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Evaluation of Blood Compatibility of Plasma Deposited Heparin-like Films and SF_6 Plasma Treated Surfaces

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In devices used in open-heart surgery and dialysis, blood must be continuously processed using extracorporeal circuits composed of peristaltic pumps and active components such as specific filters and oxygenators. Several procedures have been employed to avoid blood coagulation induced by contact with the artificial surfaces of such devices. Often heparin, a bioactive protein able to prevent clot formation, is employed. In this work, we have used heparin-containing gas plasmas to evaluate the possibility of depositing adherent anticoagulant films onto PVC and glass surfaces. The films were produced by radiofrequency plasma enhanced chemical vapor deposition from heparin/isopropanol and heparin/hexamethyldisiloxane solutions. In addition, the effects of exposure to SF_6 plasmas on the compatibility of such surfaces have also been investigated. The blood compatibility was evaluated through the determination of the density of platelets and fibrinogen and activated partial thromboplastin (APTT) and prothrombin times (PT) of human blood freshly collected and after contact for 2.5 hours with different surfaces. The deposited films were also characterized by infrared spectroscopy, contact angle and surface energy measurements. The coagulation time of blood, placed in contact with glass substrates coated by PECVD films of heparin/isopropanol mixtures, and in contact with SF_6 plasma-treated PVC, increased by about 60 and 20%, respectively, compared to the values measured with untreated samples.

Keywords: *PECVD, plasma treatment, biomaterials, blood compatibility, heparin*

1. Introduction

In modern medicine, artificial materials are increasingly employed to improve quality of life. In many situations, haemocompatibility is a property of paramount importance for artificial devices intended to substitute or operate in contact with live organs. The adhesion of blood proteins onto the material can give rise to formation of a thrombus, which can obstruct arteries leading to ischemia or embolism. The reduction in blood supply caused by thrombosis can result in severe injuries, including heart attack, and even death[1,2]. Consequently, much effort has been expended to produce surfaces with good haemocompatibility. In this context, heparin is considered the most appropriate biomolecule to suppress thrombus formation in the initial stages of blood contact with an artificial material. Owing to this, many investigations of the immobilization of heparin on polymer surfaces have been undertaken. In this context, two distinct procedures are frequently employed. In the first, the polymer can be produced with functional groups (amines and carboxylic acids, for instance) that can be heparinized. In the second, the functional groups are grafted onto the surface of the material. Barbucci et al.[3], for instance, have developed a method to graft poly(amido-amine) onto polyurethane. In the same way, Marconi et al.[4] have studied heparin immobilization on ethylene-vinyl alcohol copolymer through the reaction of the polymer with ω-dialkyl amino-aldehyde. In such studies, a significant improvement in blood compatibility has been attained. A weak coupling between heparin and the surface, however, has been observed in both cases. Glow discharge plasmas provide a possible solution to such problems. Recent studies have shown that such a technique is potentially efficient in enhancing the density of immobilizing sites for heparin and other biological molecules. As an illustration, Kang et al.[5] observed that heparin was attached to polyurethane exposed to oxygen plasmas and subsequently immersed in physiological solution for more than 100 hours, indicating the stability of the immobilized heparin. Furthermore, Kim et al.[6] have verified that the immobilization of heparin reduced thrombus formation on oxygen-plasma treated PET by more than 60%.

Although good results have been obtained with plasma treatment, there are some inconveniences, which restrict its wider application. In addition to complex routes involving several solutions of different compounds, the length of each step can be excessively long. The procedure employed by Kang et al.[5], for instance, took more than 125 hours. Furthermore, the often required heating and lengthy immersions in reactive liquids may cause modification of the bulk as well as the surface properties.

Frequently, plasmas have been used only as an activating tool for subsequent surface heparinization. In contrast, this work reports for the first time (as far as we are aware) the production of heparin-like films by plasma enhanced chemical vapor deposition of heparin-containing solutions. The performances of plasma-treated glass and PVC specimens in contact with human blood have been investigated. In addition, the effects of exposure to SF_6 plasmas on the compatibility of such surfaces have also been assessed.

