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Hemocompatibility of surface-modified, silicon-incorporated, diamond-like carbon films

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Abstract

The hemocompatibility of plasma-treated, silicon-incorporated, diamond-like carbon (Si-DLC) films was investigated. Si-DLC films with a Si concentration of 2 at.% were prepared on Si (100) or Nitinol substrates using a capacitively coupled radiofrequency plasma-assisted chemical vapor deposition method using a mixed gas of benzene (C_6H_6) and diluted silane (SiH_4:H_2 = 10:90). The Si-DLC films were then treated with O_2, CF_4 or N_2 glow discharge for surface modification. The plasma treatment revealed an intimate relationship between the polar component of the surface energy and its hemocompatibility. All in vitro characterizations, i.e. protein absorption behavior, activated partial thromboplastin time measurement and platelet adhesion behavior, showed improved hemocompatibility of the N_2- or O_2-plasma-treated surfaces where the polar component of the surface energy was significantly increased. Si–O or Si–N surface bonds played an important role in improving hemocompatibility, as observed in a model experiment. These results support the importance of a negatively charged polar component of the surface in inhibiting fibrinogen adsorption and platelet adhesion.

Keywords: Diamond-like carbon; Surface treatment; Hemocompatibility; Polar component; Platelet adhesion

1. Introduction

Diamond-like carbon (DLC) film has emerged as a promising coating layer for blood-contacting applications owing to its superior mechanical properties, chemical inertness and hemocompatibility [1–7]. Comparative studies have reported that DLC is more hemocompatible than other biomaterials such as Ti, TiN, TiC, CN and polymethylmethacrylate (PMMA) [1,2,8,9]. Because biological reactions essentially occur on the surface, the effects of the atomic bond structure or the surface properties of DLC films on their hemocompatibility have been thus a major concern [1,2,10]. However, no consistent relationship has been found between hemocompatibility and the atomic bond structure of the films or the wettability of their surfaces. For example, Kwok et al. [11] and Huang et al. [12] reported that surfaces with lower wetting angles had improved blood compatibility. Ma et al. also reported the higher albumin to fibrinogen absorption ratios on surfaces with higher surface energy [13]. However, Leach et al. observed self-contradictory behavior in hydrophilically coated guide wires and catheters [14]. They reported that hydrophilically coated guide wires suppressed clot deposition while the same coating on catheters enhanced clot deposition. In contrast, Hasebe et al. observed that hemocompatibility was improved in fluorine-incorporated DLC

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films [15,16], where the surface energy decreased with fluorine incorporation. Jones et al. explored platelet attachment on Ti, TiN, TiC and DLC surfaces [17]. The greatest platelet spreading was seen on the more hydrophilic surfaces, even if these authors did not consider the chemical effects of different materials.

In this study, we investigated the hemocompatibility of surface-modified silicon-incorporated DLC (Si-DLC) films using a plasma surface treatment method. The plasma surface treatment enabled us to address the role of surface chemical bonds. It was recently reported that Si incorporation into DLC films improves both corrosion resistance in body fluid conditions and mechanical reliability, with higher interfacial toughness [18,19]. Si-DLC coating is thus a strong candidate as a protective layer for implant materials to avoid the release of metal ions. Plasma treatment of the surface using O_2, N_2 or CF_4 glow discharge resulted in a variety of surfaces ranging from hydrophilic to hydrophobic, corresponding to changes in the polar and dispersive components of the surface energy [20]. In vitro hemocompatibility tests revealed that hydrophilic surfaces with greater polar components of the surface energy suppressed fibrinogen adsorption and platelet adhesion and activation. A model experiment revealed that Si–O or Si–N bonds on the surface play an important role in improving hemocompatibility, presumably due to the negatively charged polar component.

