Protein coatings onto the polyvinylidene fluoride surface using microwave plasma tratment

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The polyvinylidene fluoride film was coating with proteins to increase the biocompatibility and to create new sensitive surface to environment. The two steps procedure to obtain such surfaces consists in the surface activation by CO_2, N_2 or N_2/H_2 plasmas leading to the attachment of short carboxyl or amino groups then in the second step, the proteins (triglycine and protein A) were physiadsorbed or chemically grafted onto the carboxyl or amino-surface. These new surfaces have been characterized by wettability measurements, ATR-FT-IR, and SEM/EDX methods. The results proved that PVDF is a good substrate for proteins coating which further lead to increased functionality and biocompatibility of the hydrophobic surface.

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1. Introduction

Immobilization of proteins onto polymer surfaces is of great interest for many applications, particularly in developing medical implant materials[1], bioseparators [2] biosensors [3] etc. Therefore, many efforts have been made to develop methods of protein immobilization [4] in order to improve some properties or to obtain films with new high performance properties as can be seen from the following examples. Whey protein films and coatings possess excellent oxygen flavor and aroma barriers as well as good oil barrier properties, comparable to those of synthetic polymer polyvinylidene chloride and ethylene vinyl alcohol films [5,6,7]. The coated surfaces were found to be most suitable for antibody immobilization [8]. Solid-phase assays that involve binding proteins or ligands immobilized on test tube surfaces or similar matrices can substantially improve the simplicity, cost effectiveness, and speed of performance of immunoassays. Approaches to attach antibodies to a surface include passive adsorption, covalent attachment, and affinity binding [9]. Directed adsorption might also be achieved by use of protein biolinkers in combination with specifically selected substrates. Certain plastics, most notably polystyrene, are favored for use as substrates in immunoassays [10].

It is well-known that the number and strength of attractive interactions across a coating-substrate phase interface determine the adhesion between the two phases. The number of attractive interactions across the interface can be increased by improving intermolecular contact between the coating and substrate. The strong hydrophobic property of PVDF restrained it from promotion and application. It has been proposed that biofunctional materials (e.g., proteins) can be deposited from solutions onto different substrates (i.e., sheets of materials) to modify the surface properties of the substrates and/or serve as a functionalized surface that can be chemically reactive [11,12]. However, many of the economically desirable substrates (e.g., substrates formed of polymers such as polyolefins) have surfaces that are unsuitable for the rapid and inexpensive deposition of biofunctional materials, especially when durable, tightly-bound coatings of satisfactory adherence are desired. It has also been proposed that surfaces of these substrates can be modified to improve the adherence of biofunctional materials. Some suggested surface modification techniques involve: 1) irradiating the surface of a polymeric material in the presence of oxygen to create active sites and then chemically grafting a polymer onto the active sites; 2) providing an organic surface coating by plasma discharge in the presence of a plasma polymerizable, halogenated hydrocarbon gas; and 3) treating (e.g., oxidizing) the surface of a substrate so that it has a hydrophilic character with a high amount of cation-exchange groups. Such treatments can be complex, expensive, environmentally unsuitable, leave trace amounts of undesirable compounds, unsuited for high-speed manufacturing processes, and/or cause degradation of the substrate. In particular, a trend toward increasing environmental awareness and government regulation in the areas of air, water, product and food quality make some of these treatments relatively unattractive. Furthermore, these treatments fail to address the need for a practical method of depositing a durable, tenacious coating of proteins on the unmodified surface (or surfaces) of a relatively inert, hydrophobic substrate. Thus, there is still a need for a simple method of producing a durable and chemically reactive protein coating on an unmodified, relatively inert, hydrophobic substrate. A need also exists for fibrous and/or apertured film-like substrates formed from a relatively inert, hydrophobic material (e.g., a polyolefin, fluoropolymers) that are

coated with a readily available, inexpensive, natural, renewable and nontoxic material, especially if such a coated material can be produced in a high-speed manufacturing process. Meeting these needs is important since it is both economically and environmentally desirable to substitute relatively complex chemical surface modification and/or functionalization of inexpensive (and often recyclable) substrates with inexpensive, readily available natural materials. Such polymers are frequently subjected to surface treatments, such as corona discharge plasma [13], to enhance their binding capacity, typically by increasing surface hydrophilicity by the addition of carbonyl groups. However, while this approach does result in increased adsorption of total protein, it may also result in a significant decrease in biological function due to distortion of the native protein structure [14].

