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Review Article

The Studies of Diamond-Like Carbon Films as Biomaterials: Review

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Abstract: Diamond-like carbon (DLC) films have been given great attention in the last twenty years as candidate biomaterials due to its super mechanical property and bio-compatibilities. In this paper, many synthesis methods of DLC films are presented, and some researches related with DLC films applied on cardiovascular biomedical materials are mentioned. Many synthesis methods of DLC films are also presented. Research theories and analysis methods of materials hemo-compatibility are also shown in detail. Two kinds of main evaluation methods aimed to two different coagulation pathways are also introduced here. It has also been illustrated that different biomedical applications about non-doped and element-doped DLC films in different studies. However, because of the uncontrollable accurate ratio of sp^3/sp^2 in the films and imperfect evaluation methods of hemo-compatibility, an unremitting effort is essential so that DLC films can be better qualified as biomaterials.

Keywords: Diamond-Like Carbon Films, Doping, Hemo-compatibility, Biomaterials

1. Introduction

Carbon can form different crystal and disorder structures because it can exist with three different kinds of hybrid modes, sp^3, sp^2 and sp^1 [1], which make research become complicated about carbon films. In the sp^3 hybrid structure, four valence electrons of a carbon atom are distributed into directional sp^3 orbit with tetrahedral structure, and more powerful σ bond is formed between a carbon atom and adjacent atom, so this kind of bonding type is defined as diamond bond. In sp^2 hybrid structure like graphite, three among four valence electrons of a carbon atom enter into triangle directional sp^2 orbit and form σ bond in a plane, the fourth electron lies in pπ orbit with a same plane of σ bond and π orbit form weak π bond with a or many adjacent atoms. But in sp^1 structure, two of among four valence electrons enter into π orbit and form σ bond along x axis respectively, and another two electrons enter into pπ orbit of y and z axis and form π bond. Diamond-like carbon (DLC) films are this type of amorphous and metastable films whose main ingredient is carbon and include certain amount of diamond bonds [2].

Doped-DLC films can be obtained by doping different element. Such as, N-DLC, DLC-Si and BCN films can be synthesized by doping nitrogen [3, 4], silicon [5], boron and nitrogen [6, 7], respectively. Even if DLC or N-DLC films, the hybridization modes of carbon atoms in the films are sp^3, sp^2 and sp^1, thus many properties are same as diamond films. DLC films have high hardness, wear resistance, low friction coefficient [8], chemical stability and electron affinity (sometime is negative), and high transmittance, which is a kind of semiconductor material with wide gap [9, 10]. DLC films also have many another advantages such as large area deposition, low cost, surface roughness *et al*., and can be prepared under low temperature (DLC films were deposited by IBM company under 77K [11]), have fairly mature technology. Till now, DLC films have been known and given great attention by many researchers and industrial communities, and have been applied widely in fine mechanics, micro-electron mechanical devices, disc storage, auto parts and components, aerospace, optical apparatus, biomedicine and other fields [12-19], are high-powered thin film materials with important applied prospects.

It has been confirmed that DLC films have good biocompatibilities. Shen *et al.* [20] have studied hemolysis of DLC films, the results showed that the hemolytic ratio of DLC films was lower than 5%, which was satisfied with the demand of biocompatibility to hemolysis. Thomson *et al.* [21], in their preliminary study, have confirmed that no inflammatory reaction emerged in vitro to DLC films by detecting the amount of release of Lysozyme-N-acetyl-D-glucosaminidase inside macrophage on the DLC-coated culture plate. Allen *et al.* [22] also have studied the effects of DLC-coated polystyrene plate to macrophage, the results showed that the macrophages on the DLC coated and uncoated polystyrene plate are very good growth. Lu and Jones *et al.* [23] have deposited DLC films on the P-35 plastic plate by ion beam assisted deposition (IBAD), and cultured ML-1 human hematopoietic stem cells and Human embryonic kidney 293 cells (HEK293) and control group of non-DLC coated, the results showed that two kinds of cells discussed above grew normally. Some research groups [21, 22] have analysed tissue toxicity by studying the effects of DLC coatings to the viability of three cell monolayer of macrophage, mechanocyte, osteoplast using Lactate dehydrogenase (LDH) detection method, whose results also showed that DLC coatings have supplied non-toxic surface and made climbing, growing and splitting with normal state. D. J. Li [24] and Amaratunga *et al.* [25] have obtained further understanding of histocompatibility of DLC-coated Polymethyl Methacrylate (PMMA) by cell adhesion test using neutrophilic granulocyte. The research results showed that DLC films have lower neutrophilic granulocyte adhesion rate compared with reference samples. The results confirmed histocompatibility of DLC films in vitro.

DLC films have been given great attention in joint prosthesis, radio knife, anti-bacterial materials, especially implantation of artificial heart valve due to its good biocompatibility, high abrasion resistant, high hardness *et al.* advantages [26]. It is one of common heart disease for congenital defect or incomplete wall of heart valve and induced heart valve disease due to rheumatic heart disease [27]. Slight heart valve disease patients can be treated by surgery, but serious ones are difficult to be cured only by surgery and need valve replacement. Only in China, the number of current patients is ten million or so. Ten percent of these patients need valve replacement. Thus, demanded quantity is very impressive. Since 1960 Starr-Edwars [28] from America firstly was embedded artificial heart valve, the research about artificial valve has been given more and more attention [20].

Existing heart valve materials mostly use low temperature isotropic pyrolytic carbon (LTIC), however the deposition temperature is up to 1300 degree, substrate only use fire-resistant materials (like graphite). Graphite materials are easily damaged while processing due to its high brittleness and bad intensity, so only valve leaflet usually use LTIC, valve ring use metal (like titanium alloy). Yet metallic valve ring have bad wear resistant and worse blood-compatibility than LTIC, postoperative patients need to take anticoagulant drugs long term, otherwise lead to blood clotting easily. If coating DLC films onto valve ring, that will solve this problem because DLC films have high wear resistance and biocompatibility. Moreover, DLC films are able to be deposited on the surface of almost all substrate materials, such as metals, ceramics, glass and plastic *et al.* and modified these due to be deposited under the condition of below 250 degrees, even suitable to surface modification of polymer materials without affecting the properties of the matrix material. Thus, DLC films prospect to substitute LTIC as new type heart valve materials. DLC films have already been synthesized using plasma chemical vapour deposition, sputtering deposition, ion beam assisted deposition, magnetic filter arc deposition, plasma immersion ion implantation *et al.* methods [29].

2. Synthesis Methods of DLC Films

Many methods have already been used to synthesize DLC films, such as magnetron sputtering [30], laser ablation [31] and RF plasma enhanced chemical vapour deposition (PECVD) [32]. DLC films, as a kind of hemo-compatibility materials with great development potential, carbon atoms bonded pattern (C-H, C-C and C=C *et al.*) and proportion of various bonded pattern of prepared DLC films will different if using different synthesis methods and carrier of carbon atoms (various kinds of carbon methane gas, graphite *et al.*), so inner structure and composition of obtained DLC films are different, even induce greater difference and cause significant qualitative difference each other, moreover its structure property rests with impact level of high speed ion in great degree while preparing films.

2.1. Magnetron Sputtering

Magnetron sputtering is a new type sputtering technology and developed quickly since 1970s, and has been given practical application in the industrial production now because deposition rate raises an order of magnitude comparing with two sputtering. Comparing traditional two sputtering, magnetic sputtering has many advantages such as high deposition rate, low substrate temperature while depositing, little damage to films, and obtain not only high deposition rate under lower work pressure but also high quality films under lower substrate temperature. The principle of increasing sputtering rate by magnetic field firstly was invented by Penning [33] 70 years ago, and then was developed by Kay and others [34-36] and manufactured sputtered gun and pole magnetic field source. In 1974, Chapin [37] also inducted planar magnetic structure.

