International Biodeterioration & Biodegradation 88 (2014) 106–117

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biomodification and biodeterioration of carbon coatings by fungal strains

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ARTICLE INFO

Article history:
Received 2 September 2013
Received in revised form
18 December 2013
Accepted 19 December 2013
Available online 11 January 2014

Keywords:
Hard carbon coatings
Biodegradation
Fungi
Enzyme activity
FTIR
XRD
XPS

ABSTRACT

Over the past years, surface protection by hard carbon coatings has been increasingly applied in many fields. This study aimed to characterize changes in the properties of carbon coatings brought about by the growth of fungi (the first report about these processes was published by Kaczorowska et al., Nanodiam, PWN, 2006, 99–116). Alterations in the structure of diamond-like carbon (DLC) and nanocrystalline diamond (NCD) coatings, examined by Raman spectroscopy, XRD, XPS, and FTIR, were found to be caused by the processes of oxidation (incorporation of oxygen atoms) and reduction (hydrogenation) occurring within graphite carbon and amorphous carbon domains (components of the surface layer of the tested coatings along with the diamond domain). Determination of the activity of selected enzymes synthesized by the studied strains of filamentous fungi revealed that the observed biomodification and biodeterioration processes involved, among others, laccase, Mn-dependent peroxidase, two catechol dioxygenases, and esterases. The biosynthesis of these enzymes by fungi was enhanced when graphite was added to their culture media. Because of the high risk of fungal infections, the quality of carbon coatings should be routinely controlled by standard microbiological tests employing suitable strains of filamentous fungi (e.g., *Aspergillus niger*) and yeasts synthesizing enzymes that attack carbon materials.

1. Introduction

Surface protection by diamond-like carbon provides many benefits and has been increasingly used in many areas. Carbon coatings are used in optics (infrared viewers), microelectronics, and computer manufacturing (e.g., hard disc matrices) (May, 2000). Their high potential has been acknowledged for the protection of surfaces of various devices used in medicine (protective coatings on implants such as joint endoprostheses, heart valves, cranium and limb prostheses, dentistry implants), electrochemistry, and biochemical analytics (electrode probes and elementary particle detectors) (Grill, 2003; Dearnaley and Arps, 2005; Roy and Lee, 2007; Grabarczyk and Kotela, 2009; Zanin et al., 2012). Also the surfaces of various mechanical tools, including cutting blades, rotors, toothed gears, micro-mechanisms, razor blades, plastic credit cards, etc., can be covered with such coatings (Mansano et al., 2003; Mitura et al., 2006b; Kržan et al., 2009).

The characteristics of carbon coatings depend on the technology of their fabrication, which in general consists of deposition of carbon from carbonaceous precursors using diverse methods, including primary ion beam deposition, physical vapor deposition (PVD), and plasma-assisted chemical vapor deposition (PACVD) (Choi et al., 1997; Dearnaley and Arps, 2005). Films produced in these processes display excellent hardness, wear resistance, electrical and chemical resistance, and biocompatibility, which is of great importance for their medical applications (Mitura et al., 1999; Dearnaley and Arps, 2005).

The quality of carbon coatings is evaluated based on their mechanical and physicochemical features (hardness, wear resistance, slickness, tribological properties, fractional content of hydrogen, etc.). Carbon coatings for medical applications have been tested for their interactions with the cells and tissues of higher animals, mainly in terms of their biotolerance determined based on the viability and morphology of interacting cells/tissues (Linder et al., 2002; Dearnaley and Arps, 2005; Mitura et al., 2006a) and

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http://dx.doi.org/10.1016/j.ibiod.2013.12.013

bioresistance, meaning chemical stability in an environment of live tissues, body fluids, and blood (Rodil et al., 2003; LaVan et al., 2005). Furthermore, several bacterial species have been investigated for adhesion to these surfaces (Jakubowski et al., 2004; Wang et al., 2004; Lackner and Waldhauser, 2010).

However, information about the stability of carbon coatings during their long-term exposure to physiologically active microorganisms and their metabolites is scarce. Such knowledge is very important because microorganisms are omnipresent and they tend to adhere to all surfaces under natural and man-made conditions. This in turn may lead to bacterial and/or fungal contamination of carbon-coated medical implants or processes of microbiologically induced corrosion (MIC) on the surface of mechanical devices covered by DLC (e.g., those designed for wood processing and/or those used in a moist atmosphere).

This study aimed to provide greater insight into the stability of carbon coatings exposed to the action of microorganisms growing on their surface. The tested carbon coatings were deposited on either medical stainless steel (AISI 316L) or silicon plates by methane activation using radio frequency plasma chemical vapor deposition (RF PCVD) (Mitura et al., 1999; Mitura, 2007). The resulting hard carbon coatings contained two typical phases, that is, nanocrystalline diamond (carbon with σsp^3 hybridization) and graphite (C σsp^2). Depending on the ratio of these phases, such carbon coatings are termed either DLC (diamond-like carbon) or NDC (nanocrystalline diamond carbon). The former are built of a mixture of amorphous and fine-crystalline carbon with electron hybridization σsp^3, σsp^2 Πp and σsp^1 Πp^2, and can also contain hydrogenated carbon (a-C:H). In NCD films more than 95% of carbon atoms have σsp^3 hybridization. The ratio of these carbon phases in carbon coatings is dictated by the conditions of their deposition (Mitura et al., 2006b; Mitura, 2007). NCD layers produced by methane decomposition in high frequency plasma show very strong adhesion to medical stainless steel (AISI 316L) and very high wear resistance (Mitura et al., 1999).

