Improvement of haemocompatibility of metallic stents by polymer coating

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An alternative to open heart surgery in treating arterial diseases causing restricted blood flow is the implantation of intracoronary metallic stents. In spite of the advances in implantation and in spite of the excellent mechanical properties of metallic stents, there are still limitations because of the thrombogenicity of the metal. We have, hence, directed our attention to the coating of metallic stents with an ultrathin polymer layer by chemical vapor deposition (CVD) polymerization of 2-chloroparacyclophan. In a second step of surface modification the poly(2-chloroparaxylylene) layer is modified by treatment with a sulfur dioxide plasma in order to obtain a more hydrophilic surface with new functional groups. The results demonstrate the stable polymer coating of the stents and the improvement of haemocompatibility after treatment with sulfur dioxide plasma. Platelet adhesion is decreased from 85% for the metal surface to 20% for the CVD-coated and sulfur-dioxide-plasma treated surface.

1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) is an accepted clinical method to treat coronary artery diseases. Its clinical efficacy, however, is limited by acute vessel occlusion in approximately 2–7% of all patients and restenosis in 15–30% during the first 6 months after application. The implantation of metallic stents is an alternative therapy to PTCA, which showed some promising results in overcoming the main limitations of PTCA [1, 2]. In spite of the advantages of the metallic stents, insufficiently high rates of subacute restenosis have still to be accepted despite a high antithrombotic pharmaceutical regime. The high thrombogenicity of metals may be explained by the positive net electrical surface potential of most of them [3, 4]. Another surface property that causes thrombogenicity is the high free surface energy of metals [5]. Furthermore, pure metallic stents show a rather rough inside surface, as shown for a Nitinol stent in Fig. 1. The high surface roughness seems to be due to the production process and, therefore, immanent for metallic stents. Some efforts have been made to coat metallic stents with polymers in order to improve their blood compatibility, e.g. Nylon [6], polyurethane [7] or silicone [8]. All these coating techniques are solvent based and therefore connected to disadvantages like pooling, meniscus and ''bridging'' effects. In addition, liquid coatings may not be applied with precise process control in particular when coating thicknesses of less than 0.5 μm are aimed at.

The chemical vapor deposition (CVD) polymerization of poly(2-chloroparaxylylene) is a vacuum-deposited polymer coating (Fig. 2), where a solid dimer, 2,2′-dichloroparacyclophan, is first vaporized at about 150 °C in a 20 Pa vacuum and the resulting gas is then heated to 600–800 °C to yield the monomer 2-chloroparaxylylene. In the last step, the monomer gas is adsorbed as it polymerizes on the substrate at temperatures of ca. 80 °C. Furthermore poly(paraxylylenes) show extraordinarily high biostability compared to common polymer coatings. Although the CVD-coating is already used for biomaterial applications [9, 10] and seems to be ideal for the coating of metallic stents there are still limitations because of its insufficient blood compatibility. The aim of this work is to functionalize poly(paraxylylene)-coated stents in order to combine their biostability with improved biocompatibility (Fig. 3). Therefore we used a sulfur dioxide plasma treatment of poly(2-chloroparaxylylene) coated metal surfaces. It is known [11] that sulfur dioxide plasma can be a powerful tool to improve critical surface characteristics of hydrophobic polymers expected to be used in direct blood contact.

2. Materials and methods

2.1. CVD polymerization

CVD was carried out in a self-designed installation, which allowed high freedom in selecting the process parameters, e.g. vaporizing, deposition and condensation temperature, system pressure, argon flow, sample rotation, deposition rate and thickness. The coatings were carried out onto stainless steel foils and self expanding Nitinol stents of Angiomed (Karlsruhe, Germany).

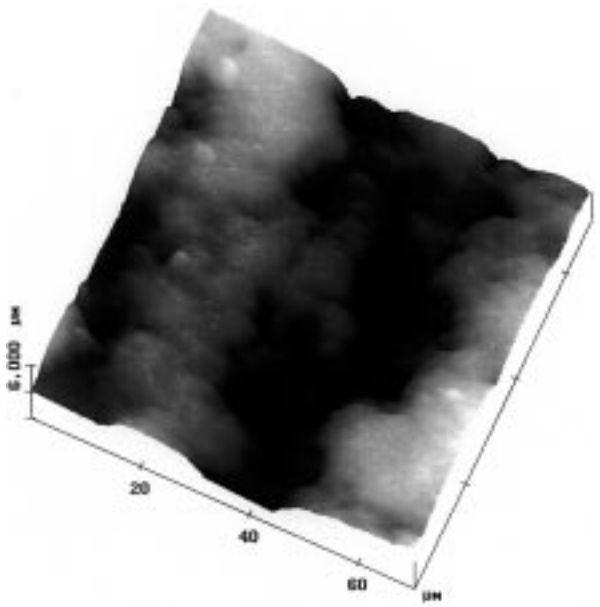


Figure 1 Atomic force micrograph of the stent surface (inside).