2. Materials and methods

2.1. Si-DLC deposition

The Si-DLC films were deposited on Si (100) or electrochemically polished Nitinol (NiTi) substrates using a radiofrequency plasma-assisted chemical vapor deposition (RF–PACVD) method. Details of the deposition system have been reported previously [18,19]. The films on the Si wafers were used for surface property characterization via wetting angle measurement and X-ray photoelectron spectroscopy (XPS) analysis. All in vitro hemocompatibility tests were performed using the films deposited on the Nitinol plates. A mixture of benzene and diluted silane (SiH_4:H_2 = 10:90) was used as the precursor gas. The substrates were initially cleaned with an argon discharge for 15 min at a bias voltage of –400 V and a pressure of 0.49 Pa. An interlayer of amorphous silicon (a-Si:H) with a thickness of nearly 5 nm was deposited on the substrates to ensure better adhesion of the Si-DLC films. The Si-DLC film was then deposited at a bias voltage of –400 V for 11 min 40 s at a pressure of 1.33 Pa. The film thickness of the Si-DLC films was 0.55 μm as measured using an alpha step profilometer. Rutherford backscattering spectrometry showed that the Si concentration in the films was 2 at.%. The structure and mechanical properties of Si-DLC film have previously been investigated in detail [18].

The Si–DLC films were then treated with RF glow discharge of various gases, i.e. such as O_2, N_2 and CF_4, in the same PACVD chamber. The plasma treatments were performed for 10 min at a bias voltage of –400 V and a pressure of 1.33 Pa. The plasma treatment did not change the film thickness significantly except for the O_2 plasma treatment. The O_2 plasma treatment etched the Si-DLC films and reduced the film thickness to 0.31 μm. The surface properties and chemical bonds of the plasma-treated Si-DLC films were reported by Roy et al. [20].

2.2. Wetting angle and XPS measurements

The surface energies of the samples were characterized according to the method of Owens [20–22]. Young's equation for wetting of a solid surface by a liquid can be expressed in terms of the dispersive and polar components of the surface energy of liquid and solid as follows:

$$1 + \cos\theta = 2\left(\frac{\sqrt{\gamma_{sv}^{d}}\sqrt{\gamma_{lv}^{d}}}{\gamma_{lv}} + \frac{\sqrt{\gamma_{sv}^{p}}\sqrt{\gamma_{lv}^{p}}}{\gamma_{lv}}\right), \quad (1)$$

where θ is the contact angle between the solid and liquid and γ_{tv}, γ_{sv} are the free energies of the liquid and solid against their saturated vapor, respectively. The superscripts d and p refer to the dispersive and polar components, respectively. The wettability of the film surfaces was characterized by measuring the θ of two different liquids with known values of dispersive and polar components of the surface energy by solving the simultaneous equations. In the present work, deionized water and formamide were used [21]. The contact angle measurements were performed using a contact angle goniometer (Rame-Hart Inc., USA). The reported contact angles in this paper correspond to an average of 10 measurements.

XPS measurements of the surface were performed using a Physical Electronics PHI 5800 ESCA system. The X-ray source used was Al K_α at 1486.6 eV and the anode was maintained at 250 W, 10 kV and 27 mA. The XPS measurements were done at a chamber pressure of 2×10^{-8} Pa. For high-resolution measurement, analyzer pass energy was 58.70 eV (energy resolution 0.125 eV). The spot size of the beam was 400 μm × 400 μm. The calibrations of peak position were done by taking the C1s peak at 284.6 eV. The curve fittings were carried out with a mixture of Gaussian and Lorentzian functions. The percentages of individual peaks were determined using the peak areas of individual peaks and the corresponding atomic sensitivity factor of the element [23].

2.3. Protein adsorption tests

Plasma protein adsorption tests were performed by treating the samples with bovine serum albumin (Sigma–Aldrich, St. Louis, MO, USA) and fibrinogen (Sigma–Aldrich) solutions and measuring the absorbance through enzyme-linked immunosorbent assay (ELISA) analysis. The proteins were dissolved separately in phosphate-buf-