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Photochemical Modification of Hydrogenated Diamond-Like Carbon Films with Acrylic Acid to Increase Antibody Immobilization

Yuqi Xue

Experiment teaching center, Sun Yat-Sen University
Zhuhai, China

Abstract

Antibody immobilization is very important for protein or antigen specific detection in immunosorbent biosensors or biochips which decide the stability and accuracy of the biosensors. Surface modification of hydrogen-DLC (DH) film is the key to increase antibody immobilization. At present, almost all modification methods of DLC films cost a long time and need acid or alkali treatment. Here, we presented a rapid and gentle photochemical process to modify the DH film on which a polyacrylic acid (PAA) film formed. After prepared by radio frequency magnetron sputtering, the DH films covalently reacted with small organic molecules acrylic acid (AA) using ultraviolet (UV) light at 254 nm within 0,5,10 and 15 min, respectively. With the UV irradiation time increasing, the surface energy increased from 27.0 to 69.9 dyn/cm contributed to the carboxyl on the films, analyzed based on the measured contact angle; while the roughness increased from 1.65 to76.7 nm, shown from the SEM images and AFM images. The peaks at 1710 cm-1, 1452 cm-1 and 1247 cm-1 in the fourier transform infrared spectroscopy (FTIR) proved the presence of carboxyl or PAA film on the surface of samples. The optical density detected by enzyme label method showed that the relative antibody adhesion were 0.924, 1.700, 1.985 and 2.363, respectively. We concluded that the antibody immobilization on the DH film is improved effectively.

Keywords: *Hydrogen-DLC, Acrylic Acid, Surface Energy, Antibody Immobilization*

1. Introduction

An antibody is a large Y-shaped protein that is known as an immunoglobulin and applied for the immune system to identify and neutralize foreign objects such as bacteria and viruses.The antibody recognizes a unique part of the foreign target, called an antigen[1]. Antibody immobilization play an important role in protein or antigen specific detection in immunosorbent biosensors or biochips[2].

Diamond-like carbon (DLC) films have attracted great interest due to their favorable nature such as chemical inertness[3],excellent smoothness[4] and optical transparency in the visible and infrared[5]. DLC film is used as a good protect film keep substrates such as GMR from corrosion and is biocompatible due to its chemical composition containing only carbon, hydrogen[6]. Chemically modified surfaces of DLC films have been used as sensor materials for bio-molecules detections[7]. A variety of CVD and PVD methods were used to deposit DLC films including magnetron sputtering, RF or DC plasma-enhanced chemical vapor deposition (PECVD), ion beam plating, ion beam sputtering, ion beam-assisted deposition and so on. RF magnetron sputtering is a high deposition rate and high purity method to prepared DLC films[8] .

Photochemical modification is a clean and safe method to initiate terminally unsaturated adsorbates reaction with hydrogen-terminated film[9]. Todd Strother et al. presented a reaction for the UV-mediated attachment of alkenes to silicon surfaces for functionalization[10] and Tami L. Lasseter et al. reported a direct covalent functionalization of Si-H and C-H with terminal vinyl group of oligomers by UV illumination at 254 nm [11]which are enlightenments for our work. At present, almost all photochemical modifications need a long time and acid or alkali treatment which could destroy the substrates like GMR chips.

In this article, we reported a functionalization of DH film via C-H with acrylic acid by UV light within 15 min. We also studied the relative antibody immobilization amounts on the films before and after photochemical functionalization..

2. Experimentals

2.1 Preparation of DH film

DLC films on Si substrates (7×7 mm^2) were deposited from graphite target (99.99 % purity) by radio frequency magnetron sputtering for 30 min in 3 Pa Argon (99.99 % purity) and sputtering power was 130 W. Then the substrate was moved from the graphite target, and hydrogen (99.99 % purity) was inlet in situ with gas pressure 10 Pa and the sputtering power was 30 W. The DLC or DH film thickness was 130nm.

2.2 Photochemical modification of DH film

In this procedure, 3μL AA liquid was dropped on the DH film surface which placed on a quartz slide (3×3 cm2). Then a second quartz slide covered on the film trapping a thin liquid film over the surface[12]. The film surface was illuminated with a UV light (254 nm, 50 W) for varying times (5, 10, 15 min) at room temperature. After the photochemical modification, the samples (DHA) were washed in acetone (3 min) and ethanol (3 min) to remove any unreacted reactants and dried with N2.

