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Haemocompatibility of carbon based thin films

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Abstract

Haemocompatibility is one of the most important properties that determine the biocompatibility of the artificial implants. The haemocompatibility testing is to look for possible undesirable effects (e.g., haemolysis, thrombus formation, alterations in coagulation) in the blood, caused by a medical device or by chemicals leaching from a device. Carbon based thin films, such as amorphous carbon (a-C) and amorphous hydrogenated diamond-like carbon (a-C:H) 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 carbon based thin films developed by magnetron sputtering under various deposition conditions, through the study of protein adsorption and platelet adhesion, by the development of a methodology. Haemocompatibility and the optical properties of carbon based thin films and the adsorbed proteins were studied by Spectroscopic Ellipsometry (SE). The number, the morphology and the phenomenon of adherent platelets were studied by Atomic Force Microscopy (AFM). The a-C:H films grown under floating conditions performed better haemocompatibility compared with those a-C films grown under ion bombardment during deposition. The results are discussed in terms of the bonding structure, surface characteristics and composition of the examined films. Protein adsorption mechanisms, which are an important aspect in the field of biomaterials, were studied by both SE and AFM, and a protein adsorption model is proposed.

Keywords: Carbon thin films; Haemocompatibility; Spectroscopic Ellipsometry; Atomic Force Microscopy; Protein adsorption; Platelet

1. Introduction

The definition of biocompatibility by the European Society for Biomaterials as "the ability of a biomaterial to induce the appropriate answer in a specific application" is very vague, since biocompatibility is defined according to the application [1]. Therefore, under the aspect of biocompatibility various tests are necessary, which must be carried out together with physical–chemical analysis methods, but they must also be performed simultaneously in direct interchange with the respective biological systems [2]. The biomaterial should be non-toxic, non-mutagenic–non-carcinogenic, not provoking an inflammatory response, should not induce allergic or immunologic reactions and should not irritate surrounding structures. The purpose of haemocompatibility testing is to look for possible undesirable changes in the blood, caused by a medical device or by chemicals leaching from a device. Undesirable effects of device materials on the blood may include haemolysis, thrombus formation, alterations in coagulation parameters and immunological changes [3].

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 [4–6]. These films have also shown good blood compatibility comparing to other organic or inorganic materials which have been already used in several biological and biomedical applications. The advantage of carbon based thin films over metal surfaces and polymers is that metals release heavy metal ions (Ni, Co etc.) which provoke a strong platelet activation (significantly higher thrombogenicity) and initiate complex inflammatory processes in the surrounding tissue, while polymer degradation products may reduce the local pH, accelerate polymer degradation rate, and/or induce inflammation [7].

Several works have been published regarding the biocompatibility of diamond-like carbon (DLC) and tetrahedral amorphous carbon (ta-C) coatings [8–19]. ta-C films, prepared by Filtered Arc Deposition, were found to be more haemocompatible, i.e. to exhibit improved coagulation properties, compared to the Low-Temperature Isotropic Pyrolytic Carbon, which is already used for biomedical applications. The density of the adherent platelets was estimated by Scanning Electron Microscopy, a widely used technique for observing platelets on

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several surfaces [20]. Diamond-like carbon thin films presented better haemocompatibility than CN_x films according to Cui and Li [8], who have calculated the ratio $\Gamma_{HSA}/\Gamma_{Fib}$. However, CN_x thin films, known as good candidate materials for orthopedic applications because of their mechanical properties, exhibited also advantages for coronary and vascular applications. High N content and lower rms roughness of CN films may increase the time needed for the creation of the clot and the platelet activation, comparing to other CN films [21]. Doping a-C:H thin films with elements such as N and Si may further improve their haemocompatible properties. Number of endothelial cells and activation of platelets on a-C:H:N prepared by Plasma Immersion Ion Implantation–Deposition has been studied by Yang et al. [22]. It was found that a-C:H:N possesses better endothelial cell growth and anti-thrombotic properties than a-C:H. When a-C:H thin films prepared by PECVD were doped with Si [23], less adherent platelets were observed on a-C:H:Si comparing to a-C:H film, which means that a-C:H:Si exhibits improved haemocompatibility.

