Applied Surface Science 258 (2012) 5166–5174

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Effect of surface structure and wettability of DLC and N-DLC thin films on adsorption of glycine

Mukhtar H. Ahmed*, John A. Byrne

Nanotechnology Integrated Bio-Engineering Centre, University of Ulster, Jordanstown, BT37 0QB, Belfast, UK

ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 4 January 2012
Accepted 29 January 2012
Available online 9 February 2012

Keywords:
Diamond-like carbon (DLC)
Wettability
Surface free energy
Glycine adsorption
Raman spectroscopy
Fourier transform infrared spectroscopy (FTIR)

ABSTRACT

Diamond-like carbon (DLC) is known to have excellent biocompatibility. Various samples of DLC and nitrogen-doped DLC thin films (N-DLC) were deposited onto silicon substrates using plasma-enhanced chemical vapour deposition (PECVD). Subsequently, the adsorption of amino acid glycine onto the surfaces of the thin films was investigated to elucidate the mechanisms involved in protein adhesion. The physicochemical characteristics of the surfaces, before and after adsorption of glycine, were investigated using Fourier transfer infrared (FTIR), Raman spectroscopy, spectroscopic ellipsometry (SE) and contact angle (θ). The Raman study highlighted decrease slightly in the ID/IG ratio at low levels of N (5.4 at.%), whilst increasing the nitrogen dopant level (>5.4 at.%) resulted in a increase of the ID/IG ratio, and the FTIR band at related to C=N. Following exposure to glycine solutions, the presence of Raman bands at 1727 cm^{-1} and 1200 cm^{-1}, and FTIR bands at 1735 cm^{-1} indicates that the adsorption of glycine onto the surfaces has taken place. These results which obtained from SE and surface free energy, show that low levels of nitrogen doping in DLC enhances the adsorption of the amino acid, while, increased doping led to a reduced adsorption, as compared to undoped DLC. Glycine is bound to the surface of the DLC films via both de-protonated carboxyl and protonated amino groups while, in the case of N-DLC gylcine was bound to the surface via anionic carboxyl groups and the amino group did not interact strongly with the surface. Doping of DLC may allow control of protein adsorption to the surface.

1. Introduction

The implantation of biomaterials into the human body allows it restructure function and hence to enhance the quality of life. The highly corrosive surroundings and the low tolerance of the body to some dissolution products restrict the materials to be used for implants [1]. Diamond-like carbon (DLC) is an excellent candidate for use as biocompatible coatings on biomedical implants [2] such as rotary blood pumps [3], artificial hearts, mechanical heart valves [4–6], coronary artery stents [7,8], hip and knee replacements [9,10], due to its remarkable properties such as high mechanical properties, high wear resistance, and chemical inertness [11,12]. Comparative studies showed that DLC has better biocompatibility and wear resistance than stainless steel [13], titanium and titanium alloys [14], poly-methyl mehtacrylate (PMMA) [15], cobalt chrome alloys, and alumina ceramics [16]. Several studies were performed to observe the dependence of hemocompatibility on the Raman D-band to G-band intensity ratio (ID/IG) of the DLC films [17]. Therefore, the structure of DLC films plays a vital role on the platelet adhesion on DLC surfaces [18].

In order to improve of it's properties, DLC films elementally modified by addition of third elements, such as nitrogen, silicon, fluorine, oxygen, and titanium [19,20]. Furthermore, nitrogen doped DLC films (N-DLC) are considered for widespread clinical use as biocompatible coatings due to their excellent mechanical properties including; surface roughness, elasticity, high hardness, infrared transparency and low friction coefficient [21,22]. It was found that hydrogen content of the DLC film as well as the ratio of sp2 to sp3 bonds can have significant effects on friction and wear [23]. The replacement of CH with NH bonds in N-DLC reduces the average coordination number and enhances the sp^2 hybridise bonding, leading to decrease in both internal stresses and the sp^3 hybridization fraction, due to presence of C=N bonds [24]. Investigations have found that the factors including nitrogen concentration, C—N film roughness and types of bond between C and N, play a significant role in clotting time and amount of adhered platelets [25].

2. Experimental details

2.1. Film deposition

Prior to film deposition, Silicon wafers 1.5 cm × 1.5 cm were washed ultrasonically in pure acetone to remove residual organic

* Corresponding author.
E-mail address: ahmed-m@ulster.ac.uk (M.H. Ahmed).

0169-4332/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2012.01.162

Table 1
Deposition of DLC film preparation process parameters employed for the PECVD.

Parameters	Samples			
	DLC	NI	NII	NIII
rf. power (W)	109	117	123	131
Ar:C_2H_2 ratio (sccm)	10:20	10:20	10:20	10:20
Gas flow rate (Torr) $\times 10^{-2}$	0.71	1.2	1.4	2.0
N_2 gas flow (sccm)	0	3	5	7
at.% N (relative to C and O) [26]	0	5.3	8.4	12.1
Film thickness (nm) by SE	170 ± 18	177 ± 16	188 ± 21	195 ± 23

Key: bias voltage 400 V, deposition time 5 min, sccm: standard centimetre cube per minute, (±) is SD for (n = 5 samples), at.% N: atomic percentage of nitrogen that were obtained in previous work [26], nm: nanometre (10^{-9} m), SE: spectroscopic ellipsometry.

contaminants followed washed with distilled water and then dried using a lint-free cloth in a flow of nitrogen gas.