The schematic diagram is shown in Figure 1. Electrons controlled by magnetic field ionize argon atom into ion, and then argon ion bombard graphite target surface and sputter carbon atoms to form films.

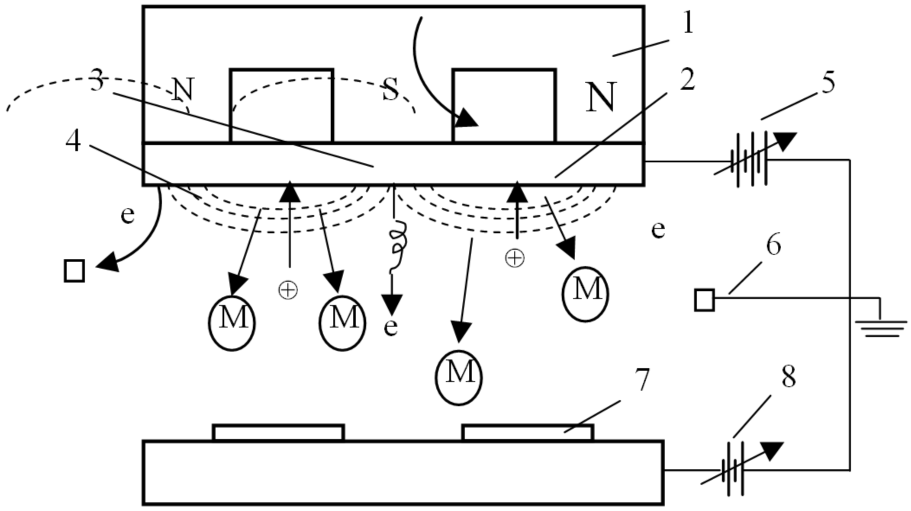


Figure 1. Schematic of magnetic sputtering 1-magnet, 2-Etching Area, 3-target, 4-magnetic line, 5-sputtered target power, 6-probe, 7- substrate, 8- Bias power, e- electron, ⊕- plus ion, Ⓜ- sputtered materials.

2.2. Laser Ablation Deposition (LAD)

Pulsed laser ablation deposition is a new type technology of films prepared and developed in the late of 1980s. Typical PLD schematic diagram is shown in Figure 2. A bound of laser beam is projected onto target by lens and make material of being irradiated area ablation, and ablative material is transmitted along target's normal direction and form a light-emitting groups looks like feathery, finally deposit on the front of underlay and from thin film. When gas is introduced, compound films with gas element can be obtained. For example, oxide films can be formed if oxygen introduced [38].

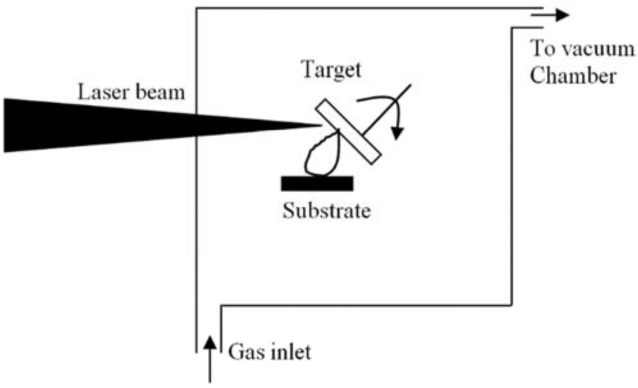


Figure 2. Schematic diagram of typical PLD device.

2.3. RF Plasma Enhanced Chemical Vapour Deposition (RF-PECVD)

RF-PCVD is the process of carbonaceous gas or mixture of several gases (one of them is carbonaceous gas) is introduced while deposition process and gas is decomposed by radio frequency, meanwhile adding negative bias voltage into substrate, and then forming DLC films. RF-PECVD is divided into two kinds of inductance and capacitance, and capacitive RF-PECVD is applied more in them. The products of RF-PECVD are different when technological condition is different [20]. The polymer composed with unsaturated C-X (X represents another gas atom bonded with C) will be obtained because there is not enough energy to open all of C-X bonds under low power density and vacuum. When the RF input density increased and the pressure reduced to an optimal value, DLC films can be gotten with diamond-like characteristic. The method is suitable to deposited films on dielectric substrate and applied widely due to its lower deposition temperature and good film quality.

2.4. Magnetically Filtered Cathodic Arc Deposition (MFCAD)

But DLC films synthesized using the methods of ref.30 and ref.32 had lower sp^3 fraction, bad hardness and wear resistant, and the DLC films by laser ablation have the problems of poor quality and nonuniformity. Compared with the methods, there are many advantages to MFCAD.

In cathodic arc deposition, arc is ignited by starting arc device and moves around target surface under the maintenance of power and driven by the magnetic field, and then carbon source is evaporated and evaporated carbon is ionized under field effect by high potential barrier before the target. Finally, carbon ions are deposited on the substrate after adjusting energy of carbon ion under negative bias voltage and superior DLC films will be obtained after carbon structure is recombined. During deposition process of vacuum cathode, large amount of carbon particles of different sizes usually are spurted from graphite target, which will affect quality and property of film. In order to obtain high quality DLC films, magnetic filter cathodic arc deposition is more and more widely used. Though its cathode spot is very small, only 1~10 μm, high current density is high, which arrived at 10^6~10^8 $A \cdot cm^{-2}$. Coll and Chhowalla [39] reported current typical two filter cathodic arc deposition system, and the schematic diagram is shown in Figure 3. In this device, using low voltage and high current as power supply, knocking at the graphite cathode by trigger electrode to ignite arc, so ion density in the field cathode is able to reach 10^{13} cm^3. Using MFCAD, DLC films with good film quality, higher sp^3 fraction and hardness can be obtained. Furthermore, a new recent plasma immersion ion implantation and deposition (PIII-D) is also developed to prepare higher sp^3 fraction DLC films using high purity graphite as plasma source.

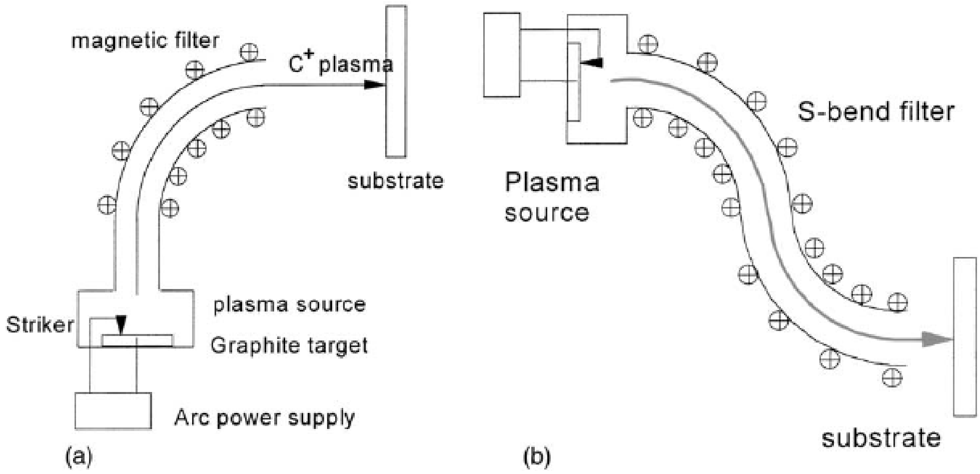


Figure 3. *Cathodic arc schematic of (a) single siphon type and (b) S type siphon.*

By for-mentioned methods, we can obtain high sp^3 bonds hydrogen-free amorphous carbon films and adjust drastically structure property of DLC films because ion energy, synthesis temperature and doped element *et al.* can be adjusted in a wide range.

