatic degradation studies, using specific enzymes, may give some information about the distribution of the components on the blend surface and such studies may be correlated with the surface properties and microstructure of the blends?

$\alpha$ -Amylase is an endo-specific enzyme, which catalyses the hydrolysis of  $\alpha$ -1,4-glycosidic linkages of starch.<sup>[36]</sup> The activity of  $\alpha$ -amylase may be assessed by measuring the reducing ends of soluble sugars released into the solution.

The starch blends (unpolished and polished samples) were incubated with  $\alpha$ -amylase enzyme in order to get some insights into the distribution of starch on the surface of the blends. The release of soluble sugars into the surrounding medium will take place only if the enzyme gains access to glycosidic linkages, in other words, if starch molecules are present at the surface (even with 'busy'

hydroxyl groups). Complication of this simple model could be expected if the components form a penetrating network. In the case of penetrating network, the enzyme will attack the starch even if it is in the bulk of the sample and the results will not be representative of the surface starch content. For example, Vikman et al.<sup>[16]</sup> have found that in the starch-PCL blends, a thin PCL layer ( $\approx 5 \mu\text{m}$ ) is formed on the upper surface of the samples during compression moulding. Because of this layer, the enzyme penetration depth as well as the rate of enzymatic hydrolysis was lower compared to the samples where the surface was cut (no PCL covering). Similarly, when starch has been blended with poly[ethylene-co-(acrylic acid)]/polyethylene<sup>[30]</sup> or with only polyethylene<sup>[37]</sup> most of starch was found to be inaccessible to the enzyme.

A release of reducing sugars (RS) was observed (Figure 9) after incubation of starch blends with  $\alpha$ -amylase.

The RS concentration increased with incubation time, although the extent of hydrolysis differs within the three studied blends. The presence of sugars in solution was due to  $\alpha$ -amylase activity (hydrolysis of starch), since for all the blends no significant RS amount was detected during the studied degradation period (7 d) when the samples were incubated only in PBS (control samples). However, it can be observed that higher amounts of RS were released from the SEVA-C blend, being almost

negligible for SPCL. No apparent difference was observed between the polished and unpolished samples for these two blends during the incubation period. The SCA blend exhibited an intermediate behaviour, in terms of enzymatic hydrolysis, but higher RS amount was detected for the polished samples. These results show that although  $\alpha$ -amylase was able to hydrolyse the starch present in the blends, the extent of degradation was distinct and this is mainly related to the degree of accessibility of the enzyme to the substrate. This, in turn, may be correlated with the chemistry of neighbour groups present in the blend, which may determine the orientation and distribution behaviour of chemical groups on the surface upon contact with aqueous solutions.

As it was mentioned before, some surface analyses, like XPS, are made under non-aqueous conditions and are not

Table 1. XPS data for SEVA-C, SPCL and SCA before and after etching.

Material	C peaks	C:CO:COO	C:O	Theoretical C:O
	eV			
SEVA-C ( $T=55^\circ$ )	285; 286.4; 288.5	1:0.42:0.09	1:0.20	1:0.2 EVA
SEVA-C ( $T=90^\circ$ )	285; 286.4; 288.1	1:0.40:0.19	1:0.24	40/60
SEVA-C, polished	285; 286.5; 287.9	1:0.83:0.27	1:0.37	1:0.8 Starch
SEVA-C, etched with 1 M NaOH	285; 286.4; 287.9	1:0.48:0.13	1:0.24	1:0.5 SEVA-C
SEVA-C, etched with 1 M HNO <sub>3</sub>	285; 286.4; 288.3	1:0.45:0.13	1:0.23	
SPCL ( $T=55^\circ$ )	285; 286.5; 288.8	1:0.65:0.30	1:0.37	1:0.3 PCL
SPCL ( $T=90^\circ$ )	285; 286.4; 288.8	1:0.80:0.35	1:0.39	1:0.8 Starch
SPCL, polished	285; 286.4; 288.8	1:0.61:0.31	1:0.35	1:0.45 SPCL
SPCL, etched with 1 M NaOH	285; 286.4; 288.9	1:0.51:0.31	1:0.33	
SPCL, etched with 1 M HNO <sub>3</sub>	285; 286.5; 288.7	1:0.58:0.32	1:0.37	
SCA ( $T=55^\circ$ )	285; 286.7; 289.0	1:1.61:0.80	1:0.57	1:0.67 CA
SCA ( $T=90^\circ$ )	285; 286.6; 288.9	1:1.48:0.88	1:0.58	1:0.8 Starch
SCA, polished	285; 286.7; 288.9	1:2.10:1.20	1:0.60	1:0.73 SCA
SCA, etched with 1 M NaOH	285; 286.8; 288.1	1:5.51:4.06	1:0.66	
SCA, etched with 1 M HNO <sub>3</sub>	285; 286.7; 289.0	1:2.06:1.17	1:0.61	

