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Incorporation of Si and SiO_x into diamond-like carbon films: Impact on surface properties and osteoblast adhesion

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Abstract

The interaction of human osteoblast cells with diamond-like carbon films incorporating silicon and silicon oxide (SiO_x, 1 6 *x* 6 1.5) and synthesized using the direct-current plasma-activated chemical vapour deposition method was investigated. Cell culture studies were performed for films with Si contents ranging from ~4 at.% to 15 at.%. Substantial differences between Si-incorporated and SiO_x-incorporated films were found for the bonding environments of Si atoms and the hybridization of underlying carbon structures. However, osteoblast-attachment studies did not show statistically significant trends in properties of cell growth (count, area and morphology) that can be attributed either to the Si content of the films or to the chemical structure of the films. The surface energy decreased by 40% as the Si content of the SiO_x incorporated DLC films increased to 13 at.%. The cell adhesion properties however did not change in response to lowering of the surface energy. The incorporation of both Si and SiO_x leads to a beneficial reduction in the residual stress of the films. The average roughness of the films increases and the hardness decreases when Si and SiO_x are added to DLC films. The impact of these changes for load-bearing biomedical applications can be determined only by carefully controlled experiments using anatomic simulators.

Keywords: Osteoblasts; DLC; Silicon; Silicon oxide; Cell adhesion

1. Introduction

Careful consideration of bulk and surface properties is required for materials used for surgical implants. These properties determine the cell attachment, proliferation and phenotypic expression. Following surgery, successful incorporation of an implant into the body depends on tissue integration and infection, which is influenced by the adherence of autologous cells and bacteria to the surface. Certain proteins are absorbed on to the surface of the prosthesis soon after implantation and mediate the bacteria and cell attachment and biological response [1–3]. It has been shown that there are important correlations between surface properties and initial cell attachment from serum-containing cultures. Surface energy, surface chemistry and surface roughness are some properties that are being widely investigated for their impacts on cell attachment [4–8]. In an earlier study, van der Valk et al. [9] identified a relationship between the polar component of surface energy and attachment of fibroblasts onto polymer surfaces. Superior cell attachment capability was found for surfaces with higher polar surface energy. Schakenraad et al. [10] found a sigmoid relationship between cells spreading and surface free energy for a range of biopolymers. Recently, Xu and Siedlecki [11] proposed water contact angle as a criterion for the adsorption of certain bovine serum proteins onto low density polyethylene surfaces. Correlations between surface free energy of protein-coated polymer surfaces

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and cell growth/proliferation are also being widely studied [12]. Experimental approaches such as combinatorial methods are now being adapted to expedite the research efforts [13].

Diamond-like carbon (DLC) is a hydrogenated amorphous form of carbon which has excellent qualities such as chemical inertness, hardness, low coefficient of friction, biocompatibility and hemocompatibility. DLC and doped DLC have been investigated extensively for possible biomedical applications [14–17]. It has been demonstrated that the doping with elements such as Si, N, Ca, P and others improve the properties of DLC such as biocompatibility, infection resistance and mechanical properties [18–24]. To the best of our knowledge a study on biocompatibility and bacterial adhesion properties of SiO_x-incorporated DLC films has not yet been published. Further, unlike for polymer materials, detailed studies of the relationship between the surface energy of DLC films and cell adhesion have not been extensively reported in the literature [20,25].

The purpose of this study was to incorporate a range of different concentrations of Si and SiO_x (1 6 x 6 1.5) into DLC films and investigate the impact on osteoblast adhesion. These modified DLC films were prepared using pulsed direct-current plasma-activated chemical vapour deposition (PACVD). The dependence of cell growth properties on the surface chemical composition, surface roughness and components of surface energy was investigated. The adhesion capability of fibroblasts/proteins and the likelihood of infection were studied using thermodynamic approaches.

2. Experimental

2.1. Pulsed DC PACVD system

The PACVD deposition system has been described in detail elsewhere [21,26,27]. Briefly, a substrate (semiconductor-grade Si, (1 0 0)) attached to a steel substrate holder/electrode inside the stainless-steel chamber was powered by a direct current (DC) pulse generator (Rübig Model MP120) operated at 415 V. Methane (precursor for DLC), argon and hydrogen were introduced into the chamber through a gas distributor using mass flow controllers (MFCs). Si-DLC films were prepared using tetramethylsilane (TMS) [21] and SiO_x-DLC films were prepared using tetraethoxysilane (TEOS). Ar was used as a carrier gas for the vapours. In the case of the preparation of SiO_x-DLC films, the glass vessel containing the metal-organic precursor was heated to 50–70 °C in a water bath, and the stainless steel tubing connecting the glass vessel containing the liquid precursor to the chamber was heated to 70–90 °C to prevent condensation.

2.2. Spectroscopic analysis

The composition of the films was determined by X-ray photoelectron spectroscopy (XPS) using a SPECS 150 system operated with Mg Ka X-ray source (10 keV and 10 mA) [28]. Spectral resolution of the instrument is ~0.1 eV. The fitting of the peaks utilized a Gaussian/Lorentzian product formula with a Shirley background [29] and was carried out using CASA-XPS V2.3.13 software. Fourier transform infrared (FTIR) spectrometry was performed using a Digilab FTS40 Spectrometer with a glow bar source, a KBr beam-splitter and a liquid-nitrogen-cooled MCT detector. The spectrometer was equipped with a Model 091-0608A universal sampling accessory to allow diffuse reflectance measurements in the mid-infrared (400–6000 cm^{-1}, or 1.7–25 lm). Raman spectroscopy was performed using Renishaw inVia confocal Raman microscope system. Specimens were illuminated with 514 nm excitation from an Ar^+ laser at an incident power of ~1 mW and a spot diameter size of approximately 1 lm.

2.3. Mechanical properties

The film thickness was measured using a step-edge profilometer (Sloane Instruments Dektak 3030). The hardness of the films was measured with a UMIS 2000 ultra micro-indentation system fitted with a diamond Berkovich indenter. Tests over a load range of 1–10 mN suggested an optimum indentation load of 5 mN. The residual stress in the films was determined by measuring the radius of curvature of the substrate before and after deposition using the profilometer [21].

2.4. Contact angles and surface roughness

Contact angles were measured using the sessile drop method with an instrument equipped with a CCD-IRIS video camera, a laser light source and Rame-Hart 2001 imaging software. Water, diiodomethane and ethylene glycol were used as the test liquids. Prior to measurements with each liquid, the samples were cleaned with acetone, 95% ethanol and deionized water. A minimum of four measurements were taken for each sample. The procedure was then repeated to obtain a total of eight readings for each sample with each liquid. The standard deviations of measurements were typically 2.0–3.0. All measurements were made at 22 °C and ~50% relative humidity. The average contact angles were used in van Oss [30] approach to obtain the dispersive component, c^{LW} (also known as Lifshitz–van der Waals component) and the polar components (acidic component/electron-acceptor component, c^+ and basic/electron-donor component, c^-) of surface energy. The polar component, c^P, and the total surface energy, c, are given by

$$c^p = 2\left[\left(c^+c^-\right)^{1/2}\right]$$

$$c = c^p + c^{LW}$$

Table 1 lists the surface energy components for the test liquids used in this study. The interfacial energy between two

ID	Title	Pages
1336	Incorporation of Si and SiOx into diamond-like carbon films: Impact on surface properties and osteoblast adhesion	7