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Haemocompatibility of amorphous hydrogenated carbon thin films, optical properties and adsorption mechanisms of blood plasma proteins

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Abstract

Haemocompatibility is one of the most important properties, together with the tissue compatibility and corrosion and wear resistance that determine the biocompatibility of the artificial implants. Carbon-based thin films, such as amorphous carbon (a-C) and amorphous hydrogenated diamond-like carbon (a-C:H or DLC) are considered as excellent candidates for use as biocompatible coatings on biomedical implants. The aim of this work is the comparative study of the haemocompatibility of the a-C:H thin films developed by magnetron sputtering under various deposition conditions, the development of a methodology in order to study the haemocompatibility of thin films, the optical properties of the adsorbed proteins (human serum albumin and fibrinogen) and their adsorption mechanisms. Haemocompatibility and the optical properties of a-C:H thin films and the adsorbed proteins were studied by spectroscopic ellipsometry (SE). The films grown under floating conditions performed better haemocompatibility compared with those deposited under application of bias voltage. In the range of vis–UV, proteins are transparent, while they present an absorption peak at higher energies, but except these characteristics, their optical functions are rather featureless. Adsorption mechanisms were studied through AFM technique too. AFM results are in accordance with those derived by SE. Combination of the two techniques gives us a more accurate description of protein adsorption mechanisms.
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Keywords: Carbon; Amorphous; Haemocompatibility; Protein adsorption

1. Introduction

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 (Logothetidis et al., 1999; Charitidis et al., 2000; Robertson, 2002). These films have also shown good blood compatibility. This is very interesting in the field of the biomaterials, which are an important aspect in the development of biomedical devices and implants.

Several works have been dealt with the biocompatibility of diamond-like carbon (DLC) and tetrahedral amorphous carbon (ta-C) coatings (Cui and Li, 2000; Hauert, 2003; Tang et al., 1995; Yang et al., 2003; Alanazi et al., 2000; Grill, 2003; Jones et al., 1999, 2000; YuU et al., 2000; Vinnichenko et al., 2004). These works conclude that the DLC (and ta-C) films show better blood compatibility than other films or materials either organic or inorganic, which have been already used in several biological and biomedical applications. However, a direct correlation between the sp^3 content and compositional properties of carbon-based films and their haemocompatible properties has not been established, yet. In addition, there is a lack on the competitive mechanisms of the protein absorption on such coatings, due to the fact that most of the studies are mainly performed in one protein solution.

The amorphous hydrogenated carbon (a-C:H) thin films studied in this work were developed with rf reactive magnetron sputtering. The sp^3 fractions of the a-C:H thin films were about 40–44%, which were found to be the optimum values, concerning the haemocompatibility of carbon based thin films (Logothetidis et al., 2005). In order to study the haemocompatibility properties of a-C:H thin films, two basic human plasma proteins that have to do with thrombus formation were used, human serum albumin (HSA) and fibrinogen (Fib). HSA is the most abundant protein in human blood plasma. It has been

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found that HSA adsorption on surfaces inhibits thrombus formation (Sugio et al., 1999; Nicholson et al., 2000). 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 (Cacciafesta et al., 2000). The main characterization technique that was applied is spectroscopic ellipsometry (SE) in the energy range from 1.5 to 6.5 eV, applied ex situ (Gioti and Logothetidis, 2003). SE is a non-destructive technique and can be applied in air as well as in liquid environment. For these reasons it is a favourable technique for the study of biological samples. First, SE was used for the fundamental characterization of the a-C:H coatings in terms of their optical and compositional properties, as well as of the protein layers formed on the a-C:H coatings. From the analysis of the SE data, using the appropriate modelling, the HSA/Fib surface concentration ratio was derived. The higher the HSA/Fib ratio is, the more haemocompatible the coating is. The results are discussed in terms of the bonding structure and composition of the examined films.