To make PVDF films hydrophilic, many researches focused on plasma treatment [15]. Surface activation has been focused on creating functional groups capable of preferential adsorption of biologically active species (proteins, enzymes, cells, drugs, etc.).

The study is focused on the adsorption/grafting of biomolecules such as proteins onto a transducer such as PVDF. In this paper, the PVDF was undergone to successive surface modification by microwave plasma activation (pre-treatment) in different atmospheres, followed by coating with proteins by both direct adsorption or by grafting. The characterization of the modified surfaces in respect with unmodified one was done by different investigation methods such as contact angle measurements, ATRFT-IR and, SEM/EDX. The chemical grafting of the biomolecules is supposed to improve the life time and the sensitivity.

2. Experimental

2.1. Materials

Polyvinylidene fluoride films (PVDF) (thickness of 0.25 mm from Goodfellow, UK) semi-crystalline, semi-opaque, density: 1.76 g/cm^3.

Protein A (purchased from Sigma Chem) is a 42 kDa MSCRAMM and pI 5.3, surface protein originally found in the cell wall of the bacteria *Staphylococcus aureus*. It is in the powder form obtained by freezing-drying and is salt free. Protein A is a very stable receptor for the cell surfaces, which exists as a single polypeptidic chain containing four repetitive domains rich in aspartic and glutamic acids free of cisteine [16].

Triglycine (Glicil-glicil-glicine) was purchased from Sigma Aldrich (with molecular structure: $C_6H_{11}N_2O_4$) is ultrapure (>99 %) and its molecular weight is 189.17

2.2. Microwave plasma treatment

Films of polyvinylidene fluoride (PVDF) have been treated in a microwave plasma, using different discharge gases (i.e. CO_2, N_2, and N_2/H_2 1:3), for the treatment conditions established as being the most appropriate for functionalizing the PVDF surface, in order to further coat/grafting it with protein layers.

The experimental set-up is given in Fig. 1.

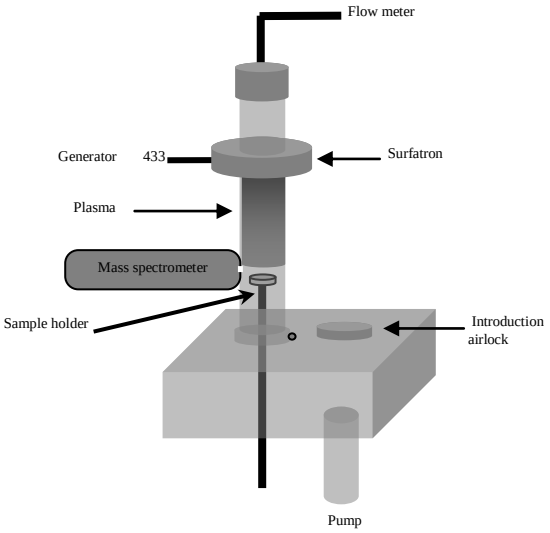


Fig. 1. The experimental set-up for plasma treatment of the PVDF surface.

A cylindrical MW plasma column is generated using a surfatron. The sample holder allows to locate the sample either in direct contact with a 2.45 GHz microwave plasma, or downstream, where only long-lived species (largely neutrals) in the plasma effluent contribute to the process chemistry. The optimal plasma parameters have been previously established [17, 18] and these are:

I) Plasma CO_2 : Q = 10 sccm, P = 50W, t = 30 sec, d = 10 cm;
II) Plasma N_2: Q = 10 sccm, P = 50 W, t = 60 sec, d = 10 cm;
III) Plasma N_2/H_2: in the ratio of 25/75: Q = 10 sccm, P = 50 W, t = 60 sec, d = 10 cm

These types of plasma activation lead to acidic, amphoteric and basic surfaces, respectively.