2.3 Antibody immobilization

The films were washed with de-ionized water and rinsed with 2-(N-Morpholino) ethanesulfonic (MES) (50 mM, pH 5.6).The surface was activated by N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride / N-Hydroxysuccinimide (EDC/NHS) (20 mM / 12 mM) solution in MES buffer for 30 min at 37 ℃. After washing with three 400μL PBS (10 mM, pH 7.4), the samples were dried with N2 and ready for goat anti rabbit IgG immobilization. The IgG solution (0.67μg/mL) was obtained by diluting the goat anti rabbit IgG stock solution (2 mg/mL, in PBS solution). 400μL IgG solution was dropped on the DHA surface incubated for 120 min at 37℃in wet box. The sample was rinsed with PBS (adding Tween20) for five times, washed with de-ionized water twice and dried with N2 for enzyme label method detection. The 3, 3', 5, 5'-Tetramethylbenzidine (TMB) color time is 10 min at 37 ℃ avoiding light with 200μL TMB mixing solution. The volume ratio of solution A and solution B was 1:1. A 96-microtitor plate was used for enzyme label method detection with 100μL TMB mixing solution. To stop the TMB color reaction, 50μL 2 M H2SO4 was dropped into the plate and then the absorbance was determined at wave length of 450 nm.

2.4 Characterizations

The FTIR spectra of the samples were acquired in transmission mode at 4 cm-1 resolution on an EQUINOX 55 FTIR spectrometer; 64 scans were averaged for both background and samples. The data were obtained on an ultrahigh vacuum system (~2.0×10-10 Pa). The film surface morphology was observed by SEM (Quanta 400F). The surface of DLC film was scanned by atomic force microscope (AFM, SPI3800N, S IISeiko, Japan) under an ambient atmosphere at room temperature. AFM measurements were performed in the noncontact mode, and an area of 500 nm × 500 nm (DH) and an area of 10μm×10μm (other samples) were scanned at a scan rate of 2.99 Hz with 256 × 256 pixel resolution. Contact angle measurements were carried out using OCA-20 contact analyser. Two test liquids, water and formamide (AR, 99.3%), were used as probes for surface free energy calculations based on Young's principle[13]. Enzyme label method detection was performed on enzyme mark instrumen (MULTISCAN MK3, Thermo SCIENTIFIC) to obtain the optical density value at 450 nm.

3. Results and Discussions

3.1 Characterization of Surface Functionalization

Figure1 shows the FTIR spectra of DH film before and after irradiation with AA for 5, 10, 15 min. After the photoreaction, the sample exhibits new peaks compared to DH film at 1710 cm-1, 1452 cm-1 and 1247 cm-1 which attributed to C=O stretch, C-O stretch and the deformation vibration of OH and aliphatic acid[14], respectively. The samples treated for different irradiation time have almost the same strength spectrum, which is shifted upward respectively in order to distinguish all the spectra of different samples in one figure. These new peaks show that COOHs are grafted to the DH film[15], polyacrylic acid (PAA) films formed, and the peaks in the FTIR spectra are almost the same as the spectra of PAA in the work of Lin et al[16]. In order to study the stability of the films, the modified samples were annealed at 300 ℃ for two hours. The FIRT spectra of the annealed samples change because of the formation of polyanhydride due to the high-temperature dehydration.The peak at 1041 cm-1 is attributed to C-O stretch vibration and the peaks at 1759 cm-1 and 1804 cm-1 are attributed to C=O stretch vibration in polyanhydride (see Figure 2). The results show that a PAA film has been stably attached to the DH film.

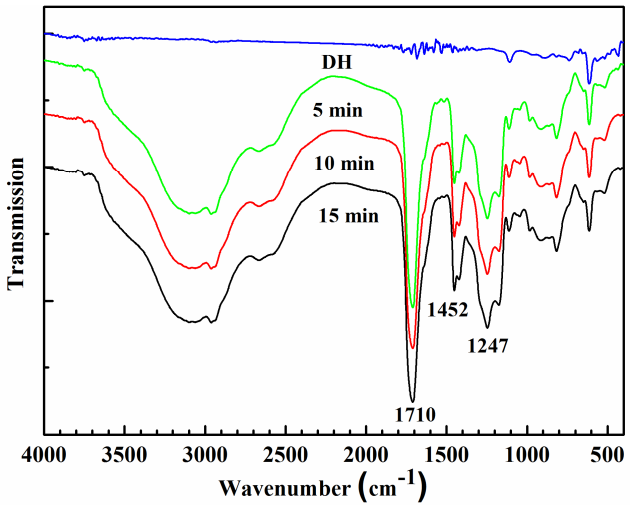

Figure 1. FTIR spectra of DH (irradiation for 0 min) and photochemical functionalization samples for 5, 10,and 15 min, respectively

