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Osteoblast interaction with DLC-coated Si substrates ☆

Feng Chai [a], Nicolas Mathis [b], Nicolas Blanchemain [a], Cathy Meunier [b], Hartmut F. Hildebrand [a,*]

[a] *Groupe de Recherche sur les Biomatériaux (GRB), Laboratoire de Biophysique, UPRES EA 1049, Faculté de Médecine, Université de Lille-2, 1 place de Verdun, 59045 Lille cedex, France*
[b] *FEMTO-ST/CREST, UMR 6174 CNRS, 4 place Tharradin, BP 71427, 25211 Montbéliard cedex, France*

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Abstract

Diamond-like carbon (DLC) coating is a convenient means of modifying material surfaces that are sensitive to wear, such as titanium and silica substrates. This work aims to evaluate the osteoblast-like cells' response to DLC-coated Si (Si-DLC), which was treated under different conditions. DLC and deuterated DLC films were deposited by plasma-enhanced chemical vapor deposition to obtain a 200-nm-thick layer on all the samples. Three types of precursor gas were applied for deposition: pure methane (CH_4), pure deuterated methane (CD_4) and their half/half mixture. All surface treatments were performed under two different self-bias voltages (V_{sb}): −400 and −600 V. The modified surfaces were characterized by X-ray photoelectron spectroscopy, Raman spectroscopy, Rutherford backscattering spectroscopy, elastic recoil detection analysis, X-ray reflectometry and the sessile-drop method. MC3T3-E1 osteoblasts were cultured on the Si-DLC wafers for 3 and 6 days. Biological tests to measure cell proliferation, cell vitality, cell morphology and cell adhesion were performed. All DLC coatings produced a slightly more hydrophobic state than non-treated Si. Certain types of amorphous DLC coating, such as the surface treated under the V_{sb} of −600 V in pure methane ($600CH_4$) or in pure deuterated methane ($600CD_4$), offered a significantly higher cell proliferation rate to Si substrate. Scanning electron microscopy observations confirmed that the optimal cell adhesion behavior, among all the treated surfaces, occurred on the surface of the $600CH_4$ and $600CD_4$ groups, which showed increased amounts of filopodia and microvilli to enhance cell–environment exchange. In conclusion, DLC coating on Si could produce better surface stability and improved cellular responses.

Keywords: DLC coatings; Surface energy; Osteoblasts response; Adhesion; Cytoskeleton

1. Introduction

Implantable biomaterials are generally intended to be used under biological constraint and in continuous contact with an aggressive environment, including water, salts and enzymes or other bioactive molecules. If the degradation of implanted materials initiates a strong foreign body reaction, such as fibrous capsules [1] or thrombus formation [2], implant failure may ensue. Indeed, when implants come into contact with any biological organism, they will generate complex body reactions, which are concomitant with the physicochemical interactions at the interface between the material and the biosystem [3,4] and play a primary role in the tissue integration.

Since the surface properties of the medical device mainly govern its biomedical applications, in most cases a surface modification is considered to be a prerequisite for better biocompatibility. In order to improve the surface reactivity, many advanced surface modification techniques via ion beam processing or coating have been proposed to study the biocompatibility of modified surfaces [5]. These surface modifications must be biocompatible, i.e. they do not generate unexpected outcomes in the cells, the tissues or the body fluids. It should also be hard, wear resistant

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* Corresponding author. Tel.: +33 320 62 69 75; fax: +33 320 62 68 54.
E-mail address: fhildebrand@univ-lille2.fr (H.F. Hildebrand).

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with a low coefficient of friction and corrosion resistant for certain applications. Examples are the medical implants for orthopeadic and cardiovascular applications, which are subject to severe external forces during the implant's lifetime in the body environment and sometimes lead to delamination and spallation of the coatings. However, commonly used modification methods have not yet eradicated this problem.

Due to its particular structure, i.e. cohabitation of the sp^2 and sp^3 phases, the diamond-like carbon (DLC) thin film possesses a number of properties, such as high hardness [6], low friction coefficient [7,8], chemical inertness [8–10] and high corrosion resistance [11,12], which make it attractive as a coating for various biomedical implants [13] such as vascular devices [14–16], biosensor [17,18], artificial joints [19] and dental prostheses [20].