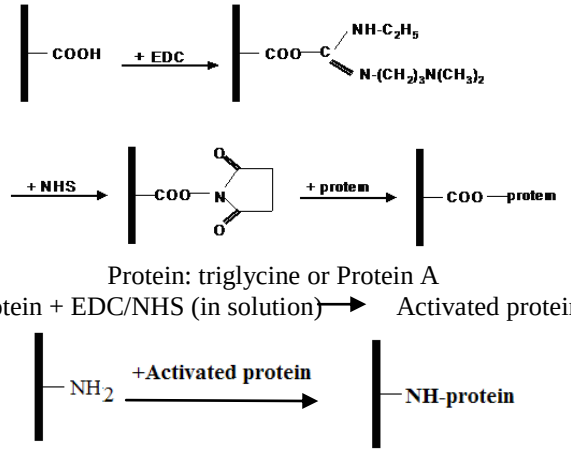
2.3. Immobilization procedures

Physisorption of the proteins

The used proteins for PVDF coating/grafting were: protein A and triglycine. After rinsing with ethanol, the PVDF film was plasma treated and then a protein (protein A or triglycine) solution (10 μL of c= 2.5 mg/mL) was spread over the entire surface and stored at 4 ^oC overnight (at least 15 h). The excess of protein was removed by rinsing with PBS.

Grafting with proteins. Untreated and plasma exposed surfaces were treated with 75 mM EDC (1-ethyl-3-(-dimethylaminopropyl) carbodiimide) + 15 mM NHS ((N-hydroxysuccinimide) and protein (10 μL of c= 2.5 mg/mL) for 1 hour to convert the terminal carboxylic groups by generation of a stable acyl amino ester intermediate. After the condensation of proteins, the aminolysis of the NHS adduct occurs. The excess of protein was removed by rinsing with PBS (pH 7.4). Before analysis all films were stored at 4 ^oC.

Grafting took place in presence of EDC (1-ethyl-3-(-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) according to the reactions:



Scheme 1. The reaction routes for the protein grafting more possible for CO_2 plasma (a) and N_2 or N_2/H_2 plasma (b).

Activation by coupling agents (EDC and NHS) enhances the stability of the coating and facilitate the formation of a suitable intermediate to condense proteins reproducible and densely leading to high sensitivity and good precision of the developed coatings [19,20].

2.4. Investigation methods

To evidence the physical adsorption/grafting of these proteins, several methods were used, such as: contact angle measurements, ATR-FT-IR, and SEM/EDX.

Contact angle measurements.

The contact angles for the polymer films were determined by the *sessile drop method*, at room temperature and controlled humidity, within 30 s, after placing 1μL drops of liquids on the film surface [21,22] using a CAM-200 instrument from KSV- Finland.

By applying the polar-dispersive theory for surface energy, the interfacial tension between blood and the film surface (γ_{SL}) was calculated with the following equation [22]:

$$\gamma_{SL} = \left[\left(\gamma_L^p\right)^{1/2} - \left(\gamma_S^p\right)^{1/2}\right]^2 + \left[\left(\gamma_L^d\right)^{1/2} - \left(\gamma_S^d\right)^{1/2}\right]^2 \qquad (1)$$

where γ_p and γ_d are the polar and, respectively, the dispersive components of the free surface energy, while *L* and *S* stand for liquid and solid, respectively. The medium-cell interfacial tension, γ_{SL}, is of 1–3 mN/m. Thus, it may be reasonably assumed that a good compatibility with a foreign surface and the mechanical stability of this interface can be assured when the blood-biomaterial interfacial tension occurs over this range [23].

To obtain reproducible results for contact angle measurements, several conditions have to be fulfilled, such as: constant temperature during determinations; the same volume of solvent drops (not higher than 1μL); evaluation of the contact angles in different points of the studied surface, the final result being the average of the obtained values. Precision in evaluating the components of the free surface energy is given by the precision in reading the contact angles between the polymer surface and the pure liquids used. The errors involved in contact angle determinations are mainly caused by surface roughness and chemical heterogeneity of the polymeric systems. The largest accepted variation in the values of the contact angles was of ± 1–2 degrees, in the case of the most heterogeneous surfaces[24]. A limitation of this approach is the heterogeneity of the surface active and ionized sites, which may not be uniformly distributed on the surface.

ATR -FT-IR

The ATR-FT-IR spectra of the films were measured at 4 cm-1 resolution on a DIGILAB Scimitar Series FT-IR spectrometer (USA) by the ATR technique, the instrument being equipped with solid cell accessories and a 45o ZnSe crystal. Samples covered the whole crystal surface (25 mm x 10 mm) and were clamped at the same pressure by adjusting the micrometer torque. Penetration thickness is about 100 mμ. For each sample, the evaluations being made on the average spectrum obtained from the three recordings. Spectra processing was done by a Grams/32 program (Galactic Industry Corp.).