2.2. Microwave plasma treatment

Plasma treatment was carried out with a hexagon plasma unit of Technics Plasma GmbH (Kirchheim, Germany). An argon plasma with a plasma power of 300 W was used for 60 s. The gas flow was 20 ml min^{-1} at a pressure of 20 Pa.

2.3. Physical and chemical surface characterization

All X-ray photoelectron spectra (XPS) were recorded on an X-Probe™ 206 spectrometer (Surface Science Instruments, Mountain View, CA). An aluminum anode producing AlK_α X-rays at 1486.6 eV was used as an X-ray source. The binding energies were referenced to hydrocarbon at 285.0 eV. The emission angle of electrons was set at 55° with respect to the sample normal, which results in an information depth of about 6 nm.

Fourier transform infrared (FTIR) spectra were obtained with a Nicolet 710 spectrometer (Offenbach, Germany) using the attenuated total reflection technique.

Contact angles were measured using the sessile drop method with pure water at room temperature on a G40 system (Krüss, Hamburg, Germany).

Fibrinogen and the corresponding monoclonal antibodies for the enzyme-linked immunosorbent assay (ELISA) studies were purchased from Sigma (Deisenhofen, Germany).

The scanning electron microscopy (SEM) images were done with a Leica S 360 microscope (Bensheim, Germany); all samples were sputtered with gold.

Atomic force microscopy (AFM) was carried out with a NanoScope III (Fa. Digital Instruments Incorporation, Santa Barbara, CA). All images were recorded using the tapping mode™.

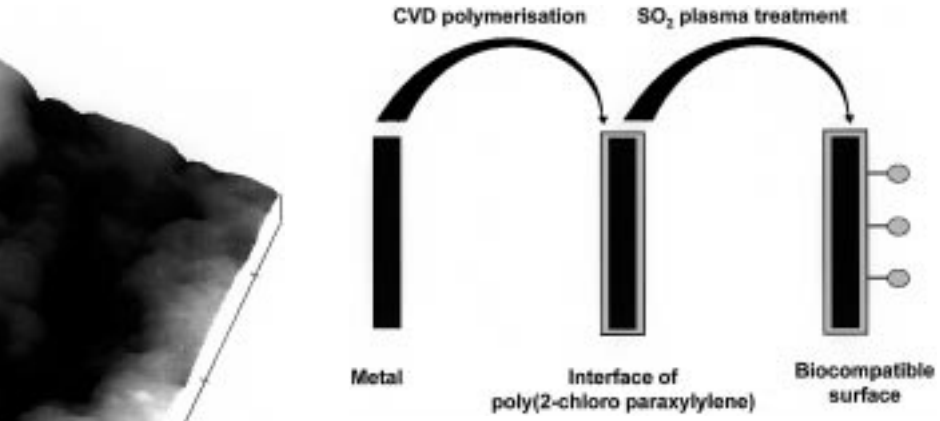
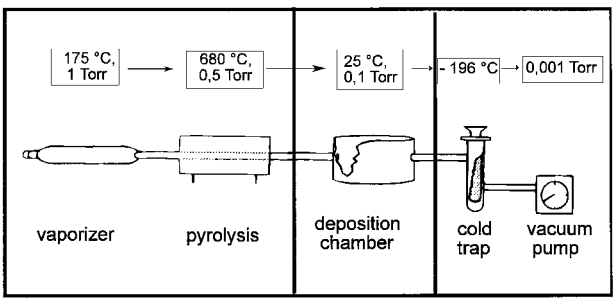


Figure 3 Concept of the metal stent modification.

