Available online at www.sciencedirect.com
ScienceDirect
Microelectronic Engineering 84 (2007) 479–485
MICROELECTRONIC ENGINEERING
www.elsevier.com/locate/mee

Optical properties of proteins and protein adsorption study

S. Lousinian, S. Logothetidis *

Aristotle University of Thessaloniki, Department of Physics, GR-54124 Thessaloniki, Greece

Available online 9 November 2006

Abstract

Haemocompatibility is one of the most important properties, together with the tissue compatibility, corrosion and wear resistance that determine the biocompatibility of the artificial implants. Carbon-based thin films, such as amorphous carbon and amorphous hydrogenated diamond-like carbon (a-C:H or DLC) are considered as excellent candidates in order to be used as biocompatible coatings on biomedical implants. The aim of this work is to develop a methodology in order to study the protein adsorption phenomenon on thin films and to explore the optical properties of two basic blood plasma proteins, human serum albumin (HSA) and fibrinogen (Fib) and their adsorption mechanisms on amorphous hydrogenated carbon (a-C:H) thin films. Two techniques advantageous for the study of biological samples are used: Vis–UV spectroscopic ellipsometry (SE) and atomic force microscopy (AFM).

a-C:H Films are grown with rf reactive magnetron sputtering. Static and real-time SE measurements are made. In the energy range of Vis–UV, proteins are almost transparent, while they present an absorption peak at higher energies. Different protein adsorption behaviour is observed on amorphous hydrogenated carbon films deposited under different conditions. This is probably due to their different surface structure, composition and topography of the surface and its interaction with the protein molecule. Adsorption phenomenon is studied through AFM technique as well. AFM results are in accordance with those derived by SE. The combination of the two techniques provides us a more accurate description of protein adsorption mechanisms.

Keywords: Amorphous hydrogenated carbon; Protein adsorption; Spectroscopic ellipsometry; Atomic force microscopy

1. Introduction

Protein adsorption has been investigated extensively during the past decades. This is an important aspect for the improvement of many applications, such as medical implants, biosensor design, etc. 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. The density, orientation, and conformation of surface-bound proteins are believed to be key factors in controlling subsequent cellular adhesion [2].

Several works have dealt with the biocompatibility of diamond-like carbon (DLC) and tetrahedral amorphous carbon (ta-C) coatings [3–12]. 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 [13–15] and have also shown good blood compatibility. This is very interesting in the field of biomaterials, which are an important aspect in the development of biomedical devices and implants. In a previous work of our research team, it was deduced that a-C:H thin films, developed with rf reactive magnetron sputtering, with sp^3 content of 42% and small amount of H_2 in plasma during deposition, exhibit good haemocompatibility [16,17].

This deduction has led to the need of investigating further the phenomenon of protein adsorption on a-C:H thin films. Two basic human plasma proteins, with function related to thrombus formation were used, Human Serum Albumin (HSA) and Fibrinogen (Fib). HSA is the most abundant protein in human blood plasma. It has been found that HSA adsorption on surfaces inhibits thrombus

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

0167-9317/$ - see front matter
doi:10.1016/j.mee.2006.10.082

formation [18,19]. 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 [20]. Two non-destructive techniques which can also be applied both in air and in liquid environment were used: spectroscopic ellipsometry (SE) and atomic force microscopy (AFM). SE has the capability of using advanced modelling procedures, which can describe complicated systems, such as the biomaterial–protein system. By the tapping mode of AFM, the oscillatory motion of the tip above the surface allows no contact with the sample and therefore no danger to drag, deform, or scratch the latter, exists. For these reasons, SE and AFM are favourable techniques for the study of biological samples. First, they were used for the fundamental characterization of the a-C:H coatings in terms of their optical, compositional and surface 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 optical properties of the two proteins, as well as their thickness change during their adsorption on the thin films, were derived. AFM technique provided information about the surface roughness of the thin films and the protein layers formed on them. The results are discussed in terms of the bonding structure and composition of the examined films.