SEM/EDX

The samples were also examined with a Scanning Electron Microscope (JEOL, JMS-6300) equipped with an energy-dispersive X-ray analyser (EDX-4, Phillips). The acceleration voltage was of 20 kV and the current of electron beam, of 300 mA. All samples were carbon-coated. The EDX analysis yields reasonably accurate quantitative results featuring all the elements present in the tested surfaces, namely, C, O, N, Na, S, Al, Si, and Cl [25].

3. Results and discussion

PVDF membranes are more expensive but have high mechanical strength, high binding capacity and are compatible with most commonly used proteins strains and immunochemical detection systems. The chemical structure of membrane offers excellent resistance to acidic and organic solvents [26].

Surface modification of the fluoropolymers is of a particular interest, as these polymeric substrates are one of the most important families of engineering polymers, well known for their physical and chemical inertness [27]. Fluoropolymers are characterized by a small surface energy, which does not allow an adequate adhesion with other layer. Gas plasma treatment under various glow discharge conditions was extensively used for this type of surface modification of fluoropolymers [28].

Comparing our previously obtained results and literature ones we can conclude that three kinds of active surfaces obtained in the optima conditions of applied treatment, already mentioned above, are undergone to covering with proteins, namely acidic surfaces (obtained

by CO_2 plasma activation, the surface roughness being increased and functionalization by implantation of oxygen functionalities mainly carboxyl groups) [29,30,31,32], basic surfaces (obtained by N_2/H_2 plasma activation [17] and amphoteric surfaces obtained by N_2 plasma activation [33,34], in all cases after air exposure the same period of time of about 1 minute.

The criterion of good adhesion is essentially a criterion of wetability. Wetting is improved when the contact angle (θ) of a coating liquid on the solid to be coated is lowered. This lowering was achieved by all kinds of plasma treatments – Figure 2 – the contact angle decreases increasing the treatment time. We considered that the 50s treatment time is enough to create a wetable surface.

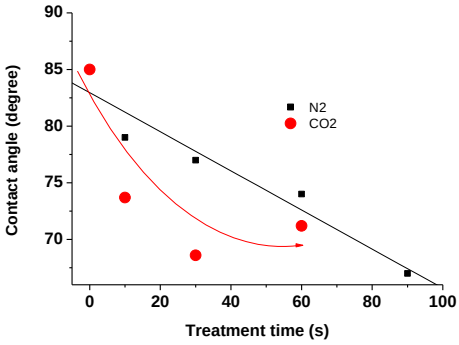


Fig 2. Contact angle between water and the PVDF surface plasma treated with different gases.

For optimum treatment conditions mentioned above, the interfacial tension with blood and tissues was evaluated using eq. (1) – Fig. 3.

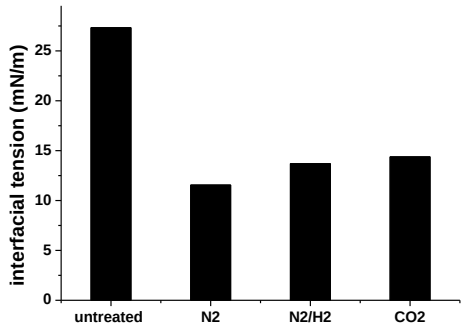


Fig. 3. Interfacial tension with blood and tissues of PVDF films untreated and treated in a microwave plasma with different discharge gases.

The interfacial tension of PVDF surfaces with blood and tissues drastically decreases after applying the plasma treatment, the obtained values tending towards the biocompatibility domain. From this point of view, nitrogen seems to be the most appropriate to be used as a discharge gas and this could be explained by the possibility of both acidic and basic groups' implantation.

Covering with proteins was achieved both by physical adsorption and by grafting according with procedures and mechanism described above.

Looking on the images of the drops deposited on the PVDF surfaces coated/grafted with proteins – Fig. 4 - different forms appear. Flatted profiles of the water drops are much frequently observed in the case of grafted surfaces.

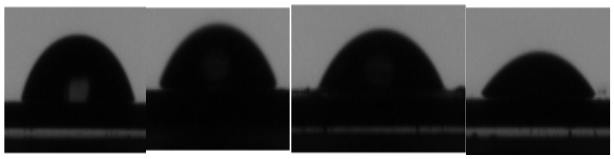


Fig. 4. Contact angle images of the plasma exposed surfaces and subsequent coated/grafted with proteins

The presence of the polar sites after plasma treatment was confirmed by contact angle titration measurements with acid and basic test solutions – Fig. 5 - which presents the dependence of the contact angle between surface and PVDF film surfaces and NaCl solution at various pHs obtained by addition of very small amount of HCl 0.1N or NaOH 0.1M to the NaCl 1M solution.

