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Surface modification of a new flexible polymer with improved cell adhesion

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An improved cell compatibility of polymers is determined by interactions with the cell surface, which are influenced by the chemical surface properties of the polymers. Of special interest are methods to change the surface properties by generating new functional groups for a better cell adhesion without affecting the bulk properties of the polymer. The study describes the surface modifications of a new flexible copolymer comprising polyvinylchloride by either wet chemical treatment or plasma treatment. In the first case the immobilization of different diols to the activated surface changes the hydrophilicity of the surface. Furthermore, for the first time the surface of the copolymer is modified by SO_2-plasma treatment to generate oxidized sulfur spezies at the surface. All modifications are characterized by X-ray-photoelectron-spectroscopy (XPS), ATR-FTIR-spectroscopy and the contact angle. The correlation between the chemical surface properties and the fibronectin adhesion indicates the surface/biological system interactions. The SO_2-plasma treated surface shows the highest value of fibronectin adsorption compared to the other surface modifications. This correlates to the results of human endothelial cell culture experiments. On the SO_2-plasma modified surface of the copolymer the cell proliferation is better than on surfaces modified by immobilization of different diols.

1. Introduction

The most important requirement of a biopolymer is unlimited biocompatibility. It is known that the chemical and physical surface structure of polymers is mainly responsible for the reactions in biological systems [1]. Therefore, instead of developing a new series of polymers, the emphasis here was laid on modifications to the surface properties, which show high cell compatibility. The main objective of this study is the surface modification of a new flexible copolymer comprising polyvinylchloride, to reach human endothelial cell seeding without affecting the bulk properties. Besides the immobilization of different diols, treatment with gas-plasma is of special interest for surface modification. Low-pressure gas-plasma is used for the modification of biomaterial precursors in a series of applications, such as improving tissue culture compability, contamination-free purification, building or removal of boundary layers, improvement of wettability and immobilization of biomolecules [2]. Until now the plasma discharge treatment of polymer surfaces for biomedical application has been carried out with gases other than SO_2 [3]. Surface modification of the new flexible copolymer by SO_2-plasma treatment is described in the present article.

2. Materials and methods

Polymer films made from a special copolymer, which consists of polyvinylchloride and the plasticizer component poly(ethen-co-vinylacetate), were used.

The first step of the wet chemical treatment was the saponification of the functional ester-groups at the surface to hydroxy groups. The activation of the OH-functionalized surface was performed with hexamethylendiisocyanate (HDI) in ether at room temperature. The immobilization reactions of ethylene glycol, 1,3-propandiol, 1,4-butandiol were carried without using a solvent.

The plasma treatments were preformed with an experimental system purchased from Technics Plasma/Kirchheim. The plasma unit consists of a hexagonal chamber with a microwave unit with a frequency of 2.45 GHz and a power of 300 W. The treatment period was 2 to 30 min for SO_2-plasma.

XPS-measurements (X-ray photoelectron spectroscopy) were carried out with a SSI 206X/Probe XPS-spectrometer (Mountain View/USA). FTIR-spectra were obtained using as attenuated reflection technique with a Germanium crystal with a Nicolet 710 spectrometer (Offenbach, Germany). The contact angle was measured using the sessile drop method with pure water at room temperature on a G40 system

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from Krüss, Germany. Fibronectin and the corresponding monoclonal antibodies for the protein adsorption experiments were purchased from Sigma, Germany. The quantitative determination of fibronectin was achieved with a fibronectin enzyme-linked immunosorbent assay (ELISA)[4].

Cell culture experiments were carried out with human umbilical vein endothelial cells (HUVEC). The polymer films were fixed to a thermanox disc with a biocompatible adhesive and sterilized by autoclaving at 116°C, 10^5 Pa for 30 min. These preparations were put into the wells of a 24-well tissue culture cluster. Cells were detached from the culture flasks using collagenase and resuspended in serum-containing culture medium. The endothelial cells were seeded into walls at a density of 10 000 cells/cm^2. After 1, 4 and 7 days adherent cells were detached from the surface using trypsin/EDTA solution (0.25%/0.25%: w/v), resuspended and counted with a coulter counter.

