Biocompatible thin films obtained from heparin-methane plasma process

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Abstract. Heparin is an appropriate molecule to suppress the thrombus formation in the initial stages of blood contact with an artificial material. Therefore the covering of a synthetic material with heparin-like molecules is a great importance issue in biomaterial science and engineering. In order to reach this goal this paper deals with the plasma deposition of thin heparin-like films on microscope slides from RF-excited heparin/methane low pressure plasmas. Plasma were excited by a RF-power supply operating on 13.56 MHz at a fixed power of 50 W. Heparin was diluted in ethanol and fed into the plasma chamber in mixtures of 50% of CH_4 (in pressure) at 10 Pa. Film's molecular structure was characterized by Fourier transform infrared spectroscopy (FTIR here in). Molecular spectra presented absorption bands due C-H, O-H and C-O stretching and bending modes. Film's surface wettability was investigated by contact angle measurements. The experimental results show values varying from 65^0 to 20^0. Surface's optical microscopy showed the occurrence of heparin islands distributed almost uniformly over the film. The blood's coagulation time placed in contact with glass substrate covered by plasma deposited heparin/methane films was measured by thrombosis time and activated thromboplastin.

1. Introduction

In the early 80's it appeared that biomedical engineering had achieved the goal of a workable total artificial human heart [1,2]. Although the heart met mast of the stringent mechanical requirements it had a fatal how. The material that contacted the blood, although in many ways the best available material did not have the necessary biocompatibility. Blood coming into contact with a segmented polyurethane would recognize it as a foreign surface and form thrombi. In human patients anticoagulants were administered chronically to inhibit clotting and thrombosis but this practice subsequently created much more problems. Despite the use of anticoagulants the patients suffered frequent strokes, believed to result from embolus of thrombi formed on the material surface. Recent efforts have led to a much greater applications of the number and complexity of processes involved in blood coagulations on foreign surfaces (5 mm). Researches shown that synthetic vascular grafts, made of Dracon^{TM} or expanded Teflon, are widely used to replace occluded or diseased arteries in humans. When used in large-diameter applications, synthetic vascular grafts, show satisfactory patency rates. In small-diameter applications (inner diameter less than 5 mm), however, graft performance is disappointing due

to stenosis and thrombus formation [3-5]. Endothelial cell seeding is an accepted approach to improve small-diameter graft performance. For successful endothelial cell seeding, a suitable substrate is required, which, is not provided by Dracon^{TM} or expanded Teflon grafts. Otherwise, it is important to mention that thermoplastic polyurethanes and plasticized poly-vinyl chloride have been widely used for various biomedical applications due to their excellent mechanical properties and proper blood compatibility [6,7]. Surface modification is an effective approach to improve the blood compatibility being the size, shape and mechanical properties of the original material maintained. Many studies have been performed on producing a blood-compatible surface by tailoring with poly-ethylene glycol, heparin, heparin-like, phospholipid polymer, and so on [6,7]. However, the aqueous solubility of some materials make them unsuitable for many biomedical applications. Good results have been obtained with plasma treatment or deposition. This work reports the production of heparin-like films using plasma polymerization process. This technique allows one to obtain materials which do not dissolve in strong acidic and basic and present physical and chemical attractive properties.

2. Experimental

Heparin diluted in isopropanol/methane mixtures (50 % in pressure) discharges were generated by a 13.56 MHz RF power supply operating in 50 W at a fixed pressure of 10 Pa. Plasmas were generated within a pyrex cylindrical reactor 190 mm of inner diameter and 150 mm long. The vacuum inside the plasma chamber is monitored by pirani^{TM} (thermocouple) and penning^{TM} (inverse magnetrom) gauges. The diffusion pump is coupled to the chamber through a gate valve and is used for cleanness purposes. The pressure is pumped down to 10^{-3} Pa, being the chamber purged with argon several times before each running of the experiment. The plasma chamber walls were heated with a temperature controlled belt in order to minimize the heparin-isopropanol condensation as well as the humidity. Heparin-isopropanol was placed inside a stainless steel bottle and was fed into the plasma chamber through a needle valve. Methane was fed into plasma chamber through needle valves and mass-flow controllers. The wettability of plasma polymerized films was investigated by contact angle measurements using a Ramé-Hart goniometer model 100 and water and diiodomethane (CH_2I_2)as probe liquids. Film's thickness were measured using an Alpha Step Tencor 100^{TM}. The film's structure was investigated by FTIR spectroscopy using a Perkin-Elmer Lambda 25^{TM} spectrometer. Surface smoothness was probed by optical microscopy. Surface's blood compatibility was evaluated by determination of platelets density, fibrinogen and prothrombin (PT), activated partial thromboplastin (APTT) and coagulation times (Tc) of a pool of eight samples of freshly collected human blood kept in contact with different samples for 2.5 hours.

3. Results and discussion

FTIR spectra of methane plasma polymer (1), heparin-isopropanol methane plasma polymer (2), sodic heparin (3) are shown in figure 1. For comparison, the principal bands of the heparin are present in the heparin-isopropanol methane plasma polymer. It suggests the preservation of the heparin structure in the films. It is important to mention that the overlapping of bands derived from bonds in the methane and isopropanol molecules do not allow the precise identification of the structure of the heparin-isopropanol methane plasma polymer. Characteristic absorption bands can be observed at 3500 to 3200 cm^{-1} (assigned to O-H stretching), 2950 to 2850 cm^{-1} (C-H stretching), 1630 cm^{-1} (O-H bending), 1440 cm^{-1} (C-H bending), 1380 cm^{-1} (C-H bending), 1280 cm^{-1} (C-O stretching), 1230 cm^{-1} and 1040 cm^{-1} (symmetrical and asymmetrical stretching of S-O in SO_3 heparin groups, respectively. The band near 1700 cm^{-1} is due to the presence of C=O groups. These results are consistent with the features of FTIR spectra of plasma polymer films deposited from methane and isopropanol discharges. The action of plasma on a material surface can promote changes in their surface energy and contact angle.

