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Optical and surface characterization of amorphous boron nitride thin films for use as blood compatible coatings

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ABSTRACT

The aim of this work is the investigation of the haemocompatibility properties of homogeneous and amorphous boron nitride (a-BN) thin films, through the adsorption of two basic blood plasma proteins, human serum albumin (HSA) and fibrinogen (Fib). The a-BN thin films were grown onto c-Si(100) substrates under different values of substrate bias voltage, employing the radio frequency (RF) magnetron sputtering technique. For the consideration of the optical, compositional and structural properties of the films, Spectroscopic Ellipsometry (SE) in the Vis–UV spectral region was used, while for the study of surface topography and surface charge distribution as well as of the wetting properties of the a-BN thin films, Atomic Force Microscopy (AFM), Electric Force Microscopy (EFM) and Contact Angle measurements were additionally employed. The properties of the thin films were correlated with their haemocompatibility, through the estimation of the ratio of HSA/Fib surface concentration. The sp^3 content of the samples does not seem to correlate with the haemocompatibility of the a-BN thin films. However, the surface properties determine the thrombogenicity potential of the studied samples. More precisely, the a-BN films with a less negatively charged surface exhibit the smallest possibility of clot formation, possibly due to the interactions between the charged chains of the Fib molecules and the a-BN surface, while slight changes in the surface roughness do not affect their haemocompatibility properties. The wetting properties determine the thickness of the adsorbed Fib as well as the ratio of HSA/Fib surface concentration.

1. Introduction

The comprehension of the interactions between plasma proteins and surfaces is crucial for the development of materials with high haemocompatibility, since plasma protein adsorption is accepted as the first event that occurs when a foreign material comes into contact with blood and the interactions of blood cells with adsorbed protein layer determine the subsequent phenomena [1].

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 [2–4] and have also shown good blood compatibility [5–10]. BN thin films are used as coatings for surgical tools [11], and the toxicity of cubic BN (c-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 [12]. The potential application of BN thin films as biocompatible coatings on implants has not been explored yet. 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 BN (h-BN), turbostratic BN (t-BN) and rhombohedral BN (r-BN) phases, with the structure and properties close to graphite, and sp^3-types – cubic BN (c-BN) and wurtzite BN (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 [13]. The BN thin films ($\sim$100 nm thickness, as determined by Vis–UV Spectroscopic Ellipsometry) 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 sp^3-BN bonds deposited at room temperature (RT), with properties similar to crystalline c-BN has been described in previous work [14]. RT is essential for the prevention of crystalline growth. This material exhibits many

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advantages against c-BN such as homogeneity (e.g. one uniform composition throughout 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 [14].

In previous works we have developed a methodology in order to study the haemocompatibility of thin films, in the sense of the least possibility of thrombus formation during material–protein interactions, the optical properties of the adsorbed plasma proteins and their adsorption mechanisms [15–17]. The aim of this work is to explore the haemocompatibility properties of a-BN thin films through the adsorption of single protein solutions of Human Serum Albumin (HSA) and Fibrinogen (Fib) (the two most relevant to thrombogenesis blood plasma proteins) and correlate their haemocompatibility properties with the different optical, structural, surface and wetting properties of the a-BN thin films. Our preliminary results provide first hints on the behaviour regarding blood plasma proteins' adsorption on the surface of a-BN thin films. The results have shown that a-BN thin films exhibit high haemocompatibility, even higher than amorphous carbon (hydrogenated or not) thin films.

2. Experimental

The sputtered a-BN films studied in this work were deposited by RF magnetron sputtering on c-Si (100) substrates at room temperature. Details about the growth of the films have been described elsewhere [14,18,19]. The energy E_i of the incident ions at the growing films surface, varied by applying a substrate negative bias voltage (V_b) of −60 V to −120 V.

For the investigation of the optical properties of the deposited films, Spectroscopic Ellipsometry (SE) measurements were performed, by an ex situ Phase Modulated Ellipsometer in the energy region 1.5–6.5 eV at an angle of incidence of 70°.