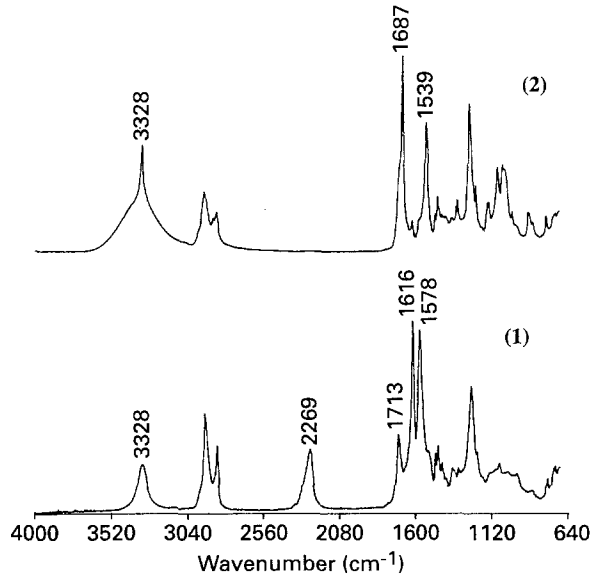
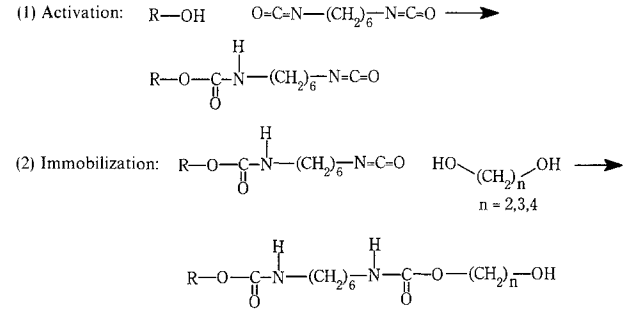


Figure 1 FTIR-spectra of the HDI activated (1) and ethylene glycol immobilized (2) copolymer film.

3. Results and discussion

Before activation of the surface the acetate groups of the plasticizer component of the flexible copolymer were saponified. The successful treatment is demonstrated by the disappearance of the O = C–O acetate photoline in the XPS C1s-spectrum. The appearance of the band in the IR-spectrum at a wavenumber of nearly 3410 cm^{-1} is due to the hydrogen bonds of the OH-groups. Additionally, the contact angle decreased from 85° for the untreated sample to 67°, which is caused by the hydrophilic OH-groups. The reaction mechanism of the activation with HDI and the immobilization of diols with different chain length is shown in Formula 1:



The activation is characterized by the appearance of the urethane-photoline in the XPS. In Fig. 1 the FTIR-spectra of the activated and ethylene glycol immobilized copolymer film is demonstrated. The band in the FTIR-spectrum at 2269 cm^{-1} is proof of the terminal NCO-groups. At 3328 cm^{-1} an absorption band of the NH-groups is observed. After immobilization of different diols the characteristic bands of the HDI-spacer molecule disappear and in the range 3410–3330 cm^{-1} an absorption band appears which is due to the hydrogen bonds of the terminal OH-groups of the immobilized diols. For preventing non-covalent bound molecules on the surface all copolymer films will be extracted by ether after HDI-activation (12 h), and by water (12 h) after diol-immobilization.

The determination of the contact angle shows that the immobilization of ethylene glycol results in a light hydrophobic surface with a contact angle of 70°. The contact angles of the surfaces with immobilized 1,3-propandiol and 1,4-butandiol were 80°–85°, which characterize a more hydrophobic surface. Probably the chain length of the diols are responsible for the different hydrophilicity of the surface. With increasing chain length of the terminal OH-groups the copolymer surface becomes more hydrophobic [5].