2. Experimental

The sputtered a-C:H films studied in this work were deposited by rf magnetron sputtering on c-Si (100) substrates at room temperature [21]. For the growth of a-C:H films, H_2 reactive gas was introduced into the vacuum chamber. One sample was deposited with negative biased voltage ($V_b = -40$ V) and the other was deposited with no substrate bias (floating). The latter one has performed good haemocompatibility properties [17]. The H_2 partial pressure in the deposition chamber during deposition was 10%. Measurements were performed with ex situ phase modulated spectroscopic ellipsometer (by Horiba Jobin Yvon, in a flow cell (by Nanofilm) in the energy region 1.5–4.0 eV) with angle of incidence 60 Å. Two separate HSA (10 mg/ml) and Fib (1 mg/ml) solutions in phosphate buffer saline (PBS, pH 7.4) were used for the study of the protein adsorption on the a-C:H films. The samples were studied by SE in the cell and their optical properties were derived. Afterwards, the protein solution was introduced into the cell and a real-time study of the protein adsorption was performed, for about 70 min, at room temperature. Complementary measurements with atomic force microscopy (AFM) (in tapping mode) (by NTMDT) after incubation time of 70 min, verified SE results and gave quantitative information about the surface morphology of the a-C:H thin films and the protein layers on them.

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 [22–27].

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

Table 1
Sample codes, bias voltage H_2 partial pressure (%) during deposition and the corresponding refractive indices n at $\omega = 0$ eV, $n_{(0)}$

Sample codes	Bias voltage, (V_b)	%H_2	Refractive index ($n_{(0)}$)
F	+10 V	10%	1.476
B	−40 V	10%	1.611

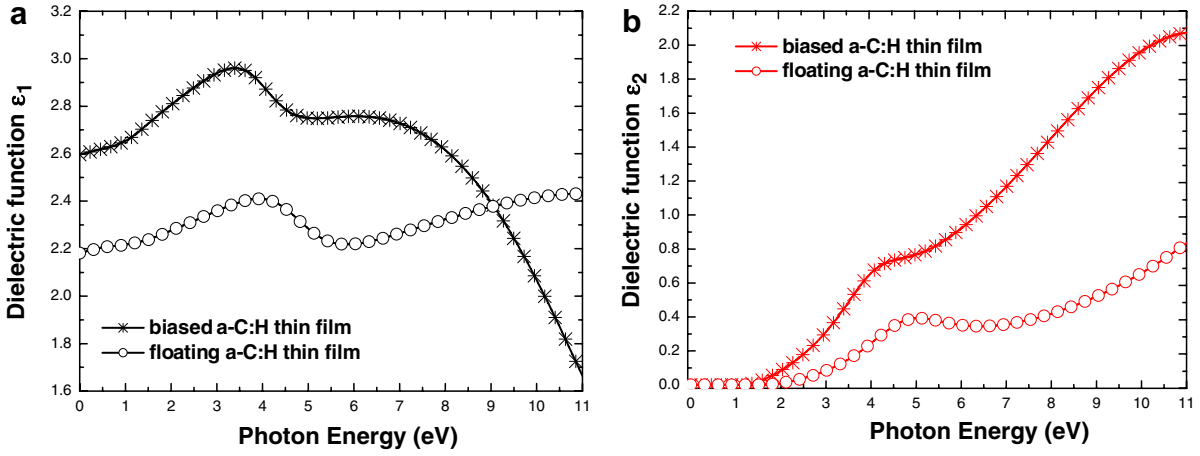

Fig. 1. The calculated bulk dielectric functions $\varepsilon(\omega)$: (a) real $\varepsilon_1(\omega)$, and (b) and imaginary $\varepsilon_2(\omega)$ parts, using the best-fit parameters for the floating and biased a-C:H films.