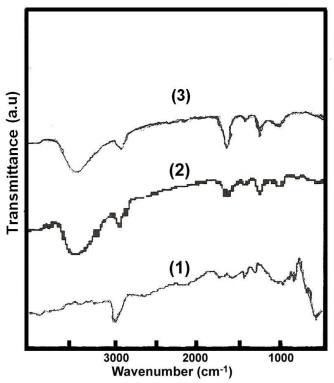

Figure 1. FTIR spectra of (1) methane plasma polymer , (2) heparin-isopropanol methane plasma polymer, (3) sodic heparin

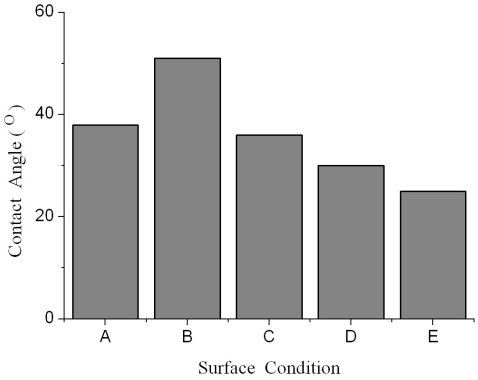

Figure 2. Contact angle of plasma deposited films for different conditions: (A) cleaned glass substratum, (B)methane plasma film, (C) heparin-isopropanol methane plasma film 40 nm thick, (D) same as (C) 10 nm thick, (E) heparin-isopropanol methane plasma film over glass covered by sodic heparin

Figure 2 shows water contact angles values for glass covered at different conditions. In (A), the glass was only cleaned in ultrasonic medium with petroleum ether and ethyl alcohol. In (B), the sample has been coated by thin films from methane plasma polymer (at 50 W and 10 Pa). In (C), the glass substrate was exposed to heparin-isopropanol methane (50% to 50%) plasma at 50 W and 10 Pa. Film thickness is approximately 40 nm. In (D) the same film as in (C) 10 nm thick. In (E), the glass substrate was covered with sodic heparin diluted in isopropanol and finally coated by heparin-isopropanol methane plasma polymer. As can be seen in this figure, the plasma treatment was profitable for obtaining hydrophilic surfaces. The different surface produced by plasma deposition have a distinctive influence on the haemocompatibility of the samples. This results can be illustrated in figure 3 that shows the measurements of coagulation times Tc. The results indicate that among all the conditions evaluated, the glass coated with methane plasma polymer and pristine glass substrate are the least haemocompatible materials. The coagulation times of these materials were up to 44% smaller than the average coagulation time of fresh blood. Figure 3 also shows that the surface produced by plasma polymerization of heparin-isopropanol methane 40 and 10 nm thick (C,D) presents quite equal coagulation times. These results are 10% lower in comparison with those obtained for fresh blood. On the other hand the surface of the sample (E), presents coagulation time higher than fresh blood.

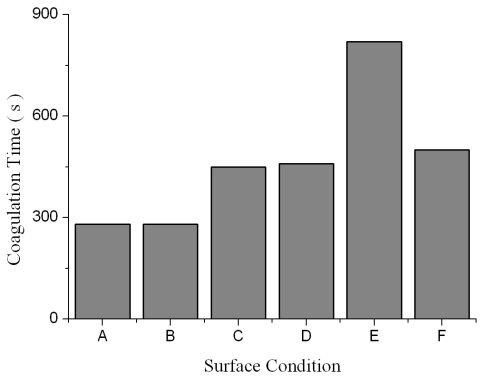

Figure 3. FTIR spectra of (1) methane plasma polymer , (2) heparin-isopropanol methane plasma polymer, (3) sodic heparin

This result can be attributed to the presence of islands of heparin on the surface of the sample that were not completely covered by the thin film from heparin-isopropanol methane plasma (micro-graphs not shown in this paper). These islands are dispersed on the surface and have irregular shapes. Another important result is also showed in this figure. The coagulation times of blood in contact of samples (C) and (D) (glass coated with heparin-isopropanol methane plasma polymers 40 and 10 nm thick respectively) are near fresh blood coagulation time. These aspects are important because they indicate an improvement in the performance of the materials most widely used nowadays in devices that operate in contact with blood. However , the material (E) (substrate coated by sodic heparin and polymer from heparin-isopropanol methane plasma) despite having high coagulation time, it is not suitable for use because it may induce or cause haemorrhages and cardiovascular complications.

4. Conclusions

It was shown that heparin-isopropanol methane plasma process could improve the haemocompatibility of glass substrates. The best results were obtained with heparin-isopropanol methane plasma polymers 40 and 10 nm thick. The coagulation times of blood in contact with such modified glass increased up to 65% compared to the values measured with pristine glass surface and glass coated by methane plasma polymer. Therefore one may conclude that the plasma surface treatment was successful for obtaining haemocompatible surfaces.

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