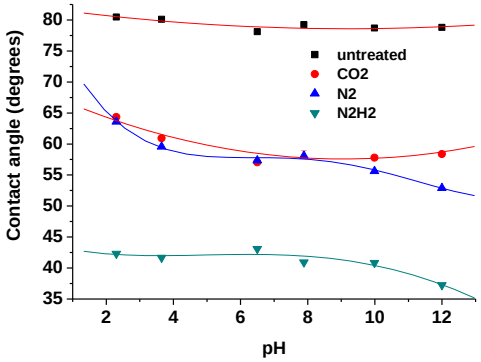


Fig. 5. Variation of the contact angle with pH for PVDF surfaces treated in microwave plasma of different gases.

The contact angle showed no dependence on the pH for the untreated surfaces, whereas for the treated surfaces, a decrease of the contact angle with increasing pH was observed. It can be noticed that the variation of the contact angle with pH of solution is much different depending on the gas used in plasma discharge, because of the different groups introduced on the surface, and consequently their neutralization occurs at different pH in function of their nature. For the surfaces treated in CO_2 and N_2 plasmas, an important decrease in the contact angle appears at acidic pH of 5, while for surfaces treated in N_2/H_2 1/3 plasma, the decrease appear at pH 10.

This diminution in contact angle values indicates a higher hydrophilicity of the treated surfaces at a pH of 10-12 because of the strong electrostatic interactions at the interface. It can supposed that acid groups were created or introduced within the surface layer by the treatments and

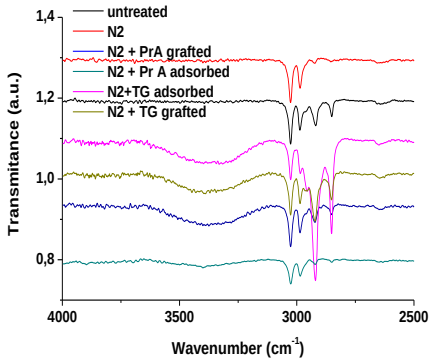
that the Cl^- and OH^- anions present in NaCl electrolyte were preferably adsorbed by these treated surfaces. After plasma treatment it is possible as ionic groups such as COOH or NH_2 to be introduced on the surfaces. The cationic groups led to a decrease of the contact angle at high pH.

ATR-FTIR analyses only a thin layer of the sample [35]. Structure of PVDF: - $(CH_2\text{-}CF_2\text{-})_n$ gives the molecular chain both high flexibility and some stereochemical constrains. As a consequence, a PVDF based material can present many types of molecular and crystal structures depending on its history (preparation, storage, etc.). Three main crystalline forms of PVDF have distinct molecular conformations. The unit cell of form I contains polymer chains with an all trans structure, while that of form II presents trans-Gauche sequences. An intermediate conformation, consisting in sequences of three bonds separated by a gauche bond is found in the case of form III. The infrared spectra distinguish the different crystalline forms of PVDF and presence of polymer chain defects [36]. An actual PVDF sample depending on the preparation conditions may present one or more of different crystalline structures, in addition to a given amorphous content. For these reasons a PVDF sample presents vibrational spectra with peculiar band shapes determined by its history.