The main problem with the load-supporting implant lies in its wear and corrosion during long-term use. The debris formed as a consequence of this wear will result in tissue inflammation, osteolysis and finally loosening of the implants. In previous studies, DLC films have been deposited on several kinds of polymeric materials [9,13,15] or metal alloys [10,11]. Their good physical properties (high hardness, scratch resistance), chemical properties (corrosion resistance, relative inertness) and derivatively biological properties with respect to blood compatibility [11,21], bioresponse [21,22], toxicity [23–25] and cell adhesion [26–28] have proved the potential merits of DLC in the biomedical field.

The most common method used for DLC deposition is radio-frequency plasma-enhanced chemical vapor deposition (rf-PECVD). This technique, with methane as the precursor gas, generates an amorphous hydrogenated carbon (a-C:H) with a predominance of sp^2 bonds and a high hydrogen concentration (>40 at.%), depending on the applied self-bias voltage (V_{sb}) [29]. Indeed, hydrogen concentration and formulation (bonded or free) play a great role in the final mechanical, optical and electrical properties of the carbon layer [30]. Hydrogen, under specific conditions, can largely lower the friction, increase the sp^3 fraction and improve the optical band gap. For this reason, it will be useful to compare the effects of hydrogen in the DLC film with those of its isotope deuterium under the same experimental condition. In our previous study [31] we proved that the inclusion of deuterium in the carbon films increased the Tauc energy of 20% (1.23 eV for hydrogen DLC and 1.48 eV for deuterated D-DLC) while keeping the same film density (between 1.5 and 1.75). Moreover, considering the mechanical results, we found that the incorporation of deuterium in the surface layer can enhance hardness and apparent Young modulus under specific V_{sb} conditions (−200 to −400 V) and allows a larger hardness range [32].

However, a thorough understanding of cell–material interactions is complex because the cell responses, such as cell migration, cell adhesion or all other mechanisms involved in the cell function and cycle, are usually not easy to identify and difficult to analyze [3,4,23,24]. Despite the reports of the successful application of DLC as a coating for metallic implant materials, there is a relative paucity of information available concerning the use of DLC as a coating for nonmetallic biomaterials. Therefore, the aim of this study is to reach a better comprehension of the DLC structure and especially of the hydrogen function in the amorphous carbon thin film on Si substrate. Basing on our previous encouraging results, the biological behaviour of D-DLC-modified surfaces were investigated to evaluate their cytocompatibility. Consequently, this information might offer an insight into the suitability and performance of DLC as a coating for orthopeadic biomaterials.

2. Materials and methods

2.1. Diamond-like carbon deposition

DLC and D-DLC films were deposited with rf-PECVD (13.56 MHz). The working power was adapted to obtain values of V_{sb} between −50 and −600 V for each treatment type. Such V_{sb} was applied on the cathode in a one-plate geometry to accelerate ionic species onto the substrate. To acquire high-purity films, we used a vacuum of at least 5×10^{-5} Pa. The working pressure was 1 Pa to promote deposition. To attain a 200 nm thick film on all samples, the gas flow was kept constant at 20 sccm, and the deposition time was monitored as well [33]. The substrate used for coating was silicon wafer (ITME, Brussels, Belgium) with low electrical resistivity (0.008–0.02 $\Omega\,cm^{-1}$) and a size at 15 × 1 mm (diameter × thickness). We used three types of precursor gas: pure methane (CH_4), pure deuterated methane (CD_4) and a 1:1 mixture of both (CH_4/CD_4). Each treatment type was performed under two different V_{sb}: –400 and –600 V, to determine whether the V_{sb} variation derived difference in chemical composition exerts any influence on the cytocompatibility of the layer.

2.2. Chemical characterizations of DLC films

Chemical properties, such as density, hydrogen/deuterium rate or sp^2 character, could play a role in the biological response of osteoblast-like cells. Therefore, the qualitative values for the sp^2-bonded carbon content in the various films were determined by Raman spectroscopy and X-ray photoelectron spectroscopy (XPS). The results obtained from these techniques were qualitative and would allow comparison of the threefold carbon bond environment variations for all tested samples.

Raman spectroscopy has proven to be a powerful method for characterizing DLC coatings due to its high sensitivity to carbon. The Raman spectra of the hydrogenated DLC films can exhibit broad Raman peaks centered at the positions ranging from 1500 to 1550 cm^{-1} and shouldered features at 1320–1350 cm^{-1}, which represents, respectively the graphitic carbon peak (G peak) and the disordered graphitic carbon peak (D peak). Our measurements were performed with a Labram Raman system (Horiba–Jobin Yvon Inc., Edison, USA), which was equipped

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