For the first time SO_2-plasma was used to modify the saponified copolymer surface. In Fig. 2 the S2p-spectrum of the SO_2-plasma-treated saponified copolymer surface is shown. XPS-investigations indicate the successful introduction of sulphonic acid and sulfate groups into the surface because of the characteristic photoline in the S2p-spectrum [6, 7]. The total sulphur at the surface amounts to 6.8 at %. After SO_2-plasma treatment the contact angle was comparable to the saponified polymer surface, 67°.

Of special interest are the fibronectin adsorption studies, which indicate the surface/biological system interactions. The SO_2-plasma-treated surface shows a higher value of fibronectin adhesion than the other surface modifications. The value amounts of 98% compared to the positive standard (TCPS). The immobilization of the different diols leads to a fibronectin adsorption of 65% in the case of immobilized ethylene glycol, while on the surfaces which are modified with the other diols values in the range 20–25% are reached (Fig. 3).

These results correlate with the human endothelial cell culture experiments. In Fig. 4 the cell proliferation of HUVEC on untreated and modified surfaces is demonstrated compared to the positive control thermanox.

The initial adhesion of the endothelial cells to the unmodified surface is significantly less than to the modified and control surface. Cell density increases with prolonged incubation time, both on the control surface and on the modified surfaces. Cells hardly

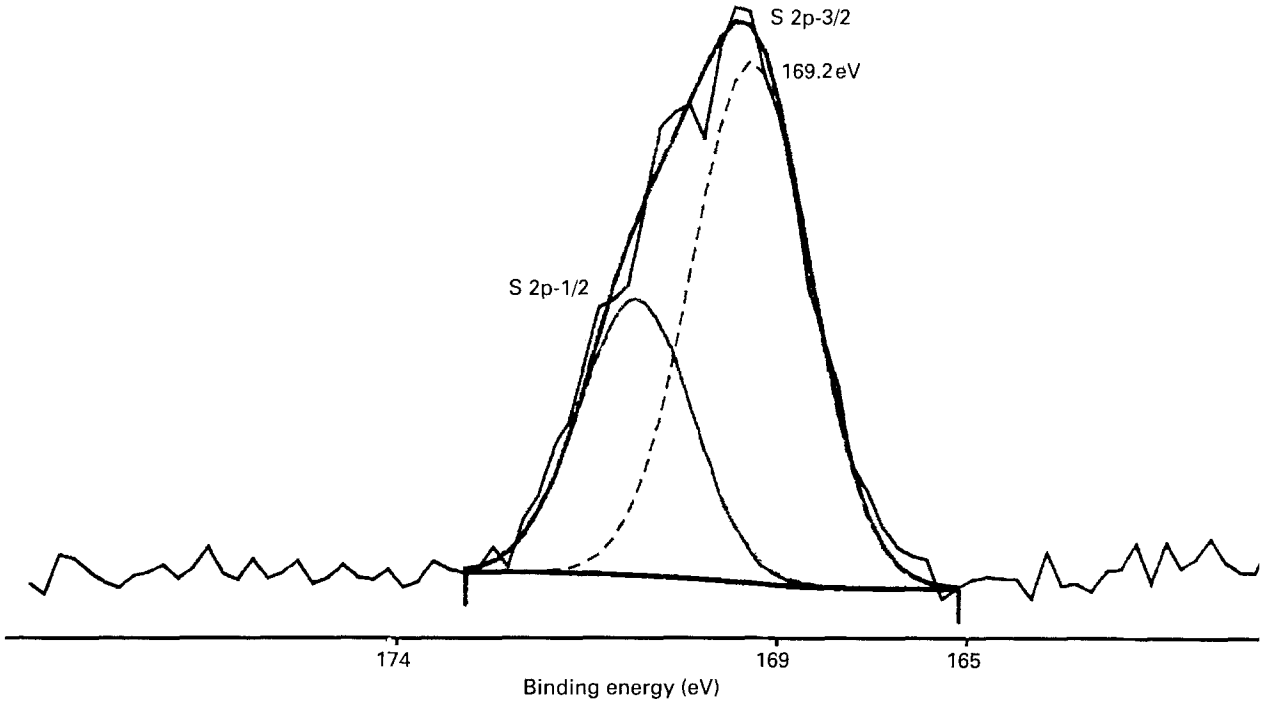


Figure 2 S2p-spectrum of the SO_2-plasma-treated copolymer surface (treatment time: 6 min.).

