Materials Science and Engineering B 152 (2008) 12–15

Contents lists available at ScienceDirect

Materials Science and Engineering B

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

Albumin and fibrinogen adsorption on boron nitride and carbon-based thin films

S. Lousinian, N. Kalfagiannis, S. Logothetidis*

Laboratory for Thin Films – Nanosystems and Nanometrology (LTFN), Department of Physics, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

ARTICLE INFO

Keywords:
Amorphous boron nitride
Amorphous carbon
Protein
Haemocompatibility
Spectroscopic ellipsometry

ABSTRACT

The haemocompatibility (in the sense of the least possibility of thrombus formation/thrombogenicity potential) of homogeneous and amorphous BN (a-BN) thin films through the adsorption of two basic blood plasma proteins, human serum albumin (HSA) and fibrinogen (Fib) is explored in this work. A comparative study of the thrombogenicity potential of BN, amorphous carbon (a-C) and amorphous hydrogenated carbon thin films (a-C:H) is also presented. a-BN and a-C thin films were produced by radio frequency (RF) magnetron sputtering onto c-Si (1 0 0) substrates under various values of substrate bias voltage. a-C:H thin films were developed by RF Reactive MS, with various values of substrate bias voltage and under different values of H_2 partial pressure during deposition. For the consideration of the optical, compositional and structural properties of the films Spectroscopic Ellipsometry in the energy region of 1.5–6.5 eV was used, while for the study of surface topography and wetting properties Atomic Force Microscopy and Contact Angle measurements were additionally employed. The properties of the thin films were correlated with their thrombogenicity, through the estimation of the ratio of HSA/Fib surface concentration. a-BN films exhibit the smallest possibility of clot formation, with their wetting properties determining the thickness of the Fib layer formed on them as well as the ratio of HSA/Fib surface concentration. In the case of a-C thin films, the increase of % sp^3 content is crucial, while the value of the fundamental gap seems to influence the possibility for clot formation on a-C:H thin films.

1. Introduction

An important consideration, for the development of materials with extreme haemocompatibility, is the conception of the interactions between plasma proteins and surfaces. Plasma protein adsorption is accepted as the first event that occurs when a foreign material comes into contact with blood [1]. Subsequent phenomena are determined by interactions of blood cells with adsorbed protein layer. This procedure normally ends in coagulation, thrombus formation and embolization [2].

Several works have dealt with the biocompatibility of diamond-like carbon (DLC), tetrahedral amorphous carbon (ta-C) and tetrahedral amorphous hydrogenated carbon (ta-C:H) coatings [3–11]. Carbon-based thin films with an increased fraction of sp^3 bonds are known to possess high mechanical hardness, low friction coefficient, low surface roughness and chemical inertness [12–14] and have also shown good blood compatibility.

BN thin films are used as coatings for surgical tools [15], and the toxicity of cubic BN quantities that remain in the body has been examined through the assessment of human neuroblastoma cells and articular chondrocytes cell growth and survival. The results suggested that c-BN may be less toxic than tungsten carbide alloys containing cobalt [16]. The potential application of BN thin films as biocompatible coatings on implants has not been explored yet. The present work exhibits the preliminary original results regarding the thrombogenicity potential of a-BN thin films, through the investigation of human serum albumin (HSA) and fibrinogen (Fib) proteins on them. Boron nitride (BN) is a chemical compound which is isoelectronic and isostructural with carbon. BN phases can be divided according to the bond hybridization into: sp^2-types i.e. a hexagonal h-BN, turbostratic t-BN and rhombohedral r-BN phases, with the structure and properties close to graphite, and sp^3-types—cubic c-BN and wurtzite w-BN forms, similar to diamond and lonsdaleite, respectively. All types of BN are chemically inert and corrosion-resistant insulators. Both sp^3- and sp^2-type phases may be applied as composites, combining advantages of both soft and hard phases [17]. The BN thin films (~100 nm thickness) developed in this work are amorphous BN (a-BN) with mixed sp^2 and sp^3 bonded BN. A detailed analysis of a-BN films rich in

* Corresponding author. Tel.: +30 2310 998174; fax: +30 2310 998390.
E-mail address: logot@auth.gr (S. Logothetidis).

0921-5107/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.mseb.2008.06.014

sp^3-BN bonds deposited at room temperature (RT), with properties similar to crystalline c-BN has been described in previous work [18]. RT is essential for the prevention of crystalline growth. This material exhibits many advantages against c-BN such as homogeneity (e.g. one uniform composition through out the films and not c-BN formed on well oriented hexagonal BN layers) and amorphous structure as well as smoother surfaces and lower internal stresses [18].

In previous works we have developed a methodology in order to study the possibility of thrombus formation during material–protein interactions, the optical properties of the adsorbed plasma proteins and their adsorption mechanisms [19–21]. The aim of this work is to explore the thrombogenicity potential of a-BN thin films through HSA and Fib adsorption on them, and compare it with several carbon based thin films as well as to correlate them with their optical, structural, surface and wetting properties and the parameters that affect protein adsorption on the studied films.

2. Experimental

The sputtered a-BN, a-C and a-C:H films studied in this work were deposited by rf magnetron sputtering on c-Si (1 0 0) substrates at room temperature. Details about the growth of the films have been described elsewhere [18,22–24]. For the growth of a-C:H films, H_2 reactive gas was introduced into the vacuum chamber. The energy E_i of the ions (mainly Ar^+ or H^+), bombarding the growing film surface, varied by applying a substrate negative bias voltage of −60 to −120 V for the a-BN films, +10 to −200 V for the a-C films and a floating bias voltage V_b (+10 V) or a negative V_b (−40 V) for the a-C:H films. The ta-C film has been deposited using filtered cathodic vacuum arc deposition technique [25]. For the in situ investigation of the optical properties of the deposited films, SE measurements were performed, by using a Phase Modulated Ellipsometer (PME) in the energy region 1.5–5.5 eV, which is attached on the deposition system, at an angle of incidence of 70.4° [22–24].