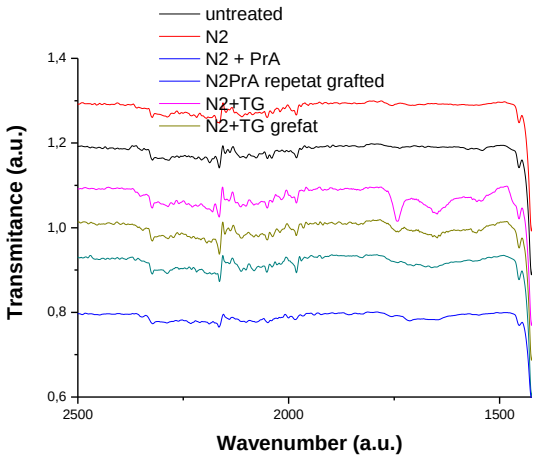
The absorption bands at 1175 and 1211 cm^{-1} in spectrum (a) shown in Fig. 6 are ascribed to the C–F stretching vibration bands [37]. The peaks and the vibration modes at 1402, 1069, 878 and 840 cm^{-1} are characteristics of pure PVDF [38], which corresponds to representative IR bands of different crystal phases (α, β, γ) [39], or due to polymer chain defects brought about by head-to-head or tail-to-tail linkages [40]. A new vibration band at 1636 cm−1 appears in any case, which relates to C=C double bonds or –COO−[41] and C=O carbonyl groups resulting from the formation of hydroperoxide radicals initiated by plasma discharge [42]. The films are practically opaque in the fingerprint region (1500-400 cm^{-1}). There are no differences between pristine polymer and treated samples in different plasmas, excepting 1500-1900 cm^{-1} region where new bands appear, their positions depending on the gas used. So, in the ATR-FTIR spectrum of the nitrogen plasma treated PVDF two weak bands are present at 1757 and 1709 cm^{-1}, in that of the CO_2 plasma treated PVDF only a band is present at 1757 cm^{-1}.

These bands are assigned to oxygen containing groups such as C=O and carboxyl groups, and nitrogen containing groups as N-H and N-O. The CO_2 plasma treatment led to the appearance of new absorption peaks with maxima at 1881 and 1725 cm^{-1}, which can be attributed to carboxylic acid fluoride COF and double bonds -CF=CF-, respectively. Characteristic bands were observed at 3200 cm^{-1} (OH str.), 1710cm^{-1} (C=O str.) and 800 cm^{-1} (COOH out-of-plane def.] in the ATR-FTIR spectrum [43]. The increase in intensity of –OH stretching vibration bonds at 3400–3500 cm^{-1} implies the formation of structure with –OH on the surface of PVDF. The microstructure profiles of the film, including crosslinking, degradation, etching and functionalization, may play an important part in the responsiveness of the surface against further functionalization. The ATR-FTIR spectra of the sample treated only with EDC/NHS presents a weak band at 3300 cm^{-1} which is assigned to the amino group indicating the fixation of EDC on this on surface while the fixation of the NHS is very weak. In presence of catalysts only the surface does not present any new bands. In the case of the spectra of the samples having adsorbed or grafted proteins the ATR-FTIR spectra of the PVDF treated with nitrogen or nitrogen/hydrogen plasma and in direct contact with a ternary solution containing EDC/NHS/protein shows new bands corresponding to the different vibration modes of amines and carbonyl of triglycine; these bands appear at 3300 and 1600-1700 cm^{-1}.

In the spectra of the samples covered/grafted with proteins appear both the bands of pristine polymer and also those corresponding to the proteins. The new bands appearing in 2500-4000 and 1500-1900 cm^{-1} regions are more intense for films grafted with Protein A. In the 1500-1900 cm^{-1} region the bands corresponding to the film coated/grafted with protein A and TG are different which evidence different types of carbonyl and amide groups present in the proteins used and also their presence on the surface.



(a)



(b)

Fig. 6. ATR-FTIR spectra of the PVDF, plasma activated and coated with protein A and TG by physiadsorption and grafting

Several characteristic bands are present in all spectra of the films covered/grafted with proteins pretreated with – Figure 6, mainly in the 1500 – 1900 cm^{-1} region Figure 6b which can be assigned to C=O and C=C bonds and amide group.

The similar observations are made from spectra of other two series of samples treated in CO_2 or N_2/H_2 plasmas. In the case of N_2 plasma treatment it is seems that the most important modification appears by adsorption of TG and grafting with Protein A.

The XPS results are in accordance with ATR-FT-IR spectra [44].

SEM/EDX

Scanning electron microscope–energy-dispersive X-ray (SEM–EDX) measurement provided the depth information about changes in PVDF surface after treatments.

Fig. 7 shows the SEM images of the PVDF substrate as reference (a), and treated in the optima microwave CO_2 plasma conditions (b) and grafted with triglycine (c) and protein A (d)

The image of the reference sample indicates the presence of the spherulites corresponding to the semi-crystalline PVDF, while the image of the PVDF treated in CO_2 plasma depends on the conditions applied. It is seems that the CO_2 plasma has a weak degradative effect on the PVDF surface but the topography of the surface is similar with the reference one. The plasma should also have an effect of smoothing. The images of the biosurfaces show a an improved contrast probably because of an increased roughness associated with heterogeneous physic-deposition of the proteins, the sherulites are not present and surface have a particular aspect after grafting with proteins. Obtaining a smoother surface by protein coating will reduce susceptibility to bacterial adhesion.