2. Experimental

Plasma enhanced chemical vapor deposition (PECVD) has been used to produce thin films on glass and medical-grade PVC. Radiof-

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requency plasmas (13.56 MHz, 100 W) were generated in a stainless steel chamber fitted with two parallel plate electrodes and evacuated by an 18 m^3/h rotary pump. Coatings produced in discharges of heparin/hexamethyldisiloxane (HMDSO) or heparin/isopropanol solutions and surfaces modified by exposure to sulfur hexafluoride (SF_6) plasmas were studied. Film properties were assessed by contact angle, θ, measurements and Fourier transform infrared spectroscopy. Blood compatibility was evaluated by the determination of the density of platelets, fibrinogen, and prothrombin (PT), activated partial thromboplastin (APTT), and coagulation times (T_c) of a pool of 10 samples of freshly collected human blood kept in contact with different specimens for 2.5 hours.

3. Results and Discussion

Figure 1(a) shows an infrared absorption spectrum of a film deposited in a heparin/HMDSO plasma. Typical absorption bands can be observed at 3450 cm^{-1} (ascribed to O-H stretching), 2960-2860 cm^{-1} (C-H stretching), 1630 cm^{-1} (O-H bending), 1420 cm^{-1} (C-H bending), 1280 cm^{-1} (C-O stretching), 1230 and 1040 cm^{-1} (symmetrical and asymmetrical stretching of S-O in SO_3 heparin groups, respectively), 1100-1000 cm^{-1} (Si-O-Si stretching) and at 850 cm^{-1} (=C-H bending).

For comparison, the Figure also shows the spectrum of sodium heparin powder (spectrum b). Note that the principal bands are present in both spectra a and b. Frequently, the absorptions at 1230 and 1040 cm^{-1} are assumed to constitute a fingerprint of heparin[5,7]. Therefore, the presence of the absorptions ascribed to sulfur-containing groups in the spectrum suggests the preservation of the heparin structure in the film. It is important to mention, however, that the overlapping of bands derived from bonds in the HMDSO molecule does not allow the precise identification of the structure of the deposited material.

Spectrum c is of a film deposited from a heparin/isopropanol mixture. Owing to the presence of OH groups in the monomer and therefore probably also in the film, a band at around 3400 cm^{-1} is expected, but is in fact broad and weak. Three peaks, at 2960, 1450 and 1380 cm^{-1}, confirm the presence of CH groups. The band centered around 1700 cm^{-1} is due to the presence of C = O groups. These observations are consistent with the features of infrared spectra of films produced from rf plasmas of isopropanol[8]. Bands characteristic of heparin (at 1230 and 1040 cm^{-1}) are very small, perhaps indicating poor preservation of the heparin in the deposited material.

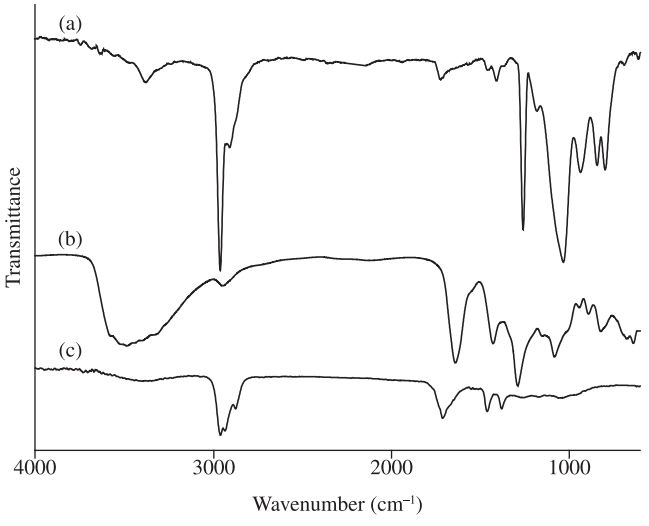

Figure 1. Infrared absorption spectra of a film deposited in heparin / HMDSO plasma (a); sodium heparin powder (b); a film deposited in heparin / isopropanol plasma (c).

Modification of the plasma conditions may produce surfaces with different characteristics[9,10]. One of the surface properties that may be greatly modified by plasma exposure is the contact angle. As an illustration, Figure 2 shows water contact angles of glass slides under different surface conditions. In A, the sample has been coated by the PECVD of heparin/isopropanol solution at an applied power of 15 W, while in B the glass was only sonicated in detergent and water baths. Condition C refers to glass surfaces also coated by PECVD of heparin/isopropanol but at an rf power of 25 W; in D, the slide was exposed to an SF_6 plasma and in E it was coated by PECVD of heparin/HMDSO solution. Clearly, plasma treatment allows the production of more hydrophilic or more hydrophobic surfaces, compared to the pristine material.