However, there is still a lack of a direct correlation between the sp^3 content, compositional properties, and doping of carbon based films with their haemocompatibility properties. In addition, there is a lack of a methodology and those techniques which can fully describe the mechanisms of the protein absorption and platelet adhesion on such coatings.

In this work, the haemocompatibility of carbon based thin films is evaluated, and a methodology is developed for studying protein adsorption and platelet adhesion on carbon based thin films. Amorphous carbon (a-C) thin films and amorphous hydrogenated carbon (a-C:H) thin films studied in this work were developed with rf magnetron sputtering (rf MS) and rf reactive MS respectively, with sp^3 fractions varying from 20 to 45%, and one ta-C thin film (sp^3 fraction 80%) was studied as well. The sp^3 fractions of the a-C:H thin films were about 40–45%, which were found to be the optimum values concerning the haemocompatibility of carbon based thin films [24]. Two basic human plasma proteins that have to do with thrombus formation were used, Human Serum Albumin (HSA) and Fibrinogen (FIB). The main characterization techniques that were applied are Spectroscopic Ellipsometry (SE) in the Vis–UV energy range [25,26] and Atomic Force Microscopy (AFM) in tapping mode [27]. They both are favourable techniques for the study of biological samples, because they are non-destructive and surface sensitive, and can be applied in air as well as in liquid environment. First, both techniques were used for the fundamental characterization of the carbon based thin films in terms of their optical and compositional properties, as well as for the characterization of the surface topography. Then the protein layers formed on the films were studied. The results are discussed in terms of the bonding structure, surface characteristics and composition of the examined films.

It is known that human blood consists of at least 150 proteins contained in the blood plasma, and of cells, i.e. red cells, leucocytes and platelets. The first event that occurs when a foreign material comes into contact with blood is plasma protein adsorption [28]. Subsequently, blood cells (e.g., platelets) interact with adsorbed protein layer [29]. In order to evaluate the haemocompatibility of a surface, in the sense of decreased possibility of thrombus formation, two parameters are studied.

The first parameter is the adsorption of two proteins that play an important role in enhancing or inhibiting thrombus formation, Fibrinogen (FIB) and Human Serum Albumin (HSA) respectively [30–32]. HSA is the most abundant protein in human blood plasma. It has been found that HSA adsorption on surfaces inhibits thrombus formation [29,31]. FIB takes part in blood coagulation, facilitates adhesion and aggregation of platelets, and is important in the processes of both haemostasis and thrombosis [31]. The adsorbed protein amount (surface concentration Γ) is calculated through Cuypers formula [33]:

$$\Gamma = 0.1 \cdot d \cdot \frac{M}{A} \cdot \frac{n_f^2 - 1}{n_f^2 + 2},$$

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. The ratio $\Gamma_{HSA}/\Gamma_{Fib}$ is used as an evaluation of the haemocompatibility of the surface (the larger the ratio is, the more haemocompatible the surface) [8,24,25].

The second parameter is the amount and the shape of the platelets adherent on the surface. There are five types of platelet morphology, from round and discoid without pseudopodia to fully spread hyaloplasm with extended, flattening pseudopodia. When platelets come in contact with a surface, they are activated, forming pseudopodia and increasing the area of contact with the surface. Formation of psedopodia and spreading hyaloplasm is indicant of the increased platelet activation which results in increased possibility of thrombus formation [34].

In the present work, we are dealing with evaluating the haemocompatibility of carbon based thin films, through the development of an appropriate methodology for investigating and describing protein adsorption and platelet adhesion phenomenon.

2. Experimental

2.1. Growth of carbon based thin films

The sputtered a-C and a-C:H films studied in this work were deposited by rf magnetron sputtering on c-Si (100) substrates at room temperature [25,34–36]. 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) or ion bombardment during deposition, and four were deposited with no substrate bias (floating). The ta-C film has been deposited using filtered cathodic vacuum arc deposition technique [37]. Measurements were performed with ex-situ phase modulated spectroscopic ellipsometer in the energy region 1.5–6.5 eV at variable angles of incidence (VASE) from 60° to 70° with a step of 5°, to get more information and for a more accurate study of the optical properties of the measured samples.