2.4. *In vitro*-measurements

For determination of platelet adhesion, four discs of each material to be tested were placed into a chamber and polypropylene was chosen as positive control. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were added to two of the samples and to each positive control, respectively. After 30 min incubation, the wells were washed. Primary antibody solution was added to all wells



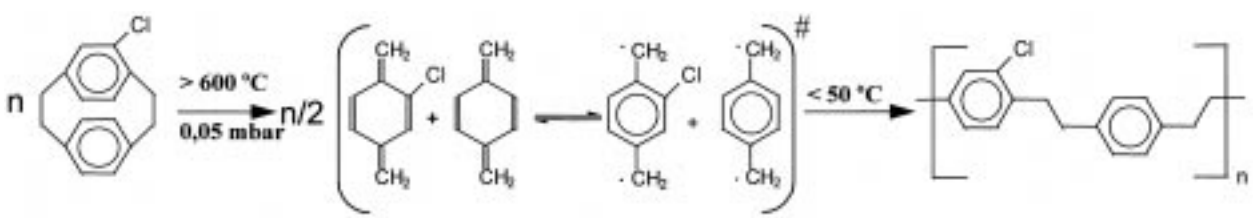


Figure 2 Mechanism of CVD polymerization.

and was incubated for 60 min. After subsequent washing the same procedure was repeated with the secondary antibody solution. Afterwards, the substrate solution was added and incubated for 10 min; upon addition of 2 M HCl the chromogenic reaction was interrupted. Optical density was determined spectrophotometrically (SLT Classic-Reader, SLT, Salzburg, Austria) at 492 nm. The values obtained from incubation with PPP were corrected using those obtained with PRP.

Incubation was performed at 37 °C and the samples were rotated at 60 U min^{-1} (Incubator 1000/Polymax 1040, Heidolph, Germany). Washing procedures were carried out three times using phosphate-buffered saline (PBS). Primary and secondary antibody solutions contained monoclonal mouse anti-CD 42b immunoglobulin G (IgG) and peroxidase-conjugated monoclonal goat anti-mouse IgG, respectively, both diluted 1:1000 in PBS containing 1% bovine serum albumin (BSA). For substrate solution, *o*-phenylenediamine dihydrochloride in citrate buffer was always freshly prepared according to manufacturer's (Sigma Immunochemicals) instructions.

For the MTT-test, the cell line L929 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) was used and maintained in RPMI 1640 medium (Bio Whittacker, Verviers, Belgium) supplemented with 10% fetal calf serum (FCS) (Bio Whittacker, Verviers, Belgium). The cells were incubated in a humified atmosphere of 7.5% CO_2 at 37 °C. Test medium was prepared by extraction in redistilled sterile water over 72 h at 37 °C, the ratio between the surface area of the material and and the volume of water being 2 cm^2 ml^{-1}. The incubation time was 24 h. 3-5(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Deisenhofen, Germany) was used in a final concentration of 0.5 mg ml^{-1} in sterile buffered saline. The solution was filtered through a 0.22 µm filter to remove any formazan crystals. After the incubation period of 2 h, reaction was terminated by adding 100 µl sodium lauryl sulfate; 400 µl of acid isopropanol was added to each well to solve the formazan dye and the optical density was measured with 570 nm test wave length and a 630 nm reference wavelength using a microplate reader.

3. Results

3.1. Adhesion behavior

The homogeneity of the CVD coating was investigated by means of SEM. Fig. 4 shows a part of a Nitinol stent compared with a stent coated by CVD polymerization. The coating shown covers the stent in its complete shape. Furthermore no bridging of sites with only little distance was found. The distance of the two blocks, shown in Fig. 4, is approximately 30 µm. The XPS element content of the poly(2-chloroparaxylylene) coating was in good accordance with the expected value.

By means of AFM the film thickness was determined to be 400 nm. The contact angle of 95° shows the hydrophobic character of the coating. The coating does not change the dilatation behavior of the stents. In order to investigate the mechanical stability of the coating upon stent dilatation the coated stents were reloaded on the detachment system and were thrown up again. The CVD coated stents as well as the CVD coated and plasma treated stents tolerate this procedure without destruction of the coating. Although superficial cracks were observed in places of high mechanical stress no deeper destruction of the polymer coating down to the metal surface was found, as shown in Fig. 5.