In order to study the protein adsorption and make an estimation on the haemocompatibility of the a-BN 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 (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 its adsorption has been found to inhibit thrombus formation [20,21]. On the other hand, Fib takes part in blood coagulation, facilitates adhesion and aggregation of platelets, and is important in the processes of haemostasis and thrombosis [22]. 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 an angle of incidence of 70°, before and after the incubation of the films in the protein solutions.

AFM tapping mode measurements were implemented for the surface characterization of the films and EFM measurements for the qualitative investigation of the surface charge distribution [23]. During EFM measurements, a voltage of 7.5 V was applied on the tip and the tip-sample distance was 10 nm. The wetting properties were studied by static contact angle measurements (with 5 μl deionized H_2O drops).

3. Results and discussion

3.1. Optical/structural properties and haemocompatibility ratio of a-BN thin films

Representative experimental SE spectra acquired before and after the incubation of an a-BN sample in Fib and HSA solutions are presented in Fig. 1, where the protein adsorption is evident due to the shift of the spectra.

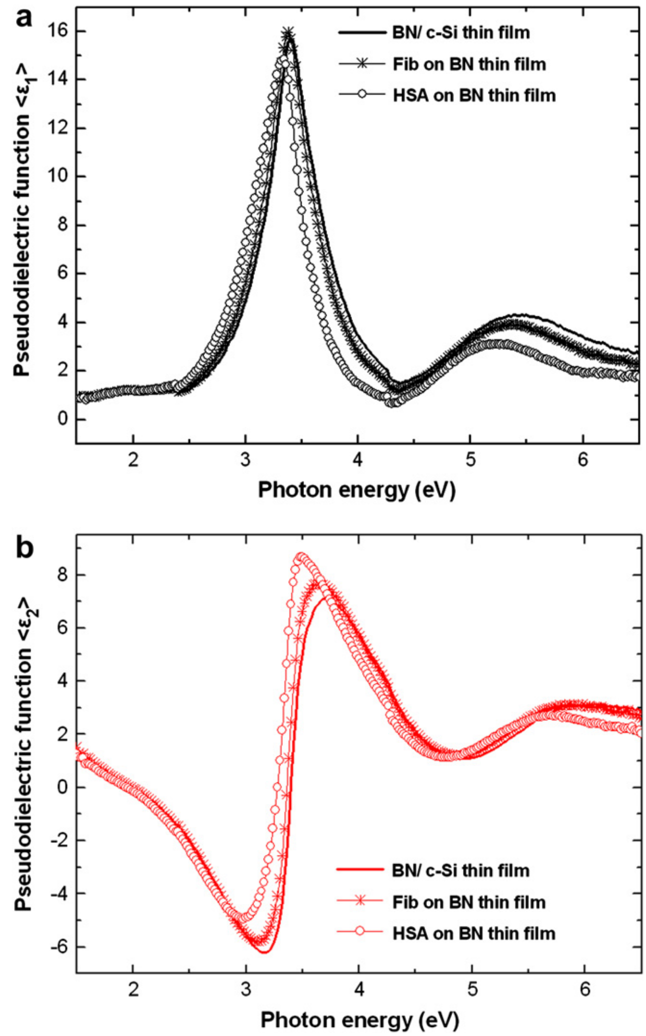

Fig. 1. Representative experimental SE data for the pseudodielectric functions $\langle\varepsilon_1\rangle$ (a) and $\langle\varepsilon_2\rangle$ (b) of a-BN thin film, HSA/a-BN thin film and Fib/a-BN thin film.

Surface concentration Γ of HSA and Fib on a-BN films was calculated by Cuypers formula [24]

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

where, A is the molar refractivity (cm^3/mol), M is the molecular weight of the protein, d and n_f are the thickness and the refractive index of the protein layer. The parameters d and n_f were determined by SE. Then, the ratio $\Gamma_{HSA}/\Gamma_{Fib}$ was calculated, as an indication of the films' haemocompatibility. Larger values of the ratio indicate smaller possibility of thrombus formation on the surface of the film, thus, a more haemocompatible film.

The optical properties of a-BN, have been described thoroughly in various studies [14,25], where the amorphous character of a-BN thin films grown in the same deposition conditions is verified by XPS and XRD measurements. The sp^3 fractions of the a-BN were estimated by Bruggeman Effective Medium Approximation (BEMA), which is described by the following equations [19,26]:

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

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