Hemocompatibility of Surface Modified Si Incorporated Diamond-like Carbon Films

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Biomedical devices composed of high quality biomaterials are desirable for effective biomedical treatment. The main problem with medical devices in cardiovascular application also lies in its thrombogenic complications, which ultimately limit the long term use of the devices. Furthermore, the existing biomaterials like stainless steel and nitinol that are commonly used in endovascular stents and heart valves are reported to release metal ions in contact with blood. It is thus required to improve the cardiovascular devices by applying hemocompatible coatings that can also prevent the release of any carcinogenic elements into blood. In this respect, diamond like carbon (DLC) film has emerged as a promising coating layer for cardiovascular applications owing to its chemical inertness and hemocompatible properties. It was reported that DLC coatings on cardiovascular implants have prevented the release of metal ions to a considerable extent [1]. Some comparative studies have revealed that DLC is more hemocompatible than Ti, TiN, TiC, CN and PMMA [2, 3].

The medical devices like stent and heart valve also experience various tensile and compressive forces during their action inside human body. A stent expands after its insertion at a selected area in the blood vessels. A heart valve undergoes millions of openings and closing. All these mechanical actions often lead to delamination and spallation of the coatings. Several attempts have been made to overcome these problems by doping the surface coatings with certain elements and use an adhesive interlayer [4]. It has been observed that Si incorporation in the a-C:H films prepared by RF plasma assisted chemical vapor deposition have improved the mechanical properties and corrosion resistance [5]. In this report, we have investigated the hemocompatibility of surface modified Si incorporated DLC (Si-DLC) films for effective coating on endovascular nitinol stents. The surface property of the film was manipulated by exposing the Si-DLC coatings to the r.f. plasma of various gases like O_2, CF_4, N_2 or H_2.

Si-DLC films were deposited on Si and nitinol substrates using capacitively coupled rf plasma assisted chemical vapor deposition (PACVD) technique. Benzene (C_6H_6) and silane (SiH_4) diluted by hydrogen (SiH_4:H_2=10:90) were used as the precursor gases. All the depositions were carried out at a bias voltage of – 400 V and a chamber pressure 1.33 Pa. Si concentration in the film is 2 at.% as measured by Rutherford backscattering spectrometry. The Si-DLC films were then treated with plasma of various gases like O_2, CF_4, N_2 and H_2 for 10 minutes at a bias voltage of – 400 V and a pressure 1.33 Pa. The plasma protein adsorption tests were performed by treating the samples with bovine serum albumin and fibrinogen solution and measuring the absorbances through enzyme linked immunosorbent assay (ELISA) analysis. The platelet adhesion tests were performed with platelet rich plasma (PRP) of citrated fresh human blood prepared by moderate centrifugation. The samples (1cm x 1cm) were initially rinsed with PBS solution and incubated in PRP solution at 37°C for 2 hours. The number of platelets of nonused control PRP solution and an equal amount of used PRP solution are measured by Coulter counter. The morphology of adherent platelet was observed by SEM after the conventional fixing procedure. Activated partial thromboplastin time (aPTT) measurements were performed by a Sysmex Instrument using platelet poor plasma (PPP) of fresh human blood. Details of the hemocompatibility test will be published elsewhere.

The plasma treated Si-DLC films showed a wide range of water contact angles starting from 13.4° to 92.1°. The O_2 plasma treated Si-DLC films have denoted the most hydrophilic surface with a water contact angle of 13.4°. On the other hand a hydrophobic surface with wetting angle larger than 100° was obtained in case of CF_4 plasma treated SiDLC films. The Si-DLC films indicated a higher dispersive component in their surface energy. The surface energies of plasma treated Si-DLC films revealed a higher polar component for the O_2 plasma treated Si-DLC films and a higher dispersive component for the H_2 and CF_4 plasma treated ones. The nitrogen plasma treated Si-DLC films showed nearly equal amounts of polar and dispersive components of surface energy.