3.2. Sulfur dioxide plasma treatment of poly(2-chlorineparaxylylene) coated metal surfaces

As shown in Fig. 3 after CVD coating with 2-chloroparacyclophan the stents were treated with sulfur dioxide plasma for 10, 30, 180 and 360 s. The sulfur dioxide plasma treatment has significant influence on the main surface properties. The contact angle shown in Fig. 6 decreases to 15° and seems to be almost independent of the plasma treatment time. The chemical surface composition is changed significantly. In particular, the sulfur content of 3.7 ± 0.4 at % is remarkably high. The high resolution S_{2p} spectrum (Fig. 7) distinguishes between the reduced and the oxidized sulfur state; the ratio of both is significantly dependent on the plasma treatment time.

Furthermore, we investigated the influence of the plasma modification on the adsorption of the human blood protein fibrinogen by means of the ELISA technique. The fibrinogen adsorption on poly(2-chloroparaxylylene) was determined to be 95% compared to tissue culture polystyrene (TCPS) as reference material and decreases to 54% after 30 s plasma treatment time (see Fig. 8). This decrease in fibrinogen adsorption seems mainly to be a consequence of the enhanced hydrophilic character of the polymer film after plasma treatment and may also be provoked by a change of the surface topology, as shown by the AFM images presented in Figs 1 and 9.

In order to exclude a potential cytotoxicity of the coatings, they were investigated in direct and indirect contact with murine fibroblasts. Cell integrity was studied by means of vital staining as well as by means of the MTT test. This tetrazolium-based colorimetric assay is designed for the spectrophotometric quantification of cell growth and vitability. Murine fibroblasts show no inhibition of their cell growth, neither in direct nor in indirect contact.

In order to investigate the influence of the CVD coating and the subsequent plasma treatment on blood compatibility, partial thromboplastin time (PTT), activated partial thromboplastin time (APTT) and platelet adhesion studies were performed, as shown in Fig. 10. The partial thromboplastin time (PTT) is the clotting time of recalcified citrated plasma after addition of partial thromboplastin, which is a phospholipid suspension usually extracted from tissue thromboplastin, the homogenate from mammalian brain or lung. Shortening of the PTT following contact with a material indicates activation of the contact phase of blood coagulation. A prolonged PTT suggests a deficiency in any of the plasma coagulation factors such as fibrinogen, factors II, V, VII, IX, X, XI or XII, but not factors VIII or XIII. Using additional activating substances the test is known as the activated partial thromboplastin time (APTT).