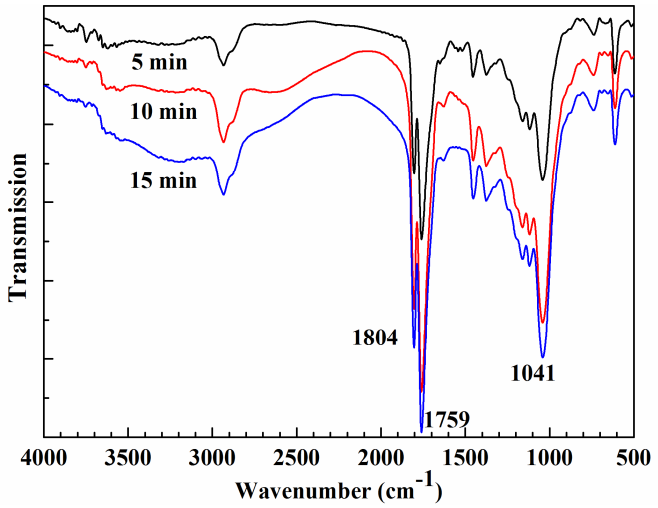

Figure 2. The FTIR spectra of samples photomodificated by UV irradiation for 5, 10, and 15 min, respectively.

3.2 The film surface morphology.

In order to study the surface morphology of the films, we used SEM and AFM to obtain 2D images and 3D images of the surface respectively. The SEM images show that the surface of DH film is compact and smooth; but becomes relatively rough after photoreaction (see Figure 3). The AFM images of sample surface show that the roughness increases from 1.65 to 76.7 nm after UV radiation, which is caused by a PAA film formed on the DH film surface (see Figure 4).

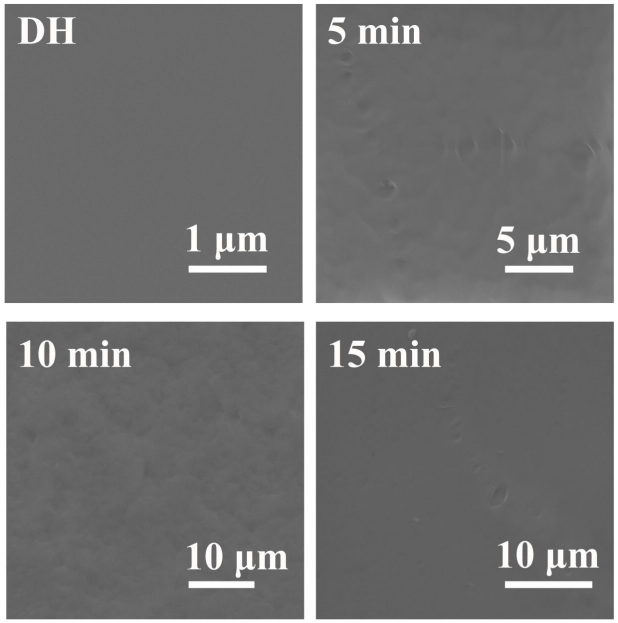

Figure 3. SEM images of surface morphology of all the samples

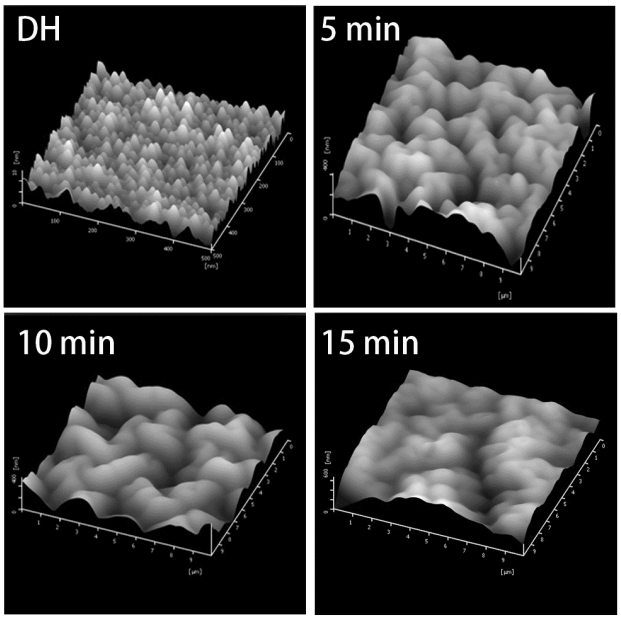

Figure 4. AFM three-dimensional images of the samples' surfaces. The x-axis and y-axis stand for horizontal scan range (500×500nm2 for S1; and 10×10μm2 for S2, S3, and S4), and the z-axis stands for vertical scan range (10 nm, 400 nm, 400 nm, and 600nm, for the image of S1, S2, S3, and S4, respectively) .