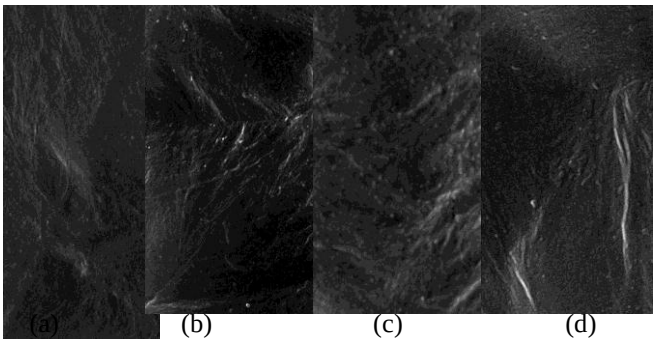


Fig. 7. SEM images of the PVDF substrate as reference (a), and treated in the optima microwave CO_2 plasma conditions (b) and grafted with protein A (c) or triglycine (d) after N_2 or N_2/H_2 microwave plasma treatment.

By electron microscopy and EDX analysis it is possible to image the surface structure and to determine the local elements distribution. Quantitative X-ray microanalysis of plasma-treated and protein coated was performed by energy dispersive spectrometry in a scanning electron microscope (SEM-EDX) in order to obtain quantitative information about area densities and spatial distributions of elements on the polymer surfaces. A typical EDX spectrum is given in Figure 8 and the cartography of the surface is presented in images of Figure 9. It contains all elements present in the components (F, C, H, N, O) and also traces of elements in PBS (Cl and Na)

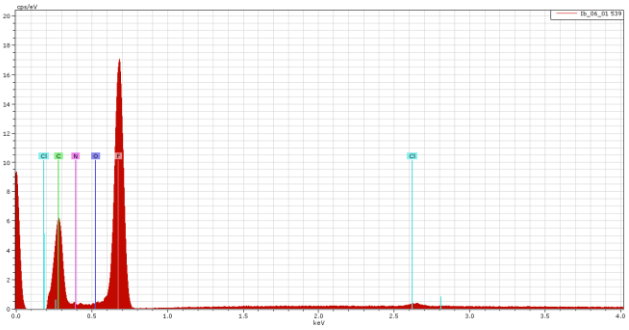


Fig. 8. Typical EDX spectra of the PVDF film surface

The cartographies confirm the homogeneous oxygen incorporation during the CO_2 plasma treatment (d). It can conclude that by plasma treatment in these conditions the degradative effect is insignificant, the distribution of the carbon (b) and fluor (c) and oxygen (d) being uniformous.

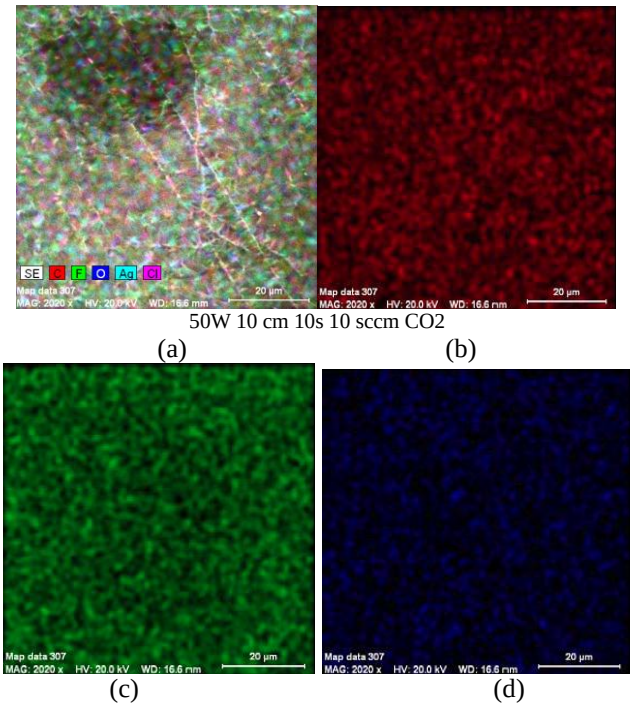


Fig. 9. EDX cartographies of entire composition (a) and in carbon (b), fluor (c) and oxygen (d) for PVDF. Film surface treated by CO_2 plasma.