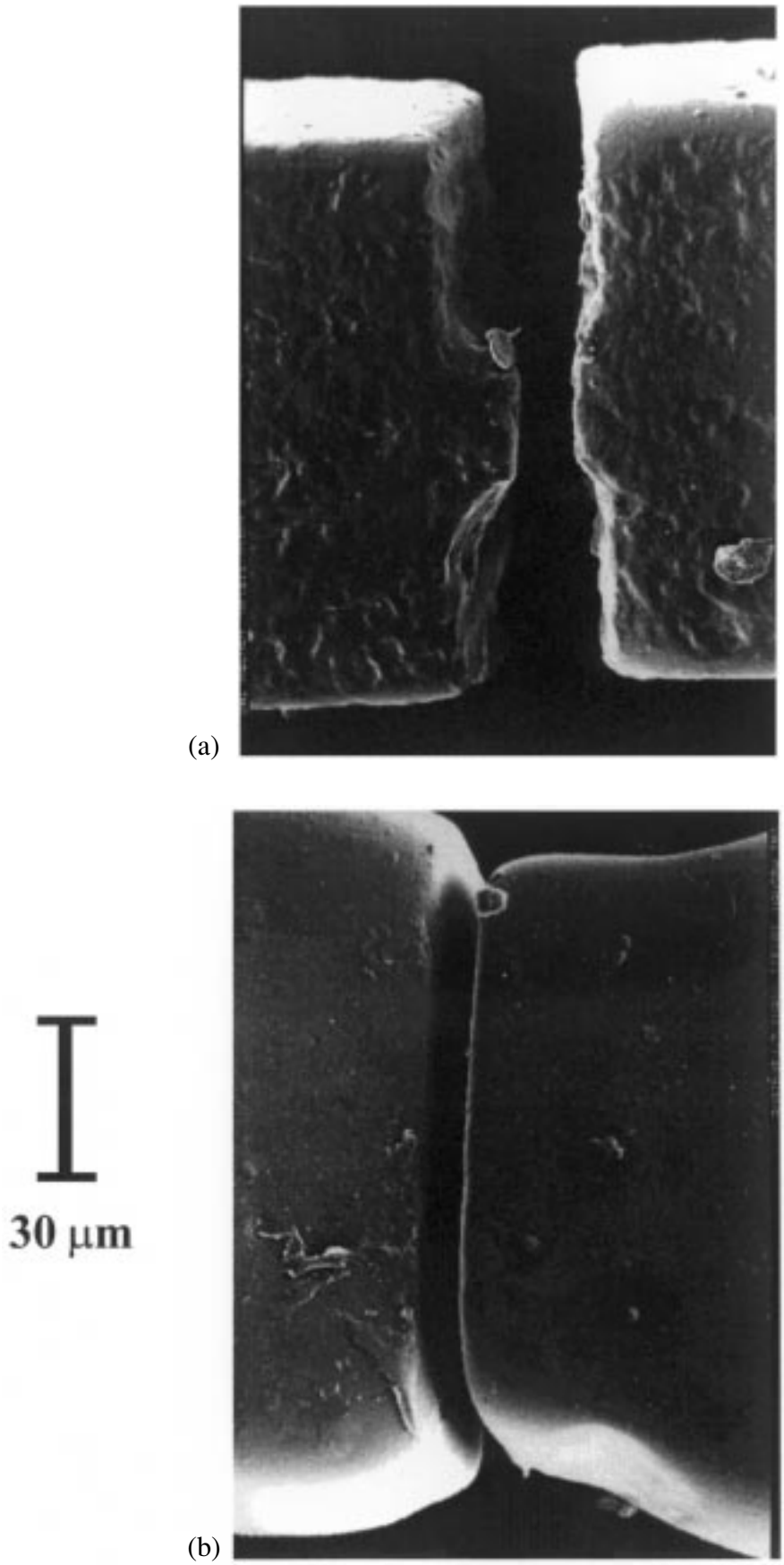


Figure 4 SEM of the (a) uncoated and (b) poly(2-chloroparaxylylene)-coated stent.

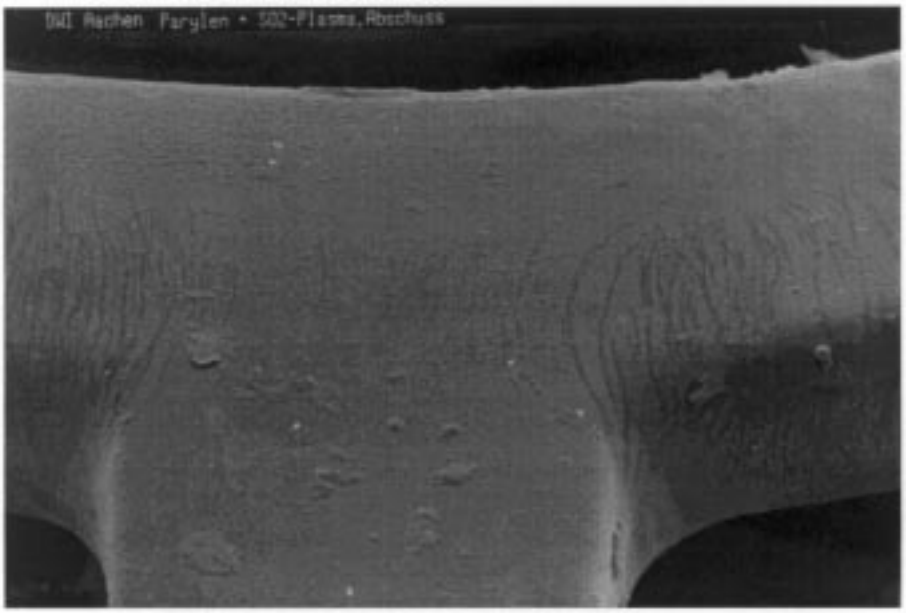


Figure 5 SEM of the poly(2-chloroparaxylylene)-coated stent after dilatation.