The results of our previous experiments concerning the influence of live microbial cells (bacteria and fungi) on carbon coatings were reported by Kaczorowska et al. (2006). It was found that these coatings, and in particular DLC ones, were strongly modified by some microorganisms. This finding prompted our research on the mechanisms of biomodification and biodeterioration of carbon coatings.

The fungal strains used in the presented experiments were selected based on the results of previous microbiological tests. They caused the most advanced degradation of the tested carbon coatings, which was visible both macro- and microscopically (Kaczorowska et al., 2002; Szczęsna-Antczak et al., 2003). Contact with the growing mycelia of these fungi affected the roughness of the carbon coatings and Raman spectra profiles (Kaczorowska et al., 2006). To identify plausible mechanisms of the observed biomodification and biodegradation processes, the chemical composition and structure of carbon coatings were analyzed by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectroscopy (in addition to Raman spectroscopy), and the surface of carbon coatings was subjected to X-ray diffraction analysis. To characterize in greater detail the impact of the tested fungal strains on carbon films, the activity of certain intra- and extracellular enzymes (selected oxidoreductases and carboxylic ester hydrolases) synthesized by these microorganisms were assayed. These enzymes were selected based on literature reports concerning enzymes involved in the biodegradation of lignin (Baldrian, 2006; Dashtban et al., 2010), brown coal (e.g., in lignite solubilization, Fakoussa and Hofrichter, 1999; Hölker et al., 1999) and aromatic compounds, including the polycyclic aromatic hydrocarbons anthracene and benzo(α)pyrene (Juhasz and Naidu, 2000; Romero et al., 2002).

The results of this study are thought to provide an insight into the mechanism/s of biomodification and biodeterioration of carbon coatings exposed to selected filamentous fungi and show some weak points of carbon layers, which make them most susceptible to biodegradation.

2. Materials

2.1. Chemicals

Graphite (*graphite flakes*) – synthetic powdered graphite (1–2 μm) was purchased from Sigma–Aldrich. Catechol, guaiacol, ABTS, veratryl alcohol, manganese sulfate (II), Azure B, p-nitrophenyl acetate (p-NPA) and horseradish peroxidase were purchased from Sigma. Tris-SO_4 and Tris–HCl were obtained from Loba Feinchemie, and the detergent Sekumatic® FC from Ecolab. Phenol red and the microbial culture media were purchased from Difco. The other chemicals were of analytical grade and purchased from POCh S.A.

2.2. Strains

The following fungal strains were used in the study: *Aspergillus niger* IBT-90 and *Mucor circinelloides* (from the pure culture collection of the ITB LUT), *Chaetomium globosum* (ŁOCK 0475), *Fusarium oxysporum* (ŁOCK 0510, strain E90), *Paecilomyces variotii* (ŁOCK 0525), *Penicillium ochrochloron* (ŁOCK 0538), *Phanerochaete chrysosporium* (Burdsal collection, ATCC 34511), and *Trichoderma viride* (ŁOCK 0570, strain E159).

2.3. Discs of medical stainless steel and silicon discs coated with carbon layer

Discs (Φ = 8 mm, h = 2.5 mm) of medical stainless steel AISI 316L (made of iron with admixtures of (%): Cr (17–19), Ni (12–14), Mo (2–3), Mn (max 2.0), Si (max 0.75), Cu (max 0.50), N (max 0.1), C (max 0.03), P (0.025), and S (0.01)) were coated with DLC and NCD layers (on both sides) by the RF PCVD method at the Institute of Materials Science and Engineering, Department of Biomedical Engineering, Lodz University of Technology. The process parameters were as follows: etching at 700 V and 10.8 Pa for 2 min (for NCD and DLC) and deposition: for DLC at 550 V and 10.8 Pa for 7 min in an atmosphere of CH_4 (flow rate: 60 cm^3/min), and for NCD: step I at 550 V and 10.8 Pa for 7 min in an atmosphere of CH_4 (flow rate: 60 cm^3/min), step II at 420 V and 10.8 Pa for 2 min in an atmosphere of CH_4 (flow rate: 60 cm^3/min).

The surface of silicon discs (pressed discs used in electronics) was coated with DLC layers under the following conditions: etching at an autopolarization potential of 700 V (electric current from a generator: 160 mA for 1 min) and deposition at an autopolarization potential of 180 V (electric current from a generator: 20 mA for 5 min, gas: CH_4).

3. Methods

3.1. Exposure of carbon coatings to the action of microorganisms (microbiological tests)

Samples of medical stainless steel and silicon protected with DLC or NCD carbon layers (as described in section 2.3) and characterized by spectroscopic methods (Raman and XRD) were sterilized by autoclaving (121 °C for 30 min) and placed in Petri dishes containing a suitable sterile solid culture medium (Czapek-Dox Agar for *T. viride*, *F. oxysporum*, *P. ochrochloron*, *C. globosum*, *A. niger*, *P. variotii*, and *M. circinelloides*, and Malt Agar supplemented with