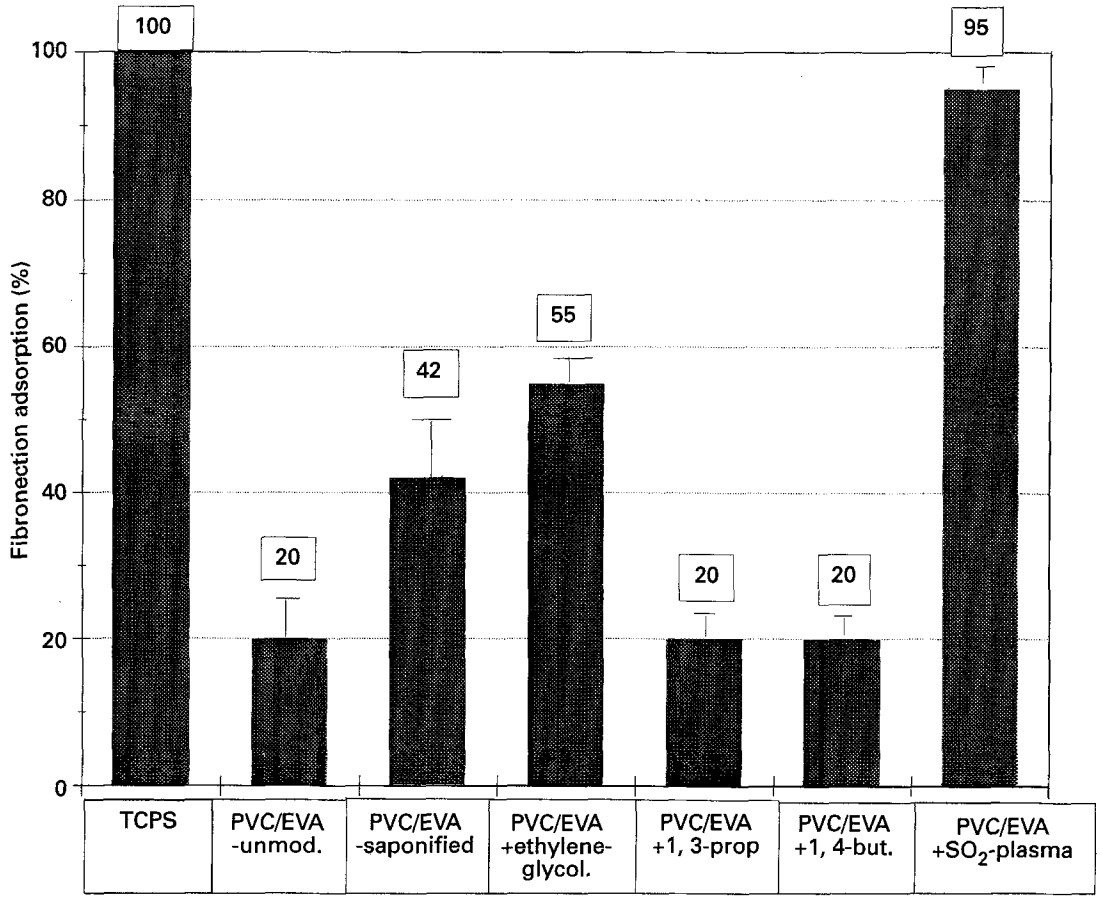


Figure 3 Fibronectin adsorption on unmodified and modified copolymer films.

proliferate on the unmodified surface. The positive control material thermanox is known for its property to promote endothelial cell adhesion and proliferation. The treatment of the copolymer film with SO_2-plasma changes the surface characteristics of the film in such a way that human endothelial cells adhere well to the modified surface. Moreover, the good interaction of human endothelial cells with the modified surface was proven by the high proliferation of the cells on such a surface, which was identical to that on the positive control and better than on the surface which had been ethylene glycol immobilized.