2. Experimental

The sputtered 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 (Gioti and Logothetidis, 2003; Gioti et al., 2000; Logothetidis, 2002). For the growth of a-C:H films, H_2 reactive gas was introduced into the vacuum chamber. Three samples were deposited with negative biased voltage ($V_b = -40$ V) and four were deposited with no substrate bias (floating). Measurements were performed with ex situ phase modulated spectroscopic ellipsometer (Uvisel p/n 23 301 909 by Horiba Jobin Yvon) in the energy region 1.5–6.5 eV at variable angles of incidence (VASE) from 60 to 70° with a step of 5° (Fig. 1), to get more information and for a more accurate study of the optical properties of the measured samples. Two different HSA (40 mg/ml) and Fib (5 mg/ml) solutions in phosphate buffer saline (PBS, pH 7.4) were used for the evaluation of the haemocompatibility of the a-C:H films. Protein concentrations and solvent pH values are very close to those of a healthy person's blood. The samples were dipped into the protein solutions for 2 h, at room temperature. Afterwards, the samples were rinsed with deionized water and dried under a N_2 flow. In order to study the protein adsorption on a-C:H films, selected samples were dipped into the protein solutions for different intervals of time (10 min–48 h) and studied by SE. Complementary measurements with atomic force microscopy (AFM) (tapping mode) verified SE results. Early stages of protein adsorption (incubation times < 10 min) have also been studied through AFM technique (Mitsakakis et al., in press) and give a more detailed description of the protein adsorption mechanisms. Finally, the bonding structure of the HSA/a-C:H and Fib/a-C:H samples has been investigated by Fourier transform infrared spectroscopic ellipsometry (FTIRSE) in the 900–4000 cm^{-1} spectral region. Measurements were made by FTIR phase modulated spectroscopic ellipsometer (Horiba Jobin Yvon). A detailed description of the FTIRSE technique has been given elsewhere (Laskarakis et al., 2001).

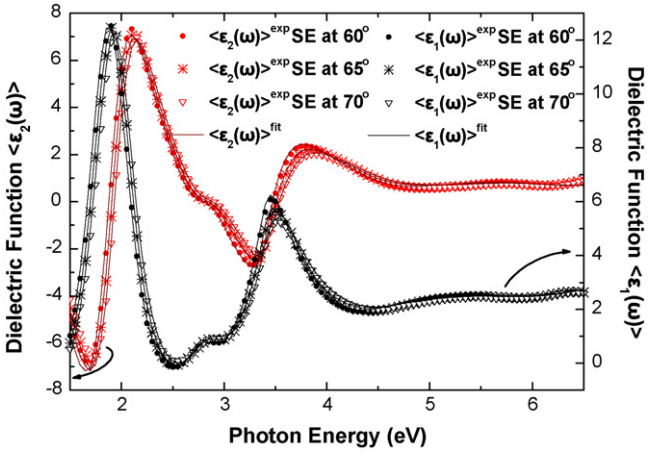

Fig. 1. The measured $\langle \varepsilon(\omega) \rangle^{exp}$ (symbols) by VASE (60–65–70°), and the calculated through the modelling and fitting procedures $\langle \varepsilon(\omega) \rangle^{fit}$ (solid lines) of an adsorbed protein layer on a-C:H film.

3. Results and discussion

3.1. Characterization of a-C:H thin films

Electronic transitions of graphite, diamond and composite carbon films, as well as the influence of the incorporation of hydrogen into the amorphous carbon matrix, have been described thoroughly in various studies (Gioti et al., 2000; Djurisic and Li, 1999; Greenway et al., 1969; Hanfland et al., 1989; Ahuja et al., 1997; Edwards and Phillip, 1985; Logothetidis et al., 1992).

In order to obtain quantitative information from the analysis of the ellipsometric data it is essential to parameterize the dielectric functions with the optical constants on the wavelength of light. In this work, we have used a recently proposed dispersion model for amorphous semiconductors namely "Tauc–Lorentz" (TL) model (Gioti et al., 2000; Jellison and Modine, 1996). This model uses a combination of the Tauc joint density of states (Tauc et al., 1966) and the quantum mechanical Lorentz oscillator model in order to describe the spectral dependence of the dielectric function. The dielectric function of amorphous carbon-based materials however, exhibits a peculiarity due to the existence of two separated contributions of interband electronic transitions; that are the $\pi \rightarrow \pi^*$ corresponding to sp^2 bonded carbon, and the $\sigma \rightarrow \sigma^*$ corresponding to both sp^3 and sp^2 bonded carbon.

The sp^3 fractions of the a-C and the a-C:H films were estimated by Bruggeman Effective Medium Theory (BEMT), which is described by the following equations (Logothetidis, 2002; Aspnes, 1982):

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

where f_i and ε are the relative volume fraction and the dielectric function of the ith component, respectively, and ε is the bulk dielectric function of the carbon film. Carbon-based thin films studied in this work are considered to be composite materials consisting of sp^3- and sp^2-bonded carbon 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. Details on the applied deposition conditions and the optical properties of a-C:H thin films studied in this work are presented in Table 1. The calculated bulk dielectric functions $\varepsilon(\omega)$, real $\varepsilon_1(\omega)$ and imaginary $\varepsilon_2(\omega)$ parts, using the best-fit parameters for the various a-C:H films are plotted in Fig. 2. The differences between the $\varepsilon(\omega)$ values for the different a-C:H samples are very

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