necessarily representative of the actual surfaces that exist in contact with the surrounding fluids. In fact, the more hydrophilic groups within the surface layer are expected to migrate to the upper surface upon exposure to water,<sup>[32]</sup>

as shown in Figure 5. That might be, to a certain extent, the case of starch blends, since the release of RS into the solution during the enzymatic degradation tests revealed that  $\alpha$ -amylase was able to hydrolyse the  $\alpha$ -1,4-glycosidic linkages of starch, that may have moved to the surface. On the other hand, it may be possible that the enzyme has gained an access to susceptible hydrolysable bonds of starch in the bulk by means of penetrating network with increasing degradation time. That phenomenon is particularly notorious for SEVA-C blend, where a significant amount of RS was detected in the first day of incubation (Figure 9). In order to gain an increased understanding about the surface chemistry of SEVA-C material, another set of enzymatic degradation tests were performed for this blend under a shorter incubation period (Figure 10). The early enzyme-mediated degradation of polymers occurs at the molecular level rather than the micron level<sup>[38]</sup> and is characterised by the hydrolytic scission of the polymer chains leading to a decrease in the molecular weight.<sup>[36]</sup>

In the first hour of incubation, no significant amount of sugars was detected (Figure 10) for both polished

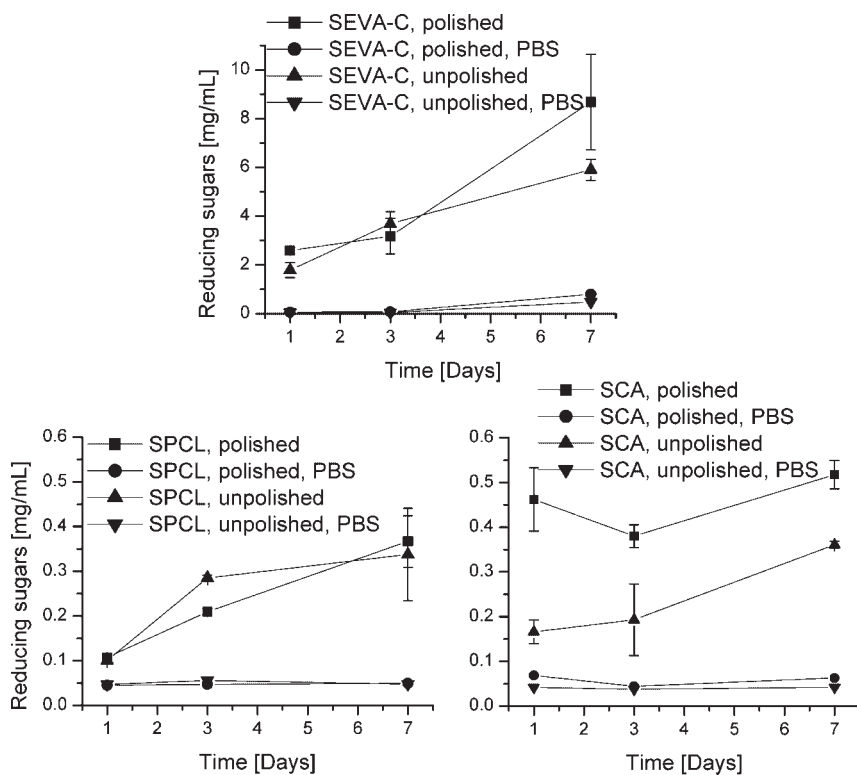


Figure 9.  $\alpha$ -Amylase activity on starch-based blends (0.1 g), measured as the concentration of RS released into the solution.



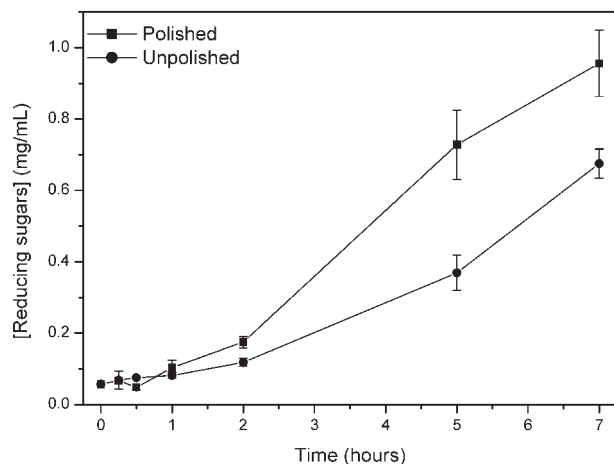


Figure 10.  $\alpha$ -Amylase activity on polished and original SEVA-C samples for a short time period.