It is interesting to note that when the rf power was increased from 15 W (condition A) to 25 W (condition C) the contact angle increased almost 75%. The mean energy of plasma electrons increases as the excitation power is increased. Consequently, greater bond fragmentation of plasma species and increased bombardment of the sample surface by energetic species are produced. Therefore, a possible explanation for the growth in the contact angle as the applied power is increased is the loss of hydrophilic species (OH, for instance) owing to greater fragmentation of heparin molecules and increased bombardment of the deposited material.

The water/glass contact angle was further increased when the samples were exposed to SF_6 (condition D) or heparin/HMDSO (condition E) plasmas. In the first case, the increase in θ results from the incorporation of fluorine into the surface, as already observed in similar situations[11]. On the other hand, the hydrophobicity of heparin/HMDSO films may have its origin in low surface polarity[12].

The variation of the contact angle of a given material can be associated with variation of its free surface energy, E_s. Such a variation is observed, for instance, in Figure 3, which shows E_s, decomposed into its polar and dispersive components, of glass surfaces under some of the abovementioned conditions. The equations used to calculate E_S are given and described in reference 11.

The trend in E_s as a function of the surface conditions is opposite to that of the contact angle. The variation in the polar and dispersive components, however, does not follow the same pattern. For instance, the polar component increases with the treatment performed under condition C. Another interesting fact, revealed by inspection of Figure 3, is that the dispersive component of the film deposited

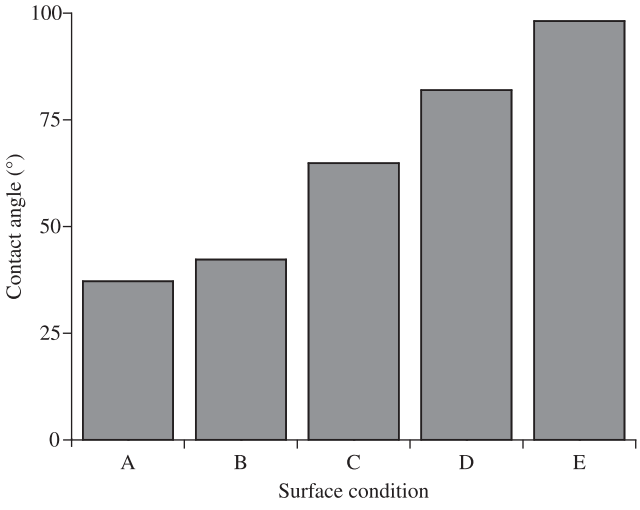

Figure 2. Contact angle of glass slides under different surface conditions. In A, the sample was coated by PECVD of heparin/isopropanol solution with an applied rf power of 15 W. In B, the glass was sonicated in detergent and water baths. Condition C refers to glass surfaces coated by PECVD of heparin/isopropanol at an rf power of 25 W; in D, the slide was exposed to SF_6 plasma; in E the slide was coated by the PECVD of heparin/HMDSO solution.

in condition E is more than five times greater than its polar component. Such an observation reinforces the interpretation that there is a causal relationship between the hydrophobicity of this film and its low surface polarity.

We have observed that PVC surfaces may also be modified by plasma treatment. As an illustration, the water contact angle was increased by up to 45% following exposure for 120 s to SF_6 discharges, reaching the value of 100.7°.

The different surfaces produced by the treatments have a distinctive influence on the haemocompatibility of the material. This can be seen in Table 1, which shows activated partial thromboplastin (APTT), and prothrombin (PT) times and the densities of fibrinogen and platelets in human blood samples freshly collected and after 2.5 hours in contact with glass slides under different surface conditions.

To better understand the results, one should recall that normal values for human blood are the following: PT ranging from 10 to 14 seconds, APTT between 25 and 36 seconds, density of fibrinogen from 150 to 370 $mg.dl^{-1}$ and 130,000 to 370,000 platelets per microliter of blood. According to these reference values, the most notable fact is the high concentration of fibrinogen in blood after contact with the as-received glass (condition B). The increase of more than 15%, compared to fresh blood, indicates advanced clot formation.

In fact, measurements of coagulation times, T_c, whose results are shown in Figure 4, indicate that, among all the conditions evaluated in this work, the pristine glass is the least haemocompatible material. In this case, T_c was up to 50% smaller than the average coagulation time of fresh blood.