The first proteins that are adsorbed on the biomaterials surface when contacting blood are albumin, fibrinogen and fibronectin. Adsorption of albumin retards the adhesion and activation of platelets, while adsorption of fibrinogen promotes platelet adhesion and activation. The adsorbed fibrinogen gets converted into insoluble fibrin polymer which finally results in the formation of thrombus. Fig. 1 shows the albumin to fibrinogen ratio of the plasma treated samples. The CF_4, O_2 and H_2 plasma treated SiDLC films showed a tendency of increase in albumin to fibrinogen ratio with time. The O_2 plasma treated Si-DLC films significantly improved the albumin to fibrinogen ratio with incubation time.

Table 1 shows the surface bonds of the Si-DLC and plasma treated Si-DLC films observed by XPS and their albumin to fibrinogen ratio.. C-CF and CF_n bonds have low polarizability. This gives rise to the low surface energy and increased hydrophobicity of CF_4 treated Si-DLC films. Si-O, O-H and C-N bonds are polar in nature, which results in higher polar component in the surface energy of O_2 and N_2 plasma treated Si-DLC films. A higher albumin/fibrinogen ratio was obtained in case of O_2 plasma treated SiDLC films followed by CF_4, N_2 and H_2 plasma treated SiDLC films. XPS analysis revealed the presence of C-C, C-O, O-H, Si-H and Si-O bonds on the surfaces of O_2 plasma treated Si-DLC films.

The aPTT determines the ability of blood to coagulate through the intrinsic coagulation mechanism. It measures the clotting time from the activation of factor XII through the formation of fibrin clot. The aPTT also governs how the biomaterial affects and influences the coagulation time. The enzymatic activities which led to clot formation are measured through aPTT. Fig 2 showed the aPTT of plasma treated Si-DLC films. The incubation time was kept fixed at 1 hour. The O_2 plasma treated Si-DLC films revealed higher aPTT compared to other plasma treated samples. It was further observed that O_2 plasma treated Si-DLC films delayed the aPTT with incubation time than Si-DLC films. It denotes that the O_2 plasma treated SiDLC films have a tendency to retard the intrinsic coagulation activities of blood compared to the other samples.

The platelet adhesion and activation on the surfaces of a biomaterial is an important test to access the hemocompatibility of the biomaterial. A low platelet adhesion and activation denotes improved hemocompatibility while a higher degree of platelet adhesion and activation results in the formation of a thrombus. Fig.3 shows the morphology of adhered platelets on Si substrates and some plasma treated Si-DLC surfaces exposed to platelet rich plasma (PRP) of fresh human blood. All the samples were incubated in PRP of fresh human blood for 2 hours. Change in morphology indicates the activation of platelets. Activated platelets loss its round shape, form pseudopodia and tends to spread on the biomaterial surface. In case of Si substrates, some crystals were observed on the PRP exposed surface. These crystals might have attached to the surface from PBS solution while treating the incubated samples with 2% glutaraldehyde in PBS solution. The N_2 plasma treated Si-DLC surfaces indicated activation of platelets on its surfaces as is evident through the formation of pseudopodia of the platelets. In case of H_2 and O_2 treated Si-DLC films, the platelets have retained its disc like shape indicating an unactivated state. Fig.4 gives the number of individual platelets adhered on the specimen surface per unit area. The O_2 plasma treated Si-DLC films were found to minimize platelet adhesion compared to N_2 plasma treated Si-DLC films and Si substrates.

The plasma treatment of Si incorporated DLC films with various gases like CF_4, H_2, N_2 and O_2 has a significant effect on its hemocompatibility. The CF_4, O_2 and H_2 plasma treated Si-DLC films have improved the albumin adsorption with time, while H_2 plasma treated samples have minimized the rate of fibrinogen adsorption. A higher aPTT was observed in case of O_2 plasma treated Si-DLC films. The O_2 plasma treated Si-DLC films also minimized platelet adhesion and activation compared to other samples. The high albumin to fibrinogen ratio seemed to explain the improved hemocompatibility of the O_2 plasma treated Si-DLC films. The O_2 plasma treated Si-DLC films can serve as effective coating on endovascular nitinol stents.