Like any protein, protein A and triglycine are composed of amino acids that exhibit significant contents of carbon and oxygen in an elemental analysis such as EDX. Therefore, carbon and oxygen in an EDX graph can be used as a protein fingerprint [45,46,47].

Table 1. The surface composition (at %) of the untreated, plasma treated and covered/grafted with proteins samples.

Sample	F	C	N	O
Untreated	71.12	25.38		3.50
CO_2	79.17	10.49	7.11	3.03
CO_2+ Pr A adsorbed	66.59	20.50	8.53	3.38
CO_2 +PrA grafted	66.68	21.72	8.55	2.73
CO_2 + TG adsorbed	66.31	22.69	8.15	2.83
CO_2 + TG grafted	68.77	19.86	8.54	2.57
N_2	65.08	23.43	8.38	2.84
N_2+ PrA adsorbed	68.43	20.07	8.78	2.71
N_2 +PrA grafted	67.01	22.88	8.47	2.93
N_2+TG adsorbed	69.18	19.89	8.18	2.74
N_2+ TG grafted	66.53	22.13	8.48	2.87
N_2/H_2	67.83	20.95	8.37	2.84
N_2/H_2+PrA adsorbed	57.78	29.81	8.87	3.54
N_2/H_2 +PrA gr4afted	66.28	21.18	8.54	3.27
N_2/H_2+TG adsorbed	67.82	20.24	8.47	3.45
N_2/H_2+ TG grafted	65.38	22.45	8.75	3.12

Figs. 7-9 show analysis by the use of SEM images and EDX analyses of the PVDF-uncoated and coated surfaces, before and after plasma treatment. The SEM images and the EDX analysis from a typical point on the untreated surface indicate the presence of carbon and oxygen. To highlight the effect of plasma treatment several points in the plasma-treated area were randomly selected for EDX analyses and the results were consistent from one surface point to another. Figure 8 shows a typical EDX profile where substantial reduction of oxygen is evident. The comparison of the EDX data before and after plasma treatment suggests that the protein coating took place. Muller et al investigated typical plasma processes such as continuous plasma, pulse plasma and plasma graft polymerisation of PVDF, concerning their effect of functionalisation with primary amino groups using a mixture of nitrogen and hydrogen, ammonia, allylamine and diaminocyclohexane. The relative amounts of surface-incorporated nitrogen and primary amino groups are determined using electron spectroscopy and the binding of a selective fluorescent probe. No correlation of the relative amount of surface-bound nitrogen, determined by ESCA, and the relative amount of primary amino groups is found. The interpretation of the chemical shift of nitrogen with ESCA is limited. This is due to the unknown ionization states of the primary amino groups resulting in peak shifts and their overlap with various other nitrogen compounds. The formation of primary amine groups is maximised when a grafting method is applied [48].

Although, SEM and EDX are inappropriate as a quantitative tool for surface protein detection some observations can be made to assess the proteins'coating on the PVDF surface. In Table 1 the surface composition is given of all treated and coated surfaces. The increase of the nitrogen content is evident for CO_2 and N_2/H_2 plasma pre-treatment and coated with proteins, in the other cases the increase in nitrogen amount on the surface is not significant. The series N_2/H_2 is mainly distinguished by an increased O content on the surface after coating with proteins.

4. Conclusions

Two new methods have been developed for polymer surface functionalization by subsequent immobilization of protein A and triglycine on PVDF surface by physiadsorption and grafting following microwave plasma treatment of various gases as CO_2, N_2 and N_2/H_2 and coating/grafting.

By using ATR-FTIR spectroscopy the formation of COF, COOH and X=C- groups after CO_2 plasma treatment and amide, amine after N_2 and N_2/H_2 plasma activation and proteins coating/grafting have been detected and characterized. Water contact angle measurements have shown a gradual decrease of contact angles after plasma activation as well as after proteins coating/grafting indicating an increase in hydrophilicity in these two steps of modification.

The purpose of these coatings is to create biocompatible surfaces for medical applications. Plasma treatment of PVDF in a microwave discharge, followed by coating/grafting with different proteins proved to be very useful for the appropriate modification of its surface properties, thus leading to a possible increase in the biocompatibility characteristics of the hydrophobic PVDF polymer.

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