Plasma assisted development of new blood compatible fluorocarbon polymer materials.

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Abstract: The various plasma-chemical techniques are used for the surface functionalization and regulation of the biological characteristics of fluorocarbon polymers to enhance their blood compatibility. Modifications of the surface morphology and chemical structure to improve thromboresistive properties of polymers by plasma etching and deposition of biocompatible materials are demonstrated and discussed.

Keywords: fluorocarbon polymers, plasma modification, immobilization of proteins, blood compatibility.

1. Introduction
Fluorocarbon polymers find expanding applications in various areas of medicine. Depending on particular applications, the biocompatibility of fluorocarbon polymers can include various requirements, which can be sometimes contradictory to each other. Thus, in the case of artificial vessels, drainages, biosensors or catheters, the interaction of the polymer with a biological medium should be minimized. In contrast, in the majority of orthopedic applications, the active interaction and integration of an implant with a tissue is required. Various plasma assisted techniques provide a wide range of target-oriented modifications of biological functions for polymer materials [1]. In this work, attention was focused on the use of plasma assisted techniques for improvement of the blood compatible properties of fluorocarbon polymers.

2. Plasma treatment and immobilization of blood compatible materials.
Plasma and vacuum ultraviolet treatment in oxygen containing gases was used to increase the wettability of fluorocarbon polymers and create the mosaic hydrophobic/hydrophilic structure on the polymer surface. As a result of this modification irreversible adsorption of blood proteins and aggregation of platelets was significantly reduced [2]. Vacuum ultraviolet (VUV) treatment (Xe plasma lamp, wavelength 147 nm) at different air pressure was used to create mosaic hydrophilic/hydrophobic structures on the surface of PTFE. Figure 1 shows the relative surface concentrations of oxygen and nitrogen on the surface of PTFE obtained after VUV modification. The maximum concentration of oxygen relatively to carbon [O]/[C] = 0.54 (about one O atom per two C atoms) was obtained after VUV irradiation in vacuum and post reactions with ambient air. According to ATR FTIR spectra a new band at 1775 cm⁻¹ was appeared after VUV irradiation of PTFE indicating the formation of hydrophilic polar groups CF₂COOH on the surface of PTFE. As a result of VUV-induced photooxidation, the hydrophilic (CF₂COOH)/hydrophobic (CF₃) mosaic structure was formed on the initially hydrophobic (CF₃) PTFE surface. The number of aggregated platelets after VUV modification at optimum conditions was found to be about twice less than that observed for untreated PTFE. It means that the thromboresistivity of PTFE was significantly improved as a result of VUV treatment.

The treatment in argon plasma was used for post grafting the Pluronic™ 120 copolymer, which is known as a blood compatible material, onto the surface of PTFE. The Pluronic™ 120 triblock copolymer (PEO/PP/PEO) was initially deposited onto the polymer surface by physical adsorption from the solution and then immobilized by treatment in a low-pressure argon plasma. Plasma-assisted techniques can be also employed for the immobilization of different PEO-polymer onto fluorocarbon surfaces. Among the procedures compared, the most promising results were obtained by O₂ plasma initiated graft polymerization of PEO - monoacrylate monomers from CHCl₃ solutions [3]. According to the XPS data this procedure leads to the formation of stable PEO layers of
about 4 nm thickness. The grafted PEO layer shows very low reeding water contact angles (from 10 to 14 degrees). Figure 2 shows the influence of the PEO-grafted fluorocarbon layer on the kinetics of protein adsorption. Fibrinogen adsorption data on the untreated fluorocarbon surface and on the fluorocarbon layer after modification by PEO are compared. The time dependent increase of the adsorbed amount was derived from ellipsometric in-situ measurements. A comparison of the adsorbed amounts clearly shows the expected efficiency of the grafted PEO layers in reducing protein adsorption.

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3. Plasma assisted structuring and deposition.

As a rule, plasma etching changes the polymer surface morphology, which plays an important role in blood compatibility [2]. For example, surface smoothing due to plasma etching upon treatment in oxygen- and fluorine containing plasmas positively affects blood compatibility because it decreases the probability of thrombosis at surface irregularities in a blood stream. We have studied the dependence of irreversibly adsorbed human serum albumin (HSA) on the mean roughness in the nanoscale region. The mean roughness ($R_q$) and contact angles ($\theta$) were changed by CF$_4$ plasma treatment of PTFE at different conditions (see Figure 3 and legend to figure).

Figure 3. SEM images of PTFE untreated, $R_q=24$ nm, $\theta=111^\circ$ (a), treated in RF plasma (13.6 MHz) in CF$_2$, 10min, $R_q=31$ nm, $\theta=138^\circ$ (b), 20min, $R_q=37$ nm, $\theta=124^\circ$ (c) and 30min, $R_q=67$ nm, $\theta=86^\circ$.

Thereby, plasma assisted structuring makes possible to minimize the irreversible protein adsorption and by that to improve the blood compatibility of polymer material on the first step of blood coagulation cascade [4].

Carbon layers doped with nitrogen and oxygen were deposited on PTFE film by plasma sputtering of graphite target in the mixture of Ar with air [5]. The surface atomic composition as well as chemical and electronic structure of carbon layers was studied by XPS, FTIR and Raman spectroscopy. Figure 6 shows XPS survey spectra of PTFE film before and after deposition of carbon layer. For untreated PTFE film the XPS spectrum consists of Fls peak (688.2 eV) and less intensive Cls peak (291.4 eV). In the XPS spectrum of the film obtained after 15 min deposition one can see intensive Cls peak (285.0 eV) as well as Ols (532.1 eV) and Nls (399.8 eV) peaks. The important point is that no significant Fls peak was observed in the XPS spectrum of the carbon layer obtained after 15 min of carbon deposition. It means that pin-hole-free carbon
coating with the thickness more then 30 Å is formed on the PTFE surface already after 15 min of graphite sputtering. The atomic surface concentrations calculated from measured integral intensities of XPS peaks for as deposited carbon film are: 72.2 at% C, 10.5 at% N and 14.0 at% O.