3. Theory Research of Materials' Hemo-Compatibility

Up to now, the mechanism about good hemo-compatibility of LTIC has still been given exact explanation, and the good hemo-compatibility has already been confirmed widely in clinic.

It is an important problem about mechanism between materials and blood. Till now, many research works have been done in order to establish effect laws of materials' surface characteristic to protein adhesion, quite representative hypothesis include as follows:

(a) From thermodynamics, it is considered that special energy state of materials surface may make fibrinogen adhesion and deformation on materials surface least. Such as Lyman *et al.* [40] proposed hypothesis of critical surface tension, the least interface energy hypothesis of Andrad [41] and proper interfacial tension (1-3dyn/cm) hypothesis of Ruckenstein [42], and matching viewpoint of dispersion force with polar force of Kaeble [43] and Ratner *et al.* [44]

(b) Micro-multiphase structure hypothesis of materials surface of Imai [45].

(c) Sawyer [46] put forward the hypothesis that anti-coagulant materials must have negative charge, and Ikada [47] improved Sawyer's hypothesis and raised the viewpoint of relationship between surface ξ potential and the anti-clotting properties.

(d) S. C. Lin [48] raised the theory that molecular structure of materials surface should maintain normal conformation of biomacromolecule inside blood.

(e) Baurschmit and Schaldach *et al.* [49] put forward the prosthesis of interact electrochemical process between materials and blood.

But, all of the viewpoints described above are put forward based on polymer materials, few researches have been developed about hemo-compatibility of inorganic materials, and it is only just a beginning for the knowledge of mechanisms of anti-coagulation to inorganic thin films.

At present, many research works about DLC films were focused on physical and mechanical properties of DLC films, but few research of anti-coagulant property has been done. We have been still lack of knowledge to mechanism of DLC films and blood each other. So, it is very significant to study mechanisms of anti-coagulation of DLC films as a kind of inorganic thin films materials.

4. Analysis Methods of DLC Films and Evaluation of Hemo-Compatibility Property

Up to now, a series of DLC films (Non-doped, nitrogen, hydrogen, fluorine, or oxygen doped DLC films) have been prepared under different process parameters using CVAD or PIII-D method. All the time, adhesion is a question to puzzle us for biomedical applications of DLC films. If thickness of DLC film is more than 100 nm, film is easy to detach from substrate due to residual stress and poor adhesion [50]. Considering the implanted effect, during the synthesis of DLC films, high frequency pulse negative bias voltage was applied to prepare a base layer by adjusting duty ratio and peak voltage and directed bias voltage was applied to the outer layer in order to achieve good adhesion [51-62].

Many advanced analysis and detection methods were employed to study physical and mechanical properties of synthesized DLC films, such as XPS, Raman, FTIR, SEM, Nano-indentation, scratching test, wear test. Here, Raman spectroscopy is a useful tool to characterize the microstructure of carbon and related materials. The surface energy (γ_s), defined as the sum of the dispersion (γ_s^d) and polar (γ_s^p) contribution, can be determined by contact angle measurements and calculated by solving Young's equations using more than three testing liquids/solid interfaces in combination with the work of adhesion [43, 63].

Usually, dynamic coagulation factor detection, platelet adhesion and fibrinogen denaturation experiments were used to study coagulant property of DLC films. Generally, there are two main pathways of coagulation. One is protein involved endogenous system, the result is fibrinogen into fibrin and leads to the formation of red thrombus, and the other is exogenous system and leads the formation of white thrombus. Figure 4 shows the coagulation pathway after biomaterials contact with blood.

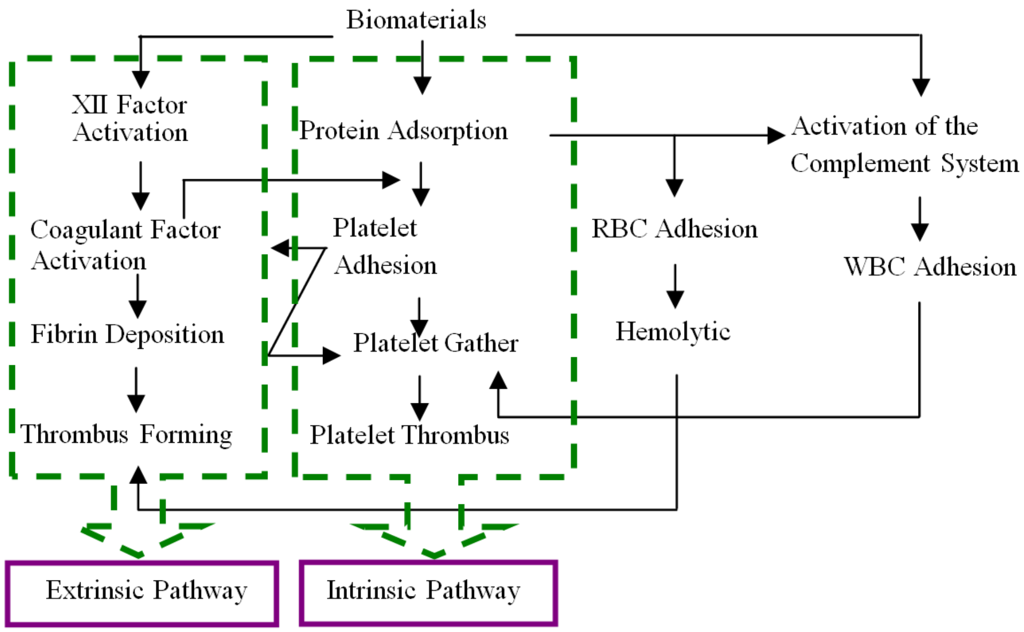


Figure 4. *Coagulation Pathway after Biomaterials contact with Blood.*

Aimed to two different coagulation pathways, two kinds of main evaluation methodology are described here.

(a) *Platelet adhesion experiment (intrinsic pathway)*: Blood is obtained from a healthy adult volunteer. Whole blood is collected in an acid citrate dextrose medium. After centrifugation, red cells and platelets are separated and platelet-rich plasma is obtained. The samples are immersed in the platelet-rich plasma (PRP) and incubated 37 °C for certain times. The samples were subsequently rinsed with a 0.9% NaCl solution to remove weakly adherent platelets. The adhered platelets were fixed in 2%, 5% glutaraldehyde solution at room temperature for 2 and 12 h, respectively, then dehydrated, and critical point dried. The specimens were then coated with a gold layer of 10~20nm thick and examined by optical microscopy and scanning electron microscopy (SEM). The quantity, morphology, aggregation, and pseudopodium of the adherent platelets are examined to investigate the surface thrombogenicity. Above twenty fields of view are often chosen at random to obtain good statistics [52, 64].

(b) *Denatured fibrinogen level testing (extrinsic pathway)* [65, 66]: It is a kind of Enzyme-linked immunoassay (ELISA) examination method for testing hemo-compatibility of biomedical materials. Here, the fibrinogen broken γ chains are exposed to and coupled with the GPIIb-IIIa series chains of platelets, stimulating the activation of platelets. Blood is obtained from a healthy adult volunteer who had been kept free of aspirin or other drugs that could have biased research results. The whole blood was anticoagulated using sodium citrate. After centrifuging, the red blood cells and platelets were separated and a platelet-poor plasma (PPP) was obtained.