In order to study the protein adsorption and make an estimation on the possibility of thrombus formation of the various carbon-based films, single HSA and Fib solutions in phosphate buffer saline (PBS, pH 7.4) were prepared with concentrations of 10 mg/ml and 1 mg/ml, respectively, with a HSA/Fib concentration ratio of 10:1, similar to that in the blood of a healthy person. HSA is the most abundant protein in human blood plasma and it has been found that its adsorption on surfaces inhibits thrombus formation [26,27]. On the other hand, Fib takes part in blood coagulation, facilitates adhesion and aggregation of platelets, and is important in the processes of both haemostasis and thrombosis [28]. The samples were dipped into the protein solutions for a total time of 2 h, at room temperature. Afterwards, the samples were rinsed with deionized water and dried under N_2 flow. Ex situ SE measurements were performed in the energy region 1.5–6.5 eV at angle of incidence of 70°, before and after the incubation of the films in the protein solutions. Surface characterization of the films was made by AFM tapping mode measurements, while the wetting properties were studied by contact angle measurements (static contact angle measurements with 5 μl deionized H_2O drops).

3. Results and discussion

3.1. Protein surface concentration ratio and optical/structural properties of carbon based thin films

Electronic transitions of graphite, diamond and composite carbon films, the influence of the incorporation of hydrogen into the amorphous carbon matrix, as well as the optical properties of a-BN, have been described thoroughly in various studies [18,29–34]. The sp^3 fractions of the a-BN, a-C, a-C:H and ta-C films were estimated by Bruggeman Effective Medium Approximation (BEMA), which is described by the following equation [24,35]:

$$\sum_i f_i \frac{\varepsilon_i - \varepsilon}{\varepsilon_i + 2\varepsilon} = 0, \quad \sum_i f_i = 1 \tag{1}$$

where, f_i and ε_i are the relative volume fraction and the dielectric function of the ith component, respectively, and ε is the bulk dielectric function of the film. Carbon-based thin films studied in this work are considered to be composite materials consisting of sp^3– and sp^2– bonded atoms and voids, with respective volume fractions of f_{sp^3}, f_{sp^2} and $f_v (= 1 - f_{sp^3} - f_{sp^2})$, and known dielectric functions. By using the above equations, f_{sp^3} and f_{sp^2} were determined. The sp^3 fractions of the studied a-BN films varied from 10 to 27%, for a-C films from 20 to 45% and those of a-C:H films were chosen to be in the range of 40–45% and were estimated by using BEMA. The accuracy of sp^2 and sp^3 content of amorphous materials, estimated by BEMA with SE-methodology, depends on the dielectric functions that are used as references. In the present work, in order to estimate the sp^2 and sp^3 volume fractions, bulk sp^2 and sp^3 bonded BN dielectric functions were used as references in BEMA. For a-C and a-C:H thin films, the dielectric functions of graphite and diamond were used as references for sp^2 and sp^3 bonds, respectively.

Surface concentration Γ of HSA and Fib on a-C:H films was calculated by Cuypers formula [36]

$$\Gamma = 0.1 d \frac{M}{A} \frac{n_f^2 - 1}{n_f^2 + 2} \quad [\mu g/cm^2], \tag{2}$$

where, A is the molar refractivity (cm^3/mol), M is the molecular weight of the protein, d and n_f the thickness and the refractive index of the protein layer (derived by SE measurements). It is reported [4,6,9] that haemocompatibility value is attributed to the ratio $\Gamma_{HSA}/\Gamma_{Fib}$, although sometimes there is disagreement between the in vitro and in vivo experiments. Then, the ratio $\Gamma_{HSA}/\Gamma_{Fib}$ was calculated, as a preliminary useful indicator of the films' haemocompatibility (thrombogenicity potential). Larger values of the ratio mean that the possibility of thrombus formation on the surface of the film is less likely, therefore the film could be considered as more haemocompatible.

The correlation between sp^3 content and protein surface concentration ratio $\Gamma_{HSA}/\Gamma_{Fib}$ for the a-BN, a-C, a-C:H and ta-C films, is presented in Fig. 1. The preliminary results in the case if a-BN thin films show that a minimum value for $\Gamma_{HSA}/\Gamma_{Fib}$ ratio appears when sp^3 content of the films is around 23–25%, while for a-C films, the ratio increases with sp^3 content. a-C:H films with around 40% of sp^3 bonding, without application of bias voltage on the substrate (floating conditions) and small H_2 partial pressure during deposition exhibit a large value of protein surface concentration ratio. According to the dispersion "Tauc–Lorentz" (TL) model for amorphous semiconductors which was employed to parameterize the dielectric functions with the optical constants on the wavelength of light [19,37,38], these films present a value of fundamental gap E_g larger than 1.8 eV, as it can be seen in the calculated bulk dielectric functions $\varepsilon(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)$, of two representative a-C:H films in Fig. 2a (inset). It has been reported that when the fundamental gap of potential biomaterials is larger than 1.8 eV, which is the fundamental gap of Fib, the electron transfer between the protein molecule and the material is not possible. Therefore, the structure of Fib molecule remains stable and no bonding rupture is observed. In this way, Fib does not change into fibrin and subsequent platelet adhesion and thrombus formation is inhibited [39]. This assumption could explain the