Inspection of Figure 4 also reveals that the surface produced by PECVD of heparin/HMDSO solution had the least influence on the coagulation factors. The coagulation time for contact with such surfaces (almost 60% higher than the value measured for contact with pristine glass) was, considering the experimental uncertainty, very close to that of fresh blood. This finding is in agreement with other authors who have shown that silicon oxide presents good blood compatibility[13, 14].

Another interesting result can be seen in Figure 5, which shows T_c as a function of water contact angle of the corresponding surfaces. As can be observed, there is a good linear correlation between θ and T_c. This linear relationship is probably due to the reduction in contact area between the blood and the surfaces as the latter become more hydrophobic. It is important to note, however, that the surface performance when in contact with blood is not determined exclusively by its contact

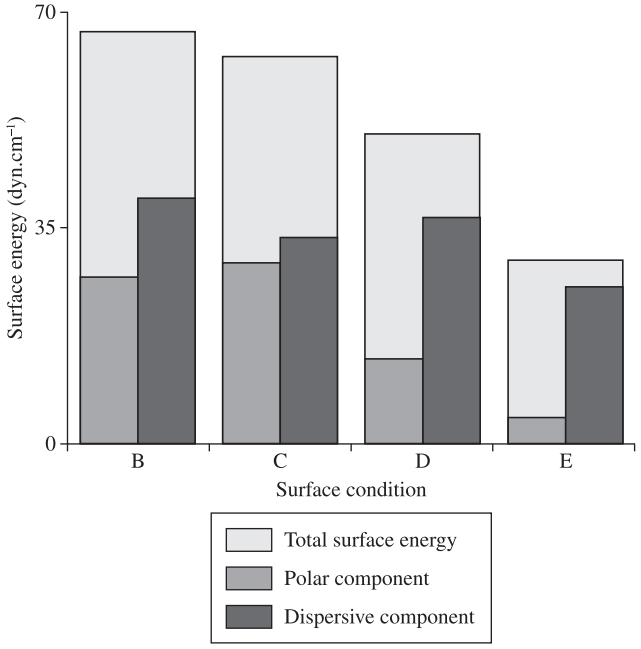

Figure 3. Free energy of glass surfaces under different conditions. In B, the surface was sonicated in detergent and water baths; in C, the surface was coated by PECVD of heparin/isopropanol at an rf power of 25 W; in D, the glass was exposed to SF_6 plasma; in E, the slide was coated by the PECVD of heparin/HMDSO solution.

Table 1. Prothrombin (PT) and activated partial thromboplastin times and densities of fibrinogen and platelets in blood samples freshly collected (A) and after 2.5 hours in contact with glass slides as-received (B), exposed to SF6 plasma (C) and coated by PECVD of heparin/isopropanol (D) and heparin/HMDSO (E) solutions.

Condition	PT (s)	APTT (s)	Fibrinogen ($mg.dl^{-1}$)	Platelets (10^3 cell.μL^{-1})
A	12.8	29.0	352.0	188.0
B	13.1	28.7	408.0	191.0
C	13.2	27.2	371.0	198.0
D	12.6	26.7	317.0	185.0
E	13.4	25.7	376.0	188.0

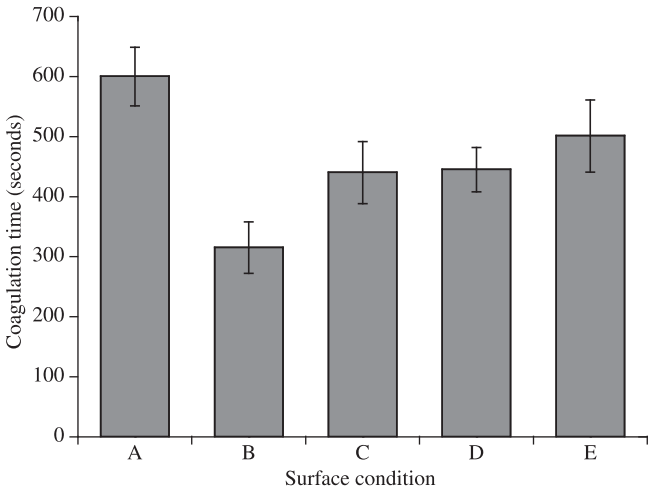

Figure 4. Average coagulation times of blood samples freshly collected (A); in contact with untreated glass slides (B); exposed to SF_6 plasmas (C); coated by PECVD in heparin/isopropanol (D); heparin/HMDSO solutions (E).