and unpolished samples. At this initial stage, the first degradation products are not small enough to become soluble. With increasing time, the molecular weight of degradation products is reduced by further hydrolysis, which allows them to diffuse from the surface to the solution. That is, in fact, what can be observed in the following hours, where increased amounts of RS are released, being higher for polished than for unpolished samples. The higher rate of enzymatic hydrolysis of polished samples in early degradation may be due to a higher amount of starch molecules exposed to the enzyme and/or to an increased access of the enzyme to the starch substrate due to the removal of the synthetic component from the outermost surface after polishing. This result may confirm the hypothesis that the synthetic component of the blend is predominant at the surface, rather than starch, but the nature of these groups may also influence the mobility of other components within the surface towards different environment conditions.

The results from contact angle measurements after chemical etching showed that SEVA-C and SPCL surfaces are quite resistant to the applied etchings. The observed decrease in the values can be related with the removal of some hydrophobic impurities from the surface. However, no differences in surface chemical composition were detected by FTIR-ATR (data not shown).

Since FTIR-ATR was not sensitive enough<sup>[39]</sup> to detect some changes on the top surface layer, contact angle titration<sup>[23]</sup> with non-buffered solutions was also performed. Surface hydrolysis processes can occur as a result of the performed chemical etching. For example, sodium hydroxide is very often used for functionalisation ( $-\text{COOH}$  groups) of PCL via hydrolysis. Carboxyl and other charged groups on the surface can be determined by contact angle titration. However, the measurements did not show the presence of any charged groups on the studied surfaces.

The obtained results from this surface structural investigation are in good agreement with the previous *in vitro* studies<sup>[40–42]</sup> on biocompatibility of these starch-based polymers. Significant cellular adhesion and proliferation (L929 mouse fibroblasts) was found<sup>[40]</sup> for both SEVA-C and SCA surfaces. However, the number of cells adherent to the SCA surface is higher than the number of cells on the SEVA-C. This is quite reasonable if one compares these results with the ones obtained from contact angle measurements and XPS. SCA is more hydrophilic and has more available hydroxyl groups which promote cell adhesion according to several studies.<sup>[43–46]</sup>

The importance of surface functionality for osteoblasts cell adhesion process was demonstrated by a comparison between the numbers of cells adhered to SPCL and SEVA-C. These two starch-based blends have similar initial hydrophilicity but different functional groups build their secondary components. SPCL surface was not as favourable for cell proliferation as SEVA-C surface. This is due to the fact that hydroxyl groups from poly[ethylene-co-(vinyl alcohol)] in SEVA-C are mobile (Figure 5) and are able to reach the surface in aqueous medium. In this way, they support cell adhesion/proliferation and the entire surface was covered by a monolayer of cells after 7 d of culture.

These differences in cell adhesion may be attributed to different protein conformations. The higher oxygen content on the surface binds the proteins more tightly, resulting in a conformation that provides a substrate, which is more favourable for cell attachment and growth<sup>[43]</sup>. Higher oxygen content and increased hydrophilicity have been observed<sup>[42]</sup> for the studied blends oxidised by potassium permanganate. The modified materials showed a significantly improved cell adhesion on both SEVA-C and SPCL. These results again confirm that oxygen-rich surfaces are preferable when trying to enhance cell adhesion and proliferation.

## Conclusion

A surface characterisation of three different starch-based biomaterials was carried out. The results showed that the bulk and surface composition for all the studied materials are quite different. Predominant presence of synthetic components at the surface, as a result of the used processing technique, is the main reason for that observation. The most significant difference was observed for the blend with poly[ethylene-co-(vinyl alcohol)]. According to the performed analyses, a migration of hydrophilic groups to the upper surface layer in aqueous environment and consecutive formation of interpenetrating network was proposed for this blend.

Surface properties, such as wettability, functionality and oxygen content, were related to cell response (attachment

and proliferation). It was found that more hydrophilic, oxygen-rich surfaces are preferable. From the functional groups present in different blends, hydroxyl ones were clearly favourable. Therefore, the biocompatibility of those materials could be improved by tailoring the hydroxyl functionalities present on the surface. This possibility together with the biocompatibility, biodegradability and the ability to drive the mechanical properties in a certain direction by alternating the synthetic component and the blending ratio, open a wide range of biomedical applications for the studied starch-based blends.

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