3.3 Contact Angle and Surface Energy of Samples.

Table1 shows the surface energy constants (the dispersive constant γLW, electron acceptor constant γ+ , and the electron donor constant γ-) of the two liquids at 25□. Table 2 shows the contact angle values and surface energy of samples at 25□. As the UV irradiation time increases from 0 to 15 min, the contact angle between with the AA modified surface and the water, or formamide, decreases; indicating the increase of the surface energy. The surface energy increases with the roughness increasing.

Table1. The surface energy constants of the test liquids at 25□.

Liquid	γ_{tol} (dyn/cm)	γ^{LW} (dyn/cm)	γ^{+} (dyn/cm)	γ^{-} (dyn/cm)
Water, H_2O	72.8	21.8	25.5	25.5

Formamide, CH_3NO	58.0	39.0	2.28	39.6

Table2. The Contact angle and the surface energy of the samples at 25□.

Samples	Irradiation time (min)	Contact angle (°)		Surface energy (dyn/cm)
		θ^W	θ^F	γ_s^{tol}
S1	0	103.0±0.0	66.2±0.9	27.0
S2	5	101.6±0.6	27.9±0.7	11.7
S3	10	27.8±0.9	11.3±4.6	64.7
S4	15	16.8±0.1	8.3±1.3	69.9

3.4 Antibody Immobilization.

Antibody immobilization on the PAA modification layer is higher than DH and increases with the increasing irradiation time (see Figure 5). The higher optical density value indicates a higher antibody immobilization. The optical density values of the DH film, the 5, 10, and 15 min modified samples are 0.924, 1.700, 1.985, and 2.363 respectively. It indicates that the antibody immobilization positively correlated with the surface energy of the films (see Figure 5) and the surface roughness (see Figure 6, R2=0.990). As a result, the antibody immobilization on the sample S2, S3 and S4 was increased by 83.9-155.7%, as compared with the DH film. The sample S2 as shown in Figure 5, has a lower surface energy than that of DH but its OD value was higher than that of DH film. This may attribute to the COOHs on the modified layer, which is easier to react with the antibody. To conclude, the antibody immobilization is related to the surface roughness and chemical composition (COOHs) on the surface.

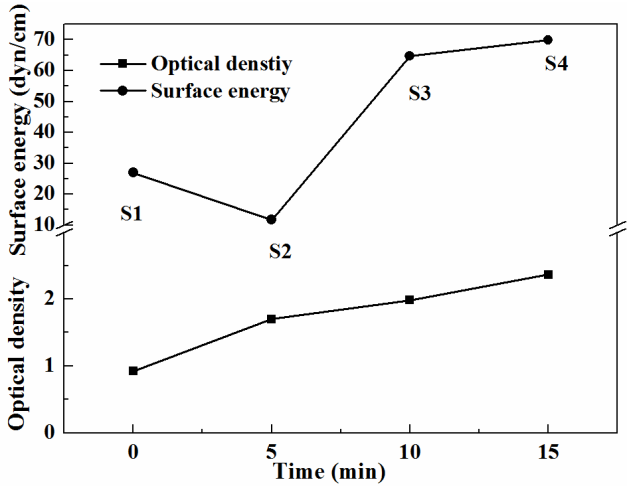

Figure5. The effect of surface energy of samples on antibody immobilization.

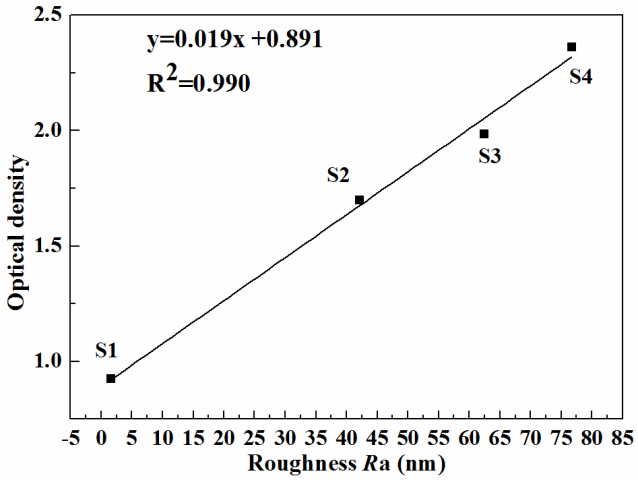

Figure 6. Antibody immobilization vs. surface roughness of the samples

4 Conclusions

We prepared DLC films on the Si substrates by RF magnetron sputtering and hydrogenated DLC films in situ. Then a modification layer (PAA films) grafted onto the DH film by photochemical reaction with acrylic acid using irradiation UV light at 254 nm. The surface energy of the samples increased with the modification time due to the COOHs grafted to the DH film, and with the increasing roughness of the samples. We concluded that the antibody immobilization on the film is improved efficiently after a PAA film grafted on the DH film.

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