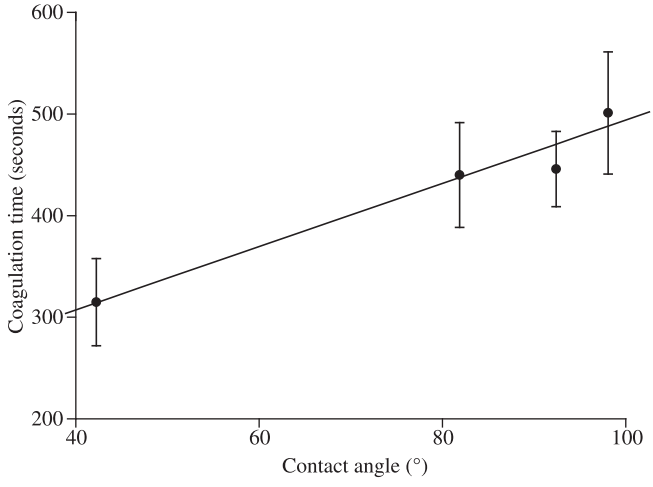

Figure 5. Coagulation time of blood in contact with glass surfaces with different contact angles.

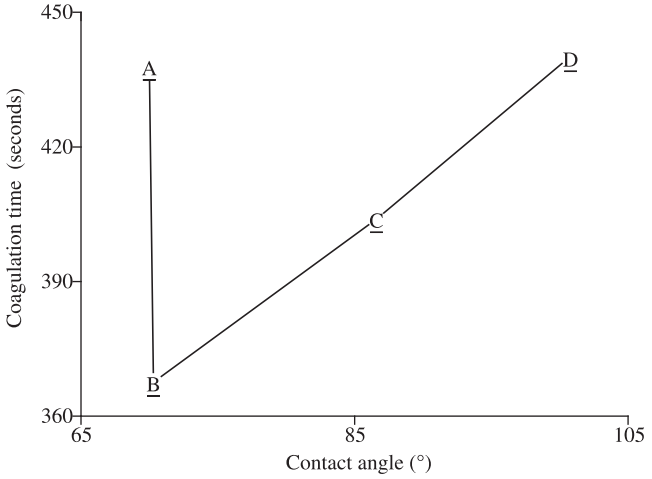

Figure 6. Coagulation time of blood in contact with surfaces with different contact angles. A indicates the value obtained from glass surfaces coated by PECVD of heparin/isopropanol solution. In B pristine PVC specimens have been immersed in blood. Condition C refers to glass slides coated by PECVD from heparin/HMDSO solution and the result in D was obtained with PVC specimens exposed to SF_6 plasmas.

angle. This becomes evident if one considers the results presented in Figure 6, which shows T_c as a function of θ for another set of experiments performed including PVC specimens as received and after exposure to SF_6 plasmas. Once more, the longest coagulation time was attained with the most hydrophobic surface and in the region between point B and D, a linear relationship is observed. In spite of this, it should be noted that under certain circumstances, corresponding to points A and B, surfaces with the same contact angle produced distinct coagulation times. While in the first case a contact angle of 70° corresponded to a T_c of 437 seconds, in B the coagulation time was 367.6 s for a contact angle of 70.3°. Such different behaviors can be attributed to the presence of heparin (in A), conferring a more anticoagulant characteristic to the surface.

Another remarkable result shown in Figure 6 is that the coagulation time of blood in contact with PVC exposed to SF_6 plasma was about 20% higher than the value obtained with the pristine polymer. This result is important because it indicates an improvement in the performance of the material most widely used nowadays in devices that operate in contact with blood. Furthermore, it reveals a procedure that enhances the polymer haemocompatibility without using coatings that may suffer surface leaching and react with the blood. Thrombocytopenia (the absence of coagulation) induced by the heparin released from coatings on tubes used in extracorporeal circulation devices and which may cause hemorrhages and cardiovascular problems[15-19] is commonly observed in clinical settings.

4. Conclusions

It was shown that different plasma treatments could improve the haemocompatibility of glass slides and PVC specimens. For the glass, the best results were obtained with PECVD of heparin/HMDSO solutions. The coagulation time of blood in contact with such modified glass increased by up to 60% compared to the values measured with untreated samples. On the other hand, SF_6 plasmas were more effective in improving the performance of medical grade PVC. In this case, it has been possible to increase by almost 20% the coagulation time of blood in contact with a material that already has good haemocompatiblity.

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