References

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Films	Bonds present on surface (XPS analysis)	Albumin/Fibrinogen ratio	
		5 mins	60 mins
SiDLC (O plasma treated)	C-C, C-O, Si-H, Si-O, O-H	0.97	1.41
SiDLC (CF_4 plasma treated)	C-C, C-CF, Si-C, F-F	0.88	1.02
SiDLC (N plasma treated)	C-C, Si-H, C-N	1.02	0.99
SiDLC (H plasma treated)	C-C, Si-H	0.82	0.92
SiDLC	C-C, Si-C	0.86	0.70

Table 1. Surface chemical bonds and protein adsorption ratio

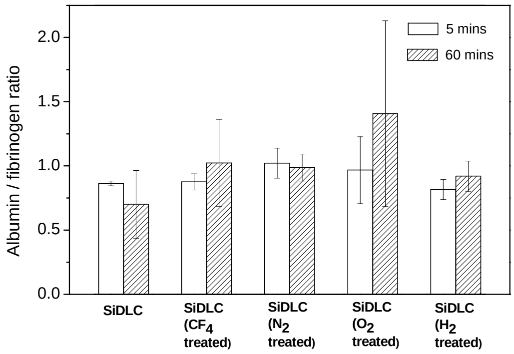

Fig. 1. Adsorbed Albumin Fibrinogen ratio

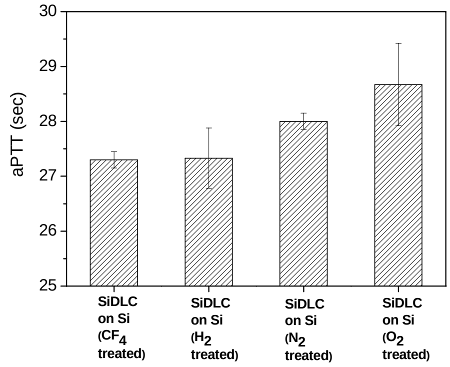

Fig. 2. aPTT time for various sampels

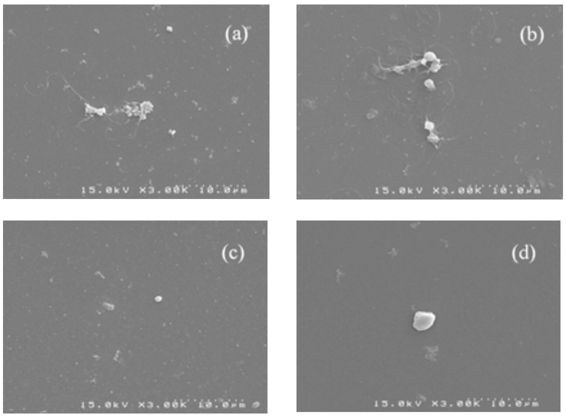

Fig. 3. Morphology of Adherent platelets
(a) Uncoated Si substrate
(b) N_2 plasma treated Si-DLC
(c) H_2 plasma treated Si-DLC
(d) O_2 plasma treated Si-DLC films

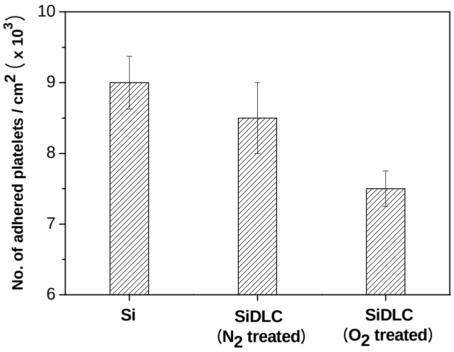

Fig. 4. Number of adherent platelets

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