Figure 6. The XPS survey spectra of PTFE film before (1) and after carbon deposition (2).

The Raman spectra of PTFE films before and after deposition of carbon layer with different thickness are shown on Figure 7. The characteristic bands of PTFE film: at 734 cm\(^{-1}\) assigned to stretching vibration of C-F, and two bands at 387 cm\(^{-1}\) and 293 cm\(^{-1}\) attributed to deformation vibration of C-C in polymer chains, are still detectable through the carbon layer with the thickness 225 nm deposited during 1 h but was not appeared in the spectra after deposition of carbon layer with the thickness 450 nm (2 h of deposition). This result is in agreement with the optical probing depth for Raman spectroscopy determined by the absorption coefficient \(a\) for carbon layer at our excitation wavelength 633 nm \(d=1/(2a)\). Taking the optical constants for different carbons we can estimate the Raman probing depth in our case as 100-200 nm that is much higher than the XPS analyzing depth (about 3 nm). The Raman spectrum of carbon coating consists of two broad bands at 1575 cm\(^{-1}\) and 1360 cm\(^{-1}\) usually detected in amorphous and diamond-like carbon films. According to Wagner's model developed for Raman scattering of carbon and polycrystalline diamond films, the peak at 1575 cm\(^{-1}\) has to be assigned to graphite-like \(sp^2\)-bonded carbon (G peak) while the scattering in low-frequency region around 1300 cm\(^{-1}\) has to be interpreted in terms of scattering by \(sp^3\)-bonded carbon plus a possible contribution of disordered \(sp^2\)-bonded carbon (D peak).

The blood compatibility of this coatings was tested by platelet adhesion analysis. Platelet adhesion patterns were investigated by SEM. All samples after platelet adhesion were decorated with copper (thickness 30 nm). For each sample 25 areas of \(400 \mu\)m\(^2\) were randomly chosen on the surface contacting with platelets rich plasma. Then we qualified the total number of platelets \(N_{\text{tot}}\) and platelet numbers \(N\) in the following four morphological classes: single-nonactivated cells (1), slightly activated deformed cells and pseudopodical cells (2), spread-fully spread platelets (3), aggregates - two or more aggregated platelets (4). The key issue for blood-compatible materials is the ability of implant surface to prevent thrombus formation. It is generally known that the platelet adhesion process controls the formation of a thrombus. The adhesion process is believed to run in several stages: platelet attachment to the surface, activation, pseudopodia formation, spreading, and aggregation. The release of intracellular components from adhered or fully spread platelets (ADP, Ca\(^{2+}\), serotonin, etc.) promotes further platelet adhesion, aggregation and finally thrombus formation. The activation of platelets can be estimated by their morphology analysis. The stronger the impact of the material on platelets, the more adhered cells are activated, spread or aggregated. According to SEM images presented in Figure 8 doped DLC coating leads to the reduction of
platelet adhesion and minimization of activation processes. In comparison with the surface of original PTFE that exhibits intensive platelet adhesion spreading and aggregation (Figure 8 (a)), DLC coating shows substantially fewer single nonactivated or slightly activated adhered platelets (Figure 8(b)).

4. Plasma assisted biological functionalization and immobilization of proteins.

Human thrombomodulin (hTM) is an endothelial cell-surface glycoprotein which has very effective anticoagulant properties. It binds to thrombin which then changes from a procoagulant protease into an anticoagulant. The cleavage of fibrinogen which leads to the formation of a fibrin clot, and the platelet-activating characteristics of thrombin, are inhibited. Microwave CO₂ plasma treatment in combination with vapor-phase grafting of poly(acrylic) acid, has been shown to be an effective preparation for the covalent coupling of human thrombomodulin [6]. The surface atomic composition and chemical of carbon layers was studied by XPS and ATR FTIR spectroscopy The data obtained by ATR FTIR spectroscopy are summarized in Figure 9.

![Figure 9 Absorption peaks after the treatments: (1) before treatment; (2) plasma treatment; (3) AAc graft polymerization; (4) hTM immobilization.](image)

New absorption peaks with maxima at 1881 and 1725 cm⁻¹ can be attributed to COF and –CF=CF– groups which were generated through the plasma treatment. The lowest process pressure led to the highest yield of COF groups after an optimum treatment time of 30 min. The characteristic bands for acrylic acid occur at 3200 cm⁻¹ (OH stretch), 1710 cm⁻¹ (C=O stretch) and 800 cm⁻¹ (COOH out-of-plane deformation). The decrease of the 1881 cm⁻¹ absorbance band probably can be attributed to a reaction of acrylic acid with COF groups. The immobilization of hTM led to the appearance on the surface amide, amine and N`R groups as it was shown by XPS analysis. The hTM immobilized on the PAAc-grafted PTFE surface exhibited the expected activity in converting thrombin from a procoagulant protease to an anticoagulant form, when assessed by a protein C activation assay. Thus, by using plasma assisted techniques we could create biologically active layers on the surface of fluorocarbon polymers.

5. Conclusions.

Plasma-assisted immobilization of biological substances in combination with other plasma modification methods provides a promising technique for the development of a new generation of blood compatible materials.

References