Human fibrinogen (Fg) was used as adhesive substrate for 96-well microtiter plates. The protein was dissolved in phosphate-buffered saline (PBS) of the following composition: 150 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, pH 7.4. The final concentration of Fg was 1 mg·ml^{-1}. The microtiter plates were incubated with the Fg solution for 10 h at 25°C. The adsorption process was stopped by washing the plates three times with PBS.

Protein adsorption experiments were performed in microtiter plates by adding 300μl PPP to each sample well containing sheets of polyurethaneureas. The samples were incubated for 10 min on a rotary plate shaker, then washed with PBS. The Fg adsorption was investigated by employing a solution of an IgG fraction of goat antiserum to human Fg diluted 1:1000 with 1% BSA in PBS. Appropriate antibody solutions (300 μl) were pipetted in four sample wells and 300 μl rabbit anti-goat IgG fraction at the same concentration was added to two sample wells as a control for non-antigen-antibody binding. The microtiter plates were incubated for 30 min on the rotary plate shaker and washed afterwards. To all sample wells 300 μl rabbit anti-goat IgG conjugated with peroxidase diluted 1:500 with 1% BSA was added and the plates incubated for 30 min on the rotary plate shaker. The samples were washed as described above. The adsorption of Fg was further monitored using a monoclonal mouse anti-human Fg directed versus the γ-chain diluted 1:1000 with 1% BSA in PBS. A quantity of 300 μl was added to four sample wells. A solution of mouse IgG at the same concentration was placed in two sample wells as nonspecific control. The incubation and washing procedure was the same as described above. Then 300 μl sheep anti-mouse IgG conjugated with peroxidase, in a 1:400 dilution, was added to all sample wells and the plates agitated for 30 min. After further washing with PBS the samples were transferred to a second plate and 300 μl OPD, 0.4 mg·ml^{-1} in 0.05 M phosphate citrate buffer with hydrogen peroxide (0.014%), was added and incubated for 10 min. At the end of the reaction 200 μl chromophore was transferred to a third plate and the optical density (OD) read at 450 nm using a microplate reader.

By analyzing the results, the interrelation among composition, structure, surface energy and coagulant of DLC films could be summarized. The blood contact mechanism of DLC films and blood also could be deduced. To coagulant property of materials, which is the important impact parameter and what parameters are close and direct? All of the problems can be inferred by research as follows in detail.

5. Research Results of DLC Films

5.1. Non-doped DLC Films

Non-doped DLC films were synthesized under different negative bias voltage at room temperature. A high purity (99.99%) graphite cathode was used to serve as the carbon plasma source [51-53].

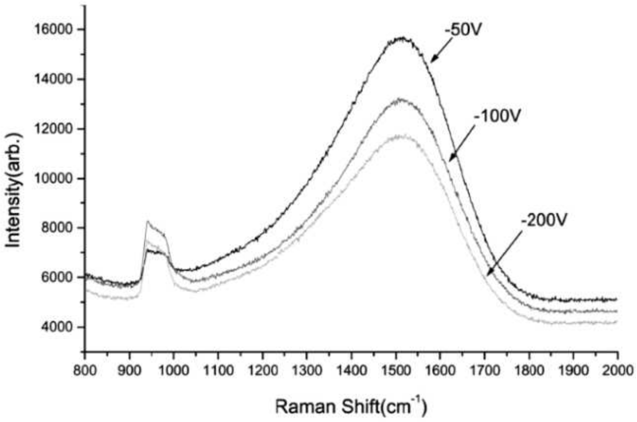


Figure 5. *Raman spectra of DLC films fabricated at different bias voltage.*

Figure 5 is typical Raman of DLC films. Compared with graphite, all the spectra show a broad peak at approximately 1540 cm^{-1} and a lower frequency shoulder at approximately 1400 cm^{-1}, commonly referred to as the G band and D band, respectively. Each spectrum was decomposed into two Gaussian line shapes. A G peak centered on 1540 cm^{-1} reflecting the zone-center E_{2g} mode of a perfect graphite crystal and a D peak centered on 1400 cm^{-1} being a zone-edge A_{1g} mode due to disorder [53]. Based on the fitting parameters, the peak positions and the ratio of the integrated areas under the D and G peaks (I_D:I_G) can be obtained. From these results, we can deduce some information of about sp^3/sp^2, sp^2 cluster quantities, bonding disorder degree and existing sp^2 chain and ring in the DLC films.

But, due to complexity of DLC inner structure, it is quite difficult to obtain a quantitative conclusion for DLC films by Raman's studying. The difficulty of the task is summarized in Figure 6. The factors of affecting displacement and direction of G and D peak and changing their intensity are also listed in the Figure 6. So, sometime the Raman results are not definitive enough to discern the DLC films. In order to obtain more structural information, XPS was often used simultaneously. XPS is quite sensitive to the characteristics of the film surface because the non-elastic scattering mean free path λ_m of the emitted photoelectron is very short [67].

Figure 7 is typical XPS spectra of the synthesized DLC films. The phenomenon showed that sp^3 bonds fraction decreased with negative voltage increase because binding energy of sp^3 bond is higher than that of sp^2 bond. Usually, for further studying composition of DLC Film, the XPS spectra were fitted with Gaussian–Lorentzian distribution (each mixture of 80% Gaussian and 20% Lorentzian) and Shirley method was employed to process the contribution of background. The asymmetric C_{1s} spectrum can be deconvolved into three peaks centered at 287.2±0.2 eV, 286.4 ± 0.2 eV and 288.5 ± 0.3 eV, which are attributed to sp^3 C–C, sp^2 C = C and C-O bonds, respectively. From the fitted XPS spectra, we can analysis semi-quantitatively the ratio of sp^3 and oxygen in the DLC films by calculating the area ratio of sp^3 to sp^2 bond and oxygen to carbon based on area of every peak. It can be seen from Figure 8 that the core level shifted to low binding energy from 287.1 eV to 286.8 eV when negative bias voltage from 0V to -200V, which show that content of sp^3 decrease with bias voltage increase. But the result is opposite to Leng's research result [53].

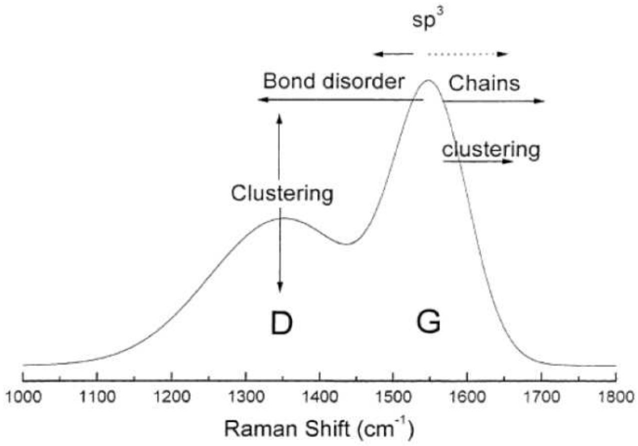


Figure 6. *Schematic of various factors of affecting G and D peak of Raman spectra for amorphous carbon.*