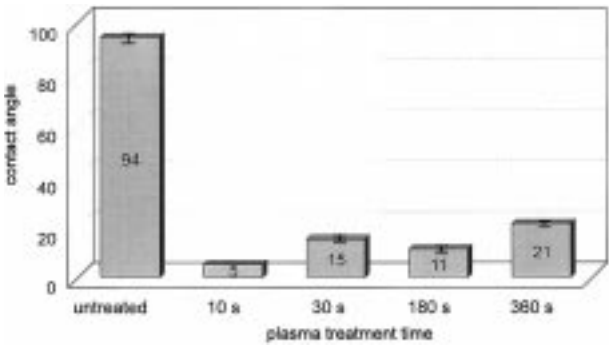


Figure 6 Contact angle of the CVD-treated and SO_2-plasma treated metal surface.

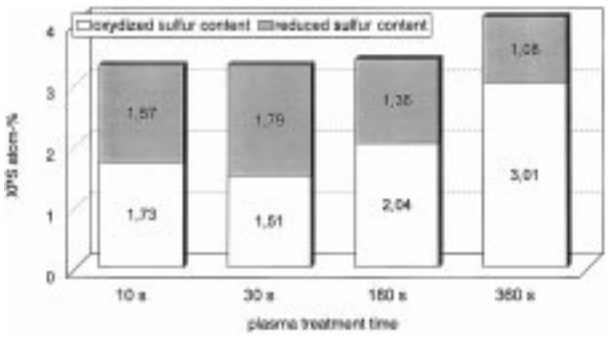


Figure 7 XPS of the sulfur content of the CVD-coated and SO_2-plasma treated metal surface.

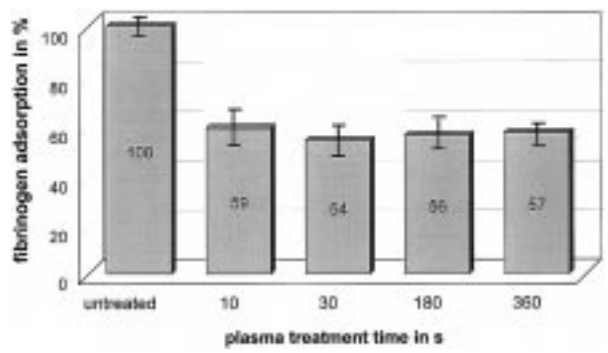


Figure 8 Fibrinogen adsorption of the CVD-coated metal surface after SO_2 plasma treatment.

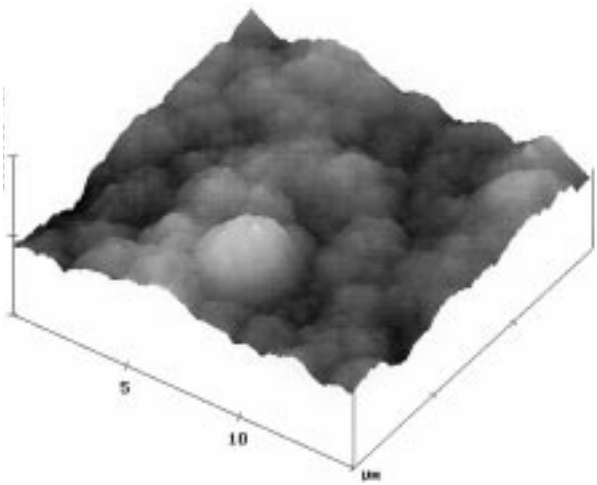


Figure 9 AFM of the poly(2-chloroparaxylylene) surface.

The APTT has a lower sensitivity in the *in vitro* evaluation of blood–material interactions because the activating substances may mask the activation caused by the device or its component materials [12]. Compared to the free metal surface the poly(2-chloroparaxylylene)-coated samples show a slight extension of APPT and PTT. Nevertheless, after 10 s sulfur dioxide plasma treatment of the CVD-coated samples, an APTT and PTT prolongation of more than 50% was found, which is slightly increased with increasing plasma treatment time. Platelet adhesion occurring on biomaterials in direct contact with blood is known to cause thrombogenic as well as restenotic processes. A minimum platelet adhesion may therefore be a critical characteristic of every potential biomaterial. The platelet adhesion, measured by means of ELISA was determined to be 85% of the poly(propylene) standard for the stainless steel surface and 98% for the poly(2-chloroparaxylylene), as shown in Fig. 11. After sulfur dioxide plasma treatment a significant decrease of the measured platelet adhesion to 20% of the poly(propylene) standard was observed.