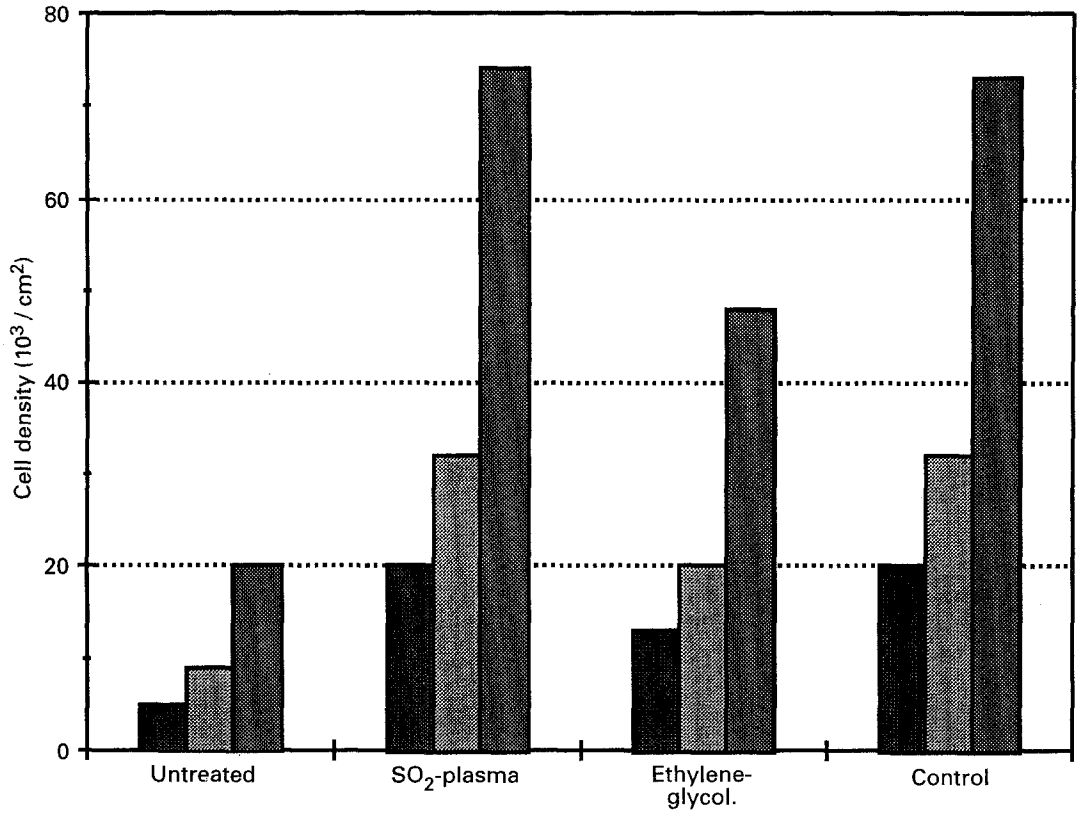


Figure 4 Proliferation of human endothelial cells on the unmodified and modified copolymer films. The positive control is thermanox. Average values of three measurements. left, day 1; middle, day 4; right, day 7

The results indicate that not only a defined hydrophilicity/hydrophobicity of the biomaterial surface but special functional groups are important for good cell compatibility. In this case the SO-functionalized surface leads to a much better cell proliferation than the OH-functionalized surface. The positive effect of oxidized sulfur species on cell adhesion has been reported [8]. The plasma treatment is an excellent method for surface modification without changing the bulk properties of the copolymer film. The observed HUVEC proliferation of the SO_2-plasma-treated copolymer indicates good tissue compatibility: an SO_2-plasma-treated copolymer surface, completely HUVEC-seeded, may also be advantageous for applications with blood contact.

Acknowledgement

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