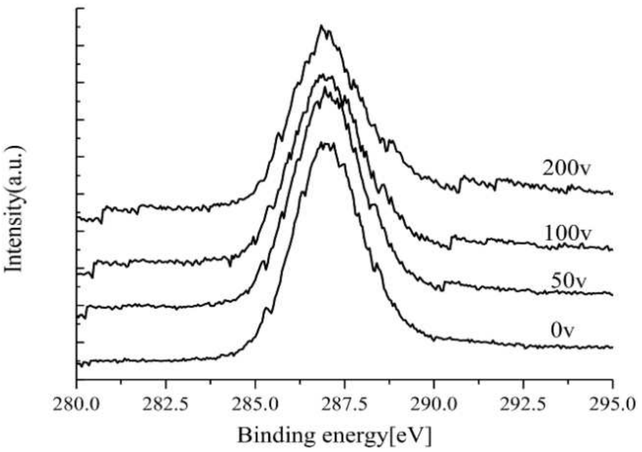


Figure 7. *XPS spectra of synthesized DLC films.*

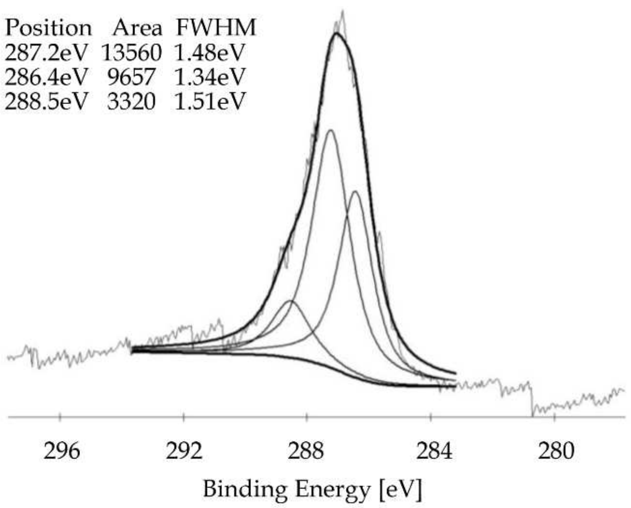


Figure 8. *Typical C_{1s} decomposition of XPS spectra using Gaussian-Lorentzian method [53].*

Wen [51] and Leng [53] have also studied mechanical properties of non-doped DLC films. The effective microhardness only increases slightly with increasing substrate bias voltage. On the other hand, the wear resistance and adhesion decreases slightly. Furthermore, thermomechanical stability of DLC films synthesized on $Cr_{17}Ni_{14}Cu_4$ stainless steel substrate was also studied [53]. After annealing at 400°C for 3 h, the effective hardness diminishes slightly by approximately 20% due to stress released, but the effective hardness and wear resistance of the annealed films are still much better than those of the $Cr_{17}Ni_{14}Cu_4$ stainless steel substrate.

Commonly, the higher the surface energy of the solid substrate relative to the surface tension of the liquids are, the better of the wettability and the smaller of the surface tension is. However, the difference in the polar contribution to the total surface energy plays a critical role in determining the wettability for a polar liquid like water. The change of substrate bias voltage affect surface energy scarcely but different to deionized water wettability due to the large difference of γ_s^p/γ_s^d. The γ_s^p/γ_s^d of deionized water decreased with substrate bias voltage, but opposite to surface tension (γ_{sw}). Increase in γ_{sw} indicated that DLC films became more and more hydrophobic with the increase of substrate bias voltage [51].

Platelet adhere experiment is often done to evaluate hemo-compatibility of synthesized DLC films from an aspect. Blood is obtained from a healthy adult volunteer. Whole blood is collected in an acid citrate dextrose medium. After centrifugation, red cells and platelets are separated and platelet-rich plasma is obtained. The samples are immersed in the platelet-rich plasma and incubated at 37°C for 30 or 120 min. After rinsing, fixing and critical point drying, the specimens with platelets on the surface are coated with a gold layer 10~20 nm thick and observed by optical microscopy and SEM. The quantity, morphology, aggregation, and pseudopodium of the adherent platelets are examined to investigate the surface thrombogenicity. Usually, twenty fields of view are chosen at random to obtain good statistics. The typical morphology (seen in Figure 9) of adhered platelets was observed by scanning electron microscopy (SEM). By observing, we can deduce the synthesized DLC film has good hemo-compatibility when substrate negative bias voltage was between -50~-100V. Certainly, further works need to be done about hemo-compatibility of DLC films.

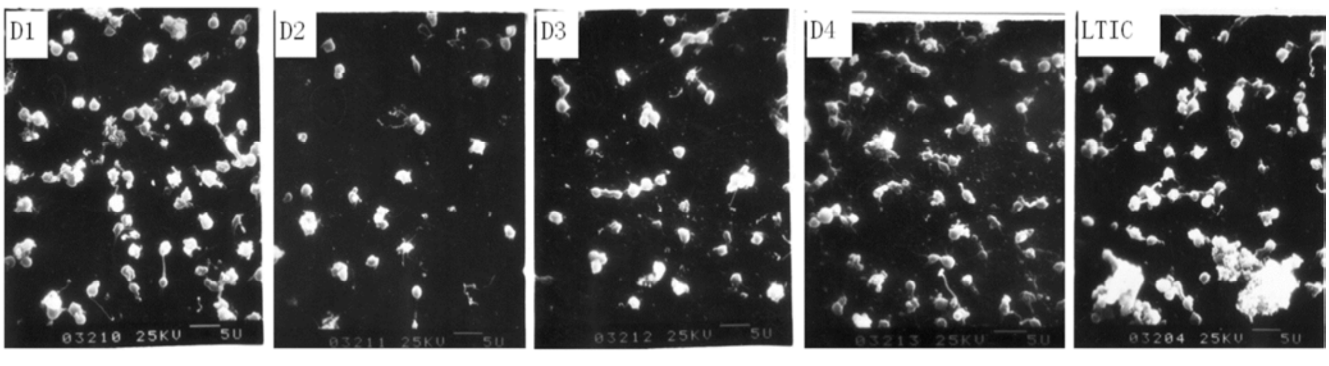


Figure 9. *Morphology of adhered platelet on DLC films under (D1) 0V, (D2) 50V, (D3) 100V, (D4) 200V substrate bias voltage, and LTIC, incubated in plasma of rich platelet (PRP) 2 hours.*

F. R. Marciano [68] studied the wettability and bactericidal activity of oxygen plasma-treated DLC films produced by plasma enhanced chemical vapor deposition technique. It was shown that DLC bactericidal activity ranged between 17% and

55%, depending on the kind of bacteria. Oxygen plasma treatment was performed in attempt to improve this activity. This treatment makes DLC surface more desorbed, rougher and superhydrophilic. However, the hydrophilic property of the treated films did not maintain itself when the films were sterilized in humid vapor (121°C, 1 atm), a routine procedure often applied to medical instruments. In addition, DLC antibacterial activity did not increased with plasma treatment. Therefore, the non-maintenance of the hydrophilic characteristics when the samples were autoclaved and the non-increase of its bactericidal activity make the use of oxygen plasma treatment in DLC films for bactericidal coating applications unsuccessful.