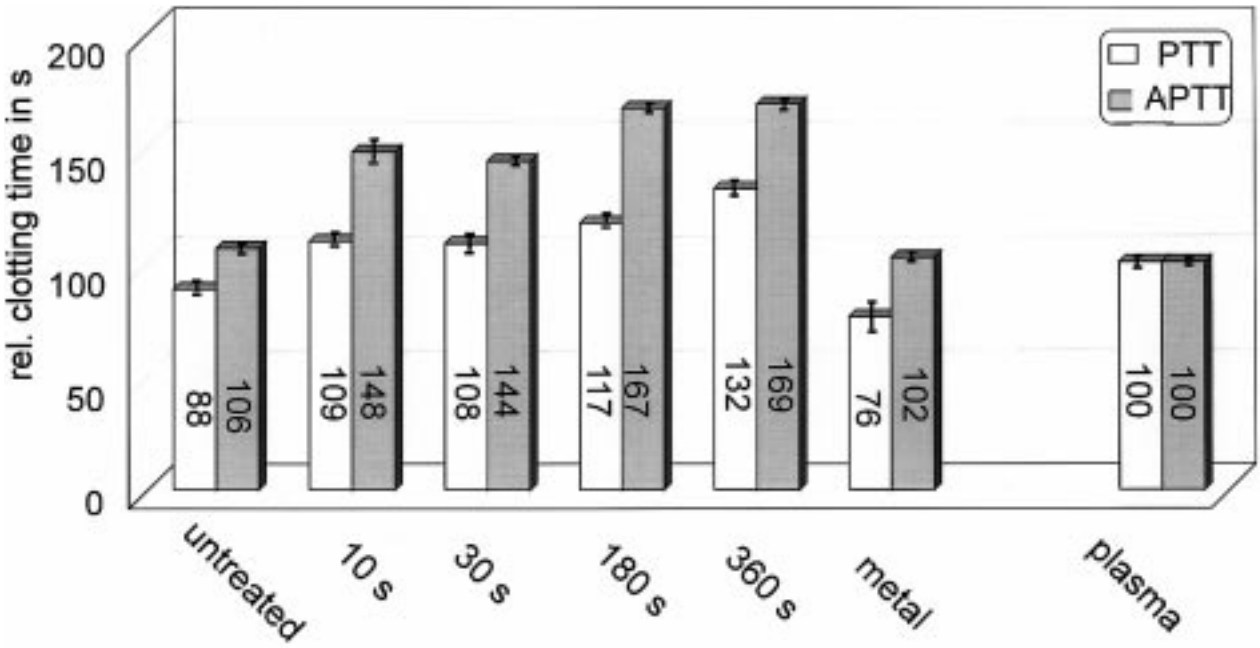


Figure 10 PTT and APTT of the CVD-coated surfaces after SO_2 plasma treatment.

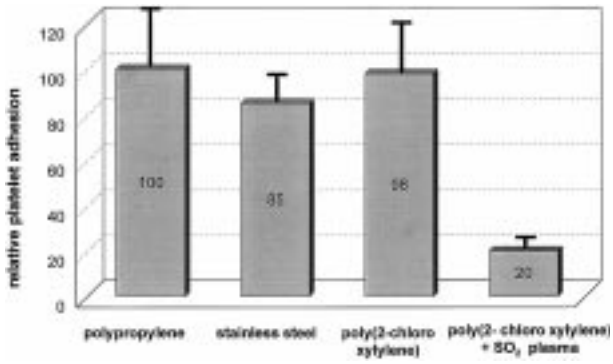


Figure 11 Platelet adhesion measured by ELISA.

4. Conclusions

This investigation shows that a coating process based on the CVD polymerization of poly(2-chloroparaxylylene) is a promising approach to introduce polymer layers onto metallic stent surfaces. The adhesion of the polymer films on the metal shows high mechanical stability in stent dilatation experiments. The CVD coating shows important features such as homogeneous coverage even in very thin applications (film thickness $< 0.5\,\mu m$) and the ability to coat complex geometries. Because the conversion from monomer gas to polymer films is a direct one, no solvents, plasticizers, catalysts, or accelerants are used. The resulting films have no cytotoxic properties. The treatment of poly(2-chloroparaxylylene)-covered surfaces with sulfur dioxide plasma leads to a change in the surface characteristics like contact angle, surface composition and topology and causes APTT and PTT prolongation as well as a decrease of platelet adhesion. These *in vitro* parameters indicate a remarkable improvement in the haemocompatibility of the metal surfaces.

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