DLC and N-DLC films were deposited on substrates by the radio frequency 13.56 MHz plasma enhanced chemical vapour deposition (rf-PECVD) using a Diavac model 320PA (ACM Ltd.), with negative electrode self bias voltages set at 400 V. The experimental equipment had been described previously in details [26]. The films were prepared under the following conditions: discharge power 109–130 W, the source gases used in present experiment was C_2H_4 while Argon (Ar) was used as a carrier gas, and nitrogen (N_2) gas was used as dopant (in case of N-DLC). The C_2H_2/Argon gas mixture flow rate was 20/10 sccm. The N_2 gas flow was varied from 0 to 7 ml min^{-1} Table 1. Prior to deposition, the vacuum chamber was evacuated to $\sim 7.5 \times 10^{-6}$ Torr.

Glycine (Sigma–Aldrich) was prepared in aqueous phosphate buffer saline (SIGMA) to give solution of 0.001 M at pH 7.4. The DLC and N-DLC coated samples were immersed in a sealed bottle containing of 25 ml of solution at room temperature for 6 h in a shaker. Following incubation, the samples were washed twice with double distilled water and dried in a flow of nitrogen gas.

3. Film characterisation

DLC and N-DLC samples were characterised using Raman FTIR spectra, spectroscopic ellipsometry (SE) and contact angle, before and after attachment of glycine. Raman spectroscopy was recorded on a ISA lab-ram model system using an Argon laser beam ~50 mW 633 nm laser diode for excitation. Prior to acquisition the spectrometer was calibrated using the zero order diffraction peak and first order peak from a silicon phonon mode from a silicon wafer sample. In this work the following parameters, confocal aperture 200 μm, spectral resolution 5 cm^{-1}, A 100× objective was employed and typical acquisition times were 5 s with 7 time repeat. This process was repeated at five different spots across the samples of DLC and N-DLC to assess uniformity of response.

The chemical bonding configurations were characterised by Fourier-transformed infrared spectrometer (FTIR). The analysis was performed at room temperature using BIORAD Excalibur (FTS 3000MX series) instrument, the spectrum was recorded in the region of 4000–400 cm^{-1} and 60 scans were accumulated at a resolution of 4 cm^{-1}. In all cases of FTIR the background spectrum was collected before the actual sample analysis using the relevant unattached coated samples with glycine and this signature was subsequently removed from the sample scan. The subsequent analysis of the attached glycine on the diamond thin film sample was performed.

Static contact angle were performed on the prepared DLC and N-DLC prior to and after glycine adsorption. The degree of wettability of the films was examined with standard solvents; distilled water (H_2O), diiodomethane (C_2I_2) and ethylene glycol $C_2H_4(OH)_2$) with known surface tensions (Table 2), and the sessile drop method using a (CAM 200 optical contact angle system (KSV instruments LTD, Finland). Drops of 5 μL of solvents were generated with a micrometric syringe and deposited on the substrate surfaces. The contact angle was read from a protector of the equipment through a microscope by a naked eye at five different places of each sample surfaces, and the values were averaged. The surface energy (γ_s) and measure of the degree of hydrophobcity/hydrophilicty (ΔG_{iwi}) were calculated according to the equation in references [27,28].

Film thicknesses were performed using spectroscopic ellipsometry (SE) (SOPRA GES-5E) in a room temperature (23 ± 2)°C. The angle of incidence φ was set to 68.0° and the laser wavelength (λ) was 532.8 nm. The refractive index (nk) and thickness (dk) of prepared samples before and after exposure of glycine can be calculated from the ellipsometric angles, Δ and Ψ, using the fundamental equation:

$$e^{i\Delta} \tan\Psi = \frac{R_p}{R_s} = f(\mathrm{nk}, \mathrm{dk}, \lambda, \varphi) \quad (1)$$

$$F = R = \frac{R_p}{R_s} = \tan\Psi \cdot \exp^{(i\Delta)} \quad (2)$$

where R_p and R_s are the complex overall reflection coefficients for the parallel and perpendicular polarised waves, respectively. They are a function of the angle of incidence φ, the radiation wavelength λ, the indices of refraction, and the thickness of each layer of the model, nk, dk.

The ellipsometric quantities ($\tan\Psi$) [relative amplitude ratio] and ($\cos\Delta$) [relative phase shift] can be derived from positions of the polariser and analyzer at the detector, which are related to the Fresnel reflection coefficients (R) for (p) and (s) polarised light [29].

4. Results and discussion

4.1. Film characterisation before attachment of glycine

4.1.1. Film thicknesses

The film thicknesses of DLC and N-DLC samples have been achieved, and the values were arranged from (170–200) nm, the growth rate of film deposition was around (36 ± 6) nm min^{-1}, Table 1.

5. Raman spectroscopy

The Raman spectra of DLC and N-DLC samples are shown in Fig. 1, in order to obtain graphite (G) and disorder (D) peaks which correspond to sp^2 hybridization. The G-peak is due to the bond stretching of all pairs of sp^2 configuration atoms in both rings and chains, whilst the D-peak is the shoulder of the G-peak at lower wave-numbers, and is due to the presence of the sp^2 aromatic rings [30].

A typical broad peak for the DLC has been observed in the range of 1000–1800 cm^{-1}, and it was deconvoluted to yield two component peaks at 1349 cm^{-1} and 1538 cm^{-1} by Gaussian curve fitting, can be attributed to disordered graphite (D peak) and pure graphite