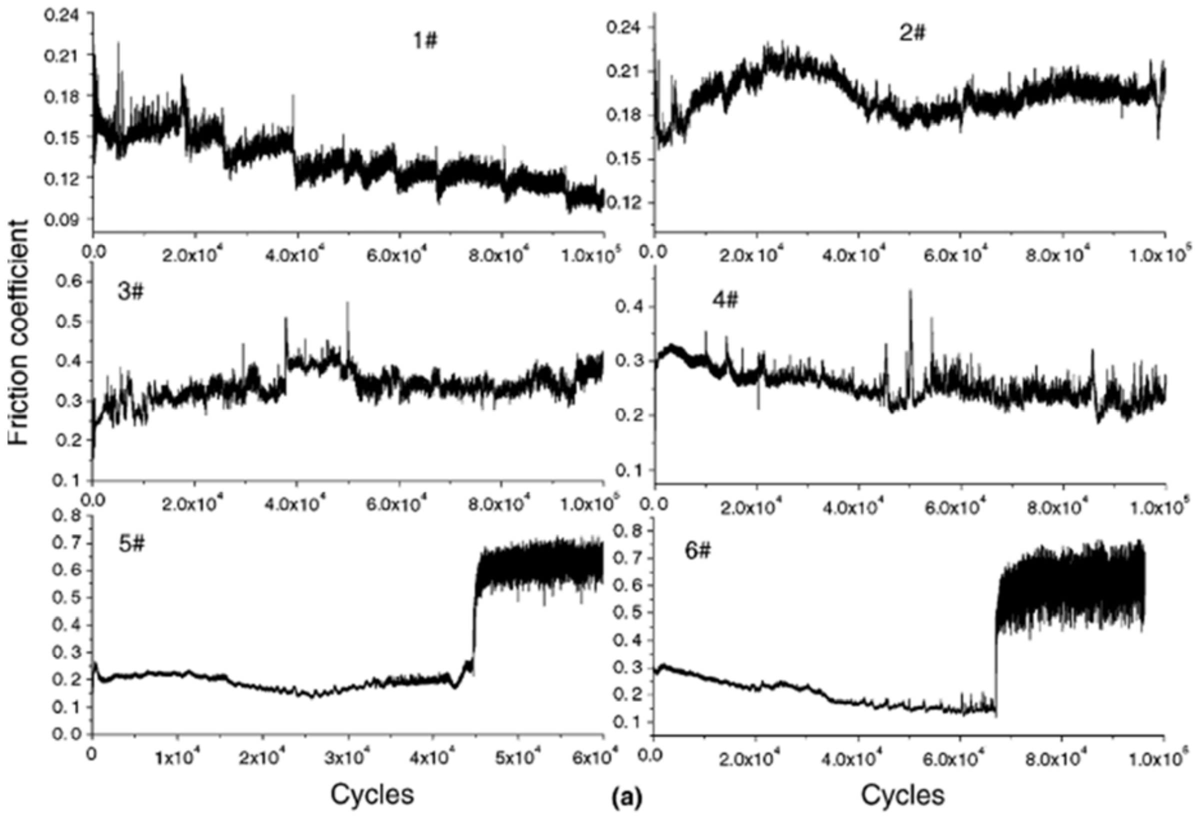
Leng [52] studied the effect of inner gas to properties of DLC films during deposition. The results showed that the DLC films have good adhesion and sp^3 carbon atom content in the DLC films decreased as the argon flow increases. The blood adhesion behavior of the DLC films is influenced by the ratio of sp^3 and sp^2. The platelet adhesion behavior of the DLC films increased with increasing argon flow. Unfortunately, the hardness and wear resistance decreased with increasing argon flow, though still much better than for Ti alloy substrates.

Leng *et al.* [69] lately investigated the biocompatibility of DLC films with different structures deposited by filtered cathodic vacuum arc (FCVA) deposition, at direct current bias voltage of 80 V and pulsed at 1000 V (duty ratio 30%, frequency 20 kHz). In this research, the biocompatibility of DLC films was investigated using macrophages, osteoblasts and fibroblasts. The relationship between bovine serum albumin adsorption and cell growth suggests that cell growth occurs in a structure-dependent manner, and that DLC films that are rich in the diamond phase exhibited outstanding biocompatibility [70, 71]. Because of the lack of repulsive forces, DLC tending to diamond structure (more sp^3 content) exhibited better biocompatibility compared with that tending to graphite structure. Their findings further prove former views that the structure (sp^2/sp^3) is an important factor in regulating the biological response of DLC and can greatly improve the biomedical application of DLC films.

5.2. Element-Doped DLC Films

The properties of DLC can be modified by incorporating other elements into the films to tailor them for specific applications.

Wen [55] synthesized nitrogen-doped DLC (N-DLC) films using Plasma immersion ion implantation and deposition (PIII-D) and studied the mechanical property of films. Nano-hardness of as-deposited films increase with the N content, and the value of the recovery R indicate that the coatings undergo not only plastic deformation but also elastic deformation during indentation testing. The results of the optical band gap and resistivity confirm the films have semi-conductor characteristics. Furthermore, wear resistant and electrical property of N-DLC films was also studied [56]. The results showed that the wear resistance of the N-DLC films becomes steadily worse with increasing N_2 pressure (Seen in Figure 10). Hall Effect measurements indicate an n-type semiconductor characteristic of N-DLC films. The resistivity and carrier mobility ratio of the films increases with increasing N_2 pressure and the carrier concentration decreases [56].



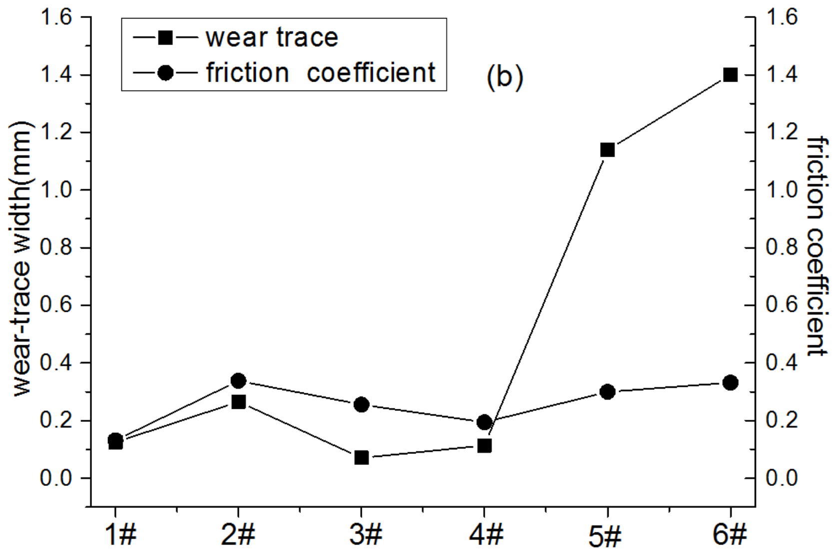


Figure 10. *(a) Friction coefficient versus wear cycles for N-DLC films on Ti6Al4V substrate, (b) Wear trace width and friction coefficient (N_2 pressure 1# 7.0×10^{-3}Pa, 2# 3.0×10^{-2}Pa, 3# 7.0×10^{-2} Pa, 4# 1.6×10^{-1}Pa, 5# 2.5×10^{-1}Pa, 6# 4.0×10^{-1}Pa).*

Yang [57-59] studied hydrogen-doped DLC (H-DLC) films systematically. The investigation of the surface energy showed that the polar part of the surface energy decreases with increasing substrate bias voltage which affected mainly by the reduction of the sp^3 content in the films. Graphitization of DLC film is promoted at higher substrate bias. The film deposited at a lower substrate bias of -75 V possesses better blood compatibility than the films at higher bias on stainless steel. The higher the bias voltage, the larger is the activation of the adherent platelets. This trend is consistent with the surface energy of the films. The effects can be attributed to the preference of albumin adsorption due to the higher adhesion work of albumin compared to that of fibrinogen and the changes of fibrinogen conformation caused by higher interfacial energy. Yang suggested two possible paths to improve the blood compatibility, suppression of the endogenic clotting system and reduction of platelet activation. One is that H-DLC film surface can suppress the contact activation path of the clotting process which is not affected very much by substrate bias voltage. The other is that H-DLC film surface may reduce the activation of platelets adhered on surface. The second process is strongly affected by substrate bias voltage.

The effects of annealing to properties of H-DLC films were also studied [58]. After annealed at 600°C in vacuum, hydrogen effusion and film graphitization are promoted. Resistivity, carrier concentration, mobility and surface energy also can be changed during annealing, which can be attributed to the increases in the content of sp^2 bonding carbon and the size of ordered sp^2 cluster. The platelet adhesion and activation of H-DLC is affected by annealing. Platelets are strongly surface activated by the H-DLC film deposited at high bias of -900 V, and the situation worsens after annealing. It can be attributed to the increase of electrical conductivity caused by appearance of nano-crystalline graphite and p-type majority carriers caused by the bond defects. In contrast, the H-DLC film deposited at -75 V bias exhibits the lower surface activation of adherent platelets, and maintains its blood-compatibility as much. It can be related to its good dielectric character and its good resistance to anneal. Furthermore, the effects are also obviously about different annealing temperature to properties of H-DLC films [59]. Hydrogen effusion and film graphitization are promoted at high annealing temperature. The physical properties, surface characteristics change with anneal temperature and the thrombogenesis of the films is affected by annealing. The results were correlated with the biological data to elucidate the blood compatibility mechanism of a-C: H films. It is believed that the possible factors affecting blood compatibility are the adhesion energy of blood plasma, band gap, carrier type and concentration. Improving the electronic structure of a-C: H films is critical to the abatement of platelet activation.

Yao [61] synthesized fluorine-doped DLC (F-DLC) films using CF_4 gas by PIII-D. Fluorinated extent was gradually improved with the increase in CF_4 flux. Etching behavior of F ions during deposition makes film become smoother. Hardness of F-DLC films is significantly reduced but still has a fairly good adhesion to the substrate with the increase of F content increased in the film. Figure 11 shows the variation of water contact angles as a function of the CF_4 flow. Improvement of the hydrophobicity of the films is obvious. With the highest CF_4 flow rate, the contact angle of distilled water on the DLC film increases by one time and is close to that of PTFE. This high hydrophobicity surface possesses good anti-adhesion property.

Compared research have been done between a-C: H: N (fabricated by PIII-D using C_2H_2+N_2 gas mixtures) and H-DLC films [54]. Incorporation of nitrogen in the amorphous carbon can increase the film hydrophilicity and roughness. Figure 12 shows that no obviously increased cell death or immediate toxicity was found in all films, while a-C: H: N films possesses better endothelial cell growth and anti-thrombotic properties than a-C: H films. This suggests that nitrogen incorporation can improve the biocompatibility of our a-C: H films.

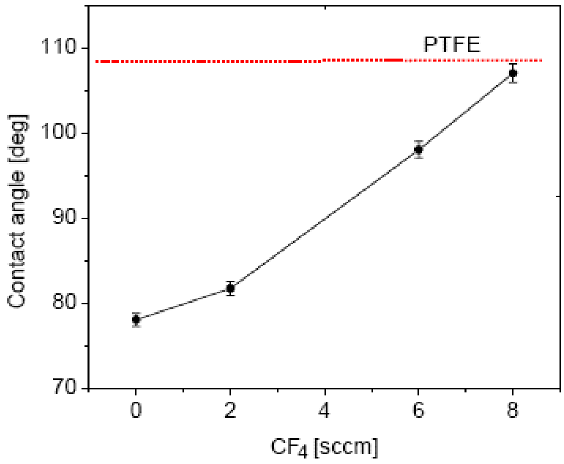


Figure 11. *Contact angles water vs. CF_4 flux.*

Korany [72] discovered that hydrogen in DLC films causes the shift of C-C bonds from sp^2 to sp^3, and generation of a larger number of C-H bonds which relieve the internal stress and produces a softer polymer-like materials. Compared with hydrogen-free DLC films, such films with a high degree of hydrogenation have low friction and wear especially when tests are performed in inert or vacuum test environments. Ronkainen *et al.* [73] evaluated the tribological performance of different DLC films in water-lubricated conditions. Their results showed that the amorphous hydrogenated carbon films could not survive in the water-lubricated conditions, and was worn through during the test, while the hydrogen-free DLC films fabricated by vacuum arc discharge exhibited the best wear resistance. However, the wear resistance of hydrogenated DLC films can be improved by doping with Si, W and Cr or by interlayers.

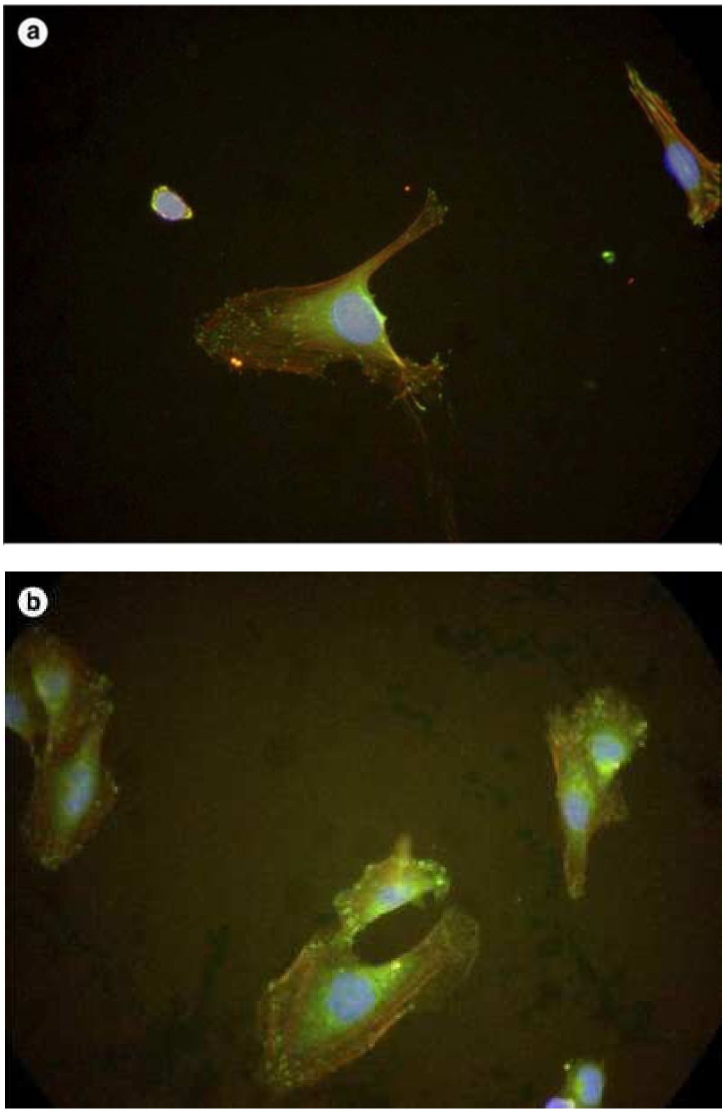


Figure 12. *Photomicrographs of human microvascular endothelial cells fluorescently stained for actin stress fibers (red), vinculin (green) and nucleus (blue) on: (a) a-C: H film and (b) a-C: H: N film. (For interpretation of the references to the color in this figure legend, the reader is referred to the web version of this article.).*

High quality TiO_2-DLC films were produced from PECVD technique [74]. The incorporation of TiO_2 nanoparticles increased hydrogen content and reduced the compressive stress and water contact angle. The antibacterial tests against *E. coli* show the increase of DLC bactericidal activity as the increasing of TiO_2 content (seen in Figure 13). The thermodynamic approach also shows the increasing in bacterial adhesion with the increasing of TiO_2 content. TiO_2-DLC films can be used in biomedical applications in general.

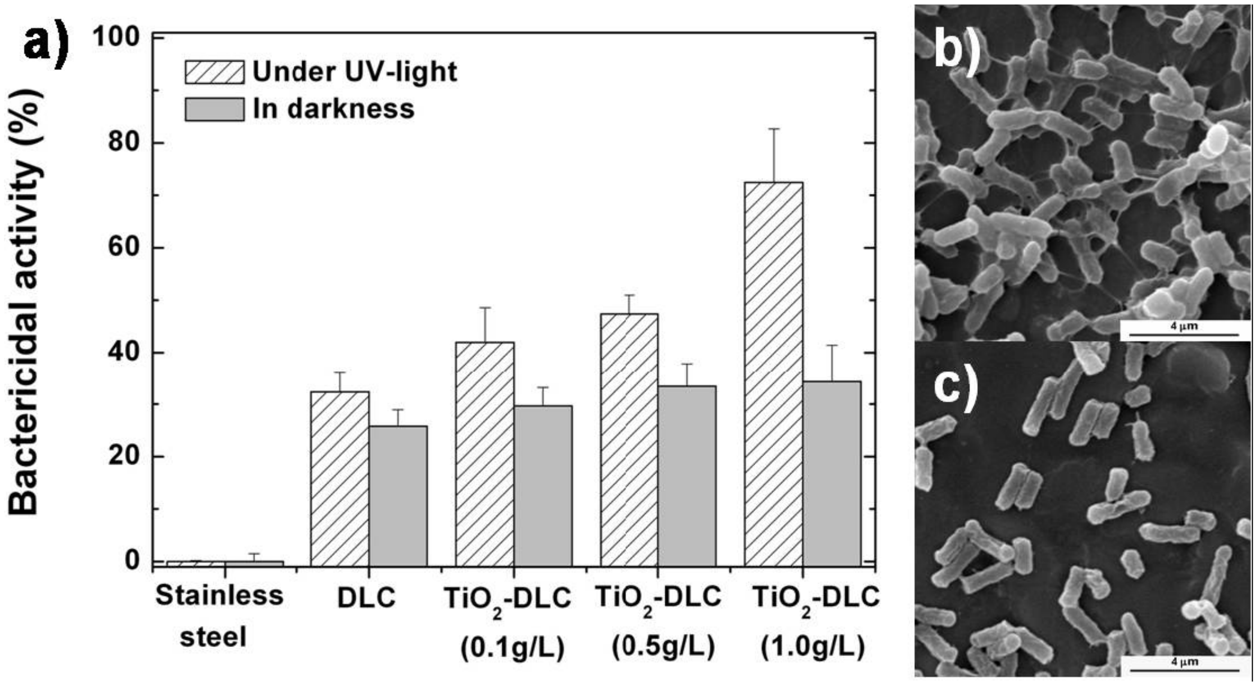


Figure 13. *a) TiO_2 nanoparticles increased DLC bactericidal activity, b) under UV-light. More bacterial cytoplasmic projections can be seen on TiO_2-DLC surface, c) compared to the pure DLC.*

Amin *et al.* [75] synthesized DLC films containing titanium oxide (DLC-TiO_x, $x \le 2$) using pulsed DC metal–organic plasma activated chemical vapor deposition (MOCVD) technique. The biomimetic growth of amorphous carbonated apatite on the DLC-TiO_x in simulated body fluid (SBF) was found and dependent on the Ti content of the film. UV light exposure prior to immersion in SBF increased the growth rate of apatite formation significantly as a result of increased hydrophilicity of the surface.

Silicon-doped DLC films have also been researched diffusely. But the articles about multi-doped DLC coatings for biomedical applications are still minority now. Lidia Swiatek *et al.* [76] obtained the Si/Ag-incorporated DLC layers deposited on titanium alloy (Ti6Al7Nb) using modified radio frequency plasma assisted chemical vapor deposition (RF PACVD) method. The biocompatibility assessment of the deposited DLC-Si/Ag coatings was performed using the LIVE/DEAD test (Live/Dead Viability/Cytotoxicity Kit, Molecular Probes, USA). In this research, good cell proliferation and low cytotoxicity could express good biocompatibility of DLC-Si/Ag films in which the content of silver and silicon is proper (seen in Figure 14). Their results demonstrated that the simultaneous doping with Ag and Si (at proper ratio) allows to obtain coatings of good biocompatibility and high antibacterial properties, which are due to the bactericidal properties of Ag and the fact of the doped Si positively influences the proliferation of endothelial cells according to the literature [77-79].

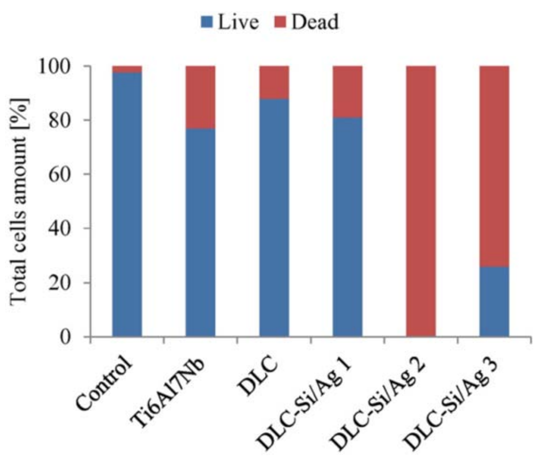


Figure 14. *Cytotoxicity of the deposited DLC-Si/Ag coatings expressed by the relative number of dead and live endothelial cells (EA. hy 926 cell line).*

6. Conclusions

Pyrolytic C is still a common valve prosthesis biomaterial but the most widely used C allotrope coating in medical prostheses and surgical instruments is DLC, a metastable form of amorphous C due to its super mechanical properties and good biocompatibilities. Diamond-like carbon shares properties of both graphite and diamond, such as high hardness and elastic modulus, chemical inertness, high electrical resistivity, infrared transparency, high refractive index and excellent smoothness. These properties are achieved in an isotropic disordered thin film with no grain boundaries, thus makes DLC much cheaper to produce when compared to diamond itself.

In this paper, many about preparations and analysis methods of inorganic biomedical DLC film materials were described, two main evaluation methods about coagulant properties of materials were also be described. In order to fully exploit the full potential of DLC films as blood contacting biomaterials, the films must have proper ratio of sp^3 to sp^2, so as to achieve good blood compatibility and mechanical properties. Doping can improve anticoagulant property of DLC films, prolong clotting times when plasma contacted with DLC films and reduce conformation change of fibrinogen adhered on the materials surface. It has been found the DLC films with higher γ_s^p/γ_s^d, lower interfacial tension possess good anticoagulant property. The results discussed in the chapter support the conclusion that the important factors on affecting the films anticoagulant property are a suitable sp^3 bonds content, γ_s^p/γ_s^d ratio and interfacial tension.

All the time, DLC films are the hotspot in the fields of biomaterials. But, it has still incompletion for grasping DLC films such as controlling accurately the ratio of sp^3 to sp^2 in deposited DLC films in the present research works, even if there are many synthesis methods. Meanwhile, evaluation methods must be reinforced, including implantation evaluation in animals, for understanding contacting behaviour of DLC films with blood in depth and obtaining complete information to evaluate hemo-compatibility of materials. Therefore, a continuous effort is essential so that we can perfectly take advantage of DLC films as biomaterials.

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