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# Influence of silicon concentration on the haemocompatibility of amorphous carbon

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**Abstract**

Amorphous carbon (a-C) has good blood compatibility and has been proposed as a coating material for blood contacting devices such as heart pumps and stents. In this study, unhydrogenated a-C films with different silicon concentrations were synthesized by magnetron sputtering, and the corresponding evolution of the surface energy and compatibility with blood were analysed. The incorporation of silicon not only decreased the $sp^2$-hybridized carbon bonding configurations, but the static evaluation of the films incubated in human platelet-rich plasma also showed a decrease in platelet adhesion. Bonding structure and surface energy were determined to be factors contributing to the improved haemocompatibility.


*Keywords:* Silicon-incorporation; Amorphous carbon; Bonding structure; Surface energy; Human platelet adhesion; Haemocompatibility

## 1. Introduction

Amorphous carbon (a-C) has good blood compatibility [1–6] and may be used to coat poorly haemocompatible materials for devices in contact with blood. It also offers good mechanical properties such as high hardness and elastic modulus and low coefficient of friction [7]. However, high intrinsic stress inherited during the synthesis cannot be avoided, as it is the by-product of the formation of the diamond-like phase in the a-C [8]. This stress poses a major challenge for the intended usage, as film delamination can occur. The intrinsic stress of a-C films can be reduced by incorporating a small amount of silicon [9,10]. Okpalugo et al. [11] have studied hydrogenated a-C films with silicon-doping levels of less than 10 at%, and showed improved haemocompatibility with decreased platelet count for the doped films. Study on unhydrogenated a-C containing silicon showed that the quantity of adhering platelets was largely influenced by the surface energy of the film [12]. Besides changing deposition parameters or post-deposition treatment, the surface energy can be tuned by altering the concentration of the dopant. In this study, we investigate the influence of silicon concentration on the surface energy of the coatings, and the resulting blood compatibility.

## 2. Methodology

### 2.1. Film deposition and surface roughness

Silicon-incorporated unhydrogenated a-C films were sputtered from graphite (99.995% purity) and silicon (99.999% purity) targets on (1 0 0) monocrystalline silicon wafers using E303A Magnetron Sputtering System. Prior to deposition, the wafers were chemically cleaned in a piranha bath (a 3:1 mixture of concentrated sulphuric acid with hydrogen peroxide for removing organic residues) and treated with Ar plasma for 20 min at a radio frequency (RF)-induced substrate bias of −300 V to remove the surface oxides. The graphite target power density was 7.4 W/cm$^2$, and the silicon target power density was 2.5 W/cm$^2$. The base pressure ($4.5 \times 10^{-3}$ Pa), process pressure ($800 \times 10^{-3}$ Pa), Ar flow rate (50 sccm) and substrate bias voltage (−10 V) all remained constant. The surface morphology was characterized by a Shimadzu 9500J2 atomic force microscope under constant force in contact mode.

### 2.2. X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy

The atomic concentration of Si and C were determined by XPS using the Kratos AXIS X-ray photoelectron spectrometer equipped with a monochromatic Al-Kα (1486.71 eV) X-ray radiation operating at 15 kV

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and with a vacuum of $10^{-6}$ Pa. The bonding of a-C(Si) was characterized by Raman spectroscopy using Renishaw Raman Spectroscope RM1000 excited with a HeNe laser at a wavelength of 633 nm and laser power of 1 mW. The peak deconvolution was performed using Gauss–Lorentz distribution function, and the fitted curves have a reduced $\chi^2$ of 1.2 or less to ensure convergence.

### 2.3. Surface energy determination

The surface free energy of the films was determined by contact angle measurements. The Lifshitz–van der Waals (LW)/acid–base (van Oss) approach [13] was employed to interpret the contact angle measurements. In this method, the surface tension is differentiated into three components: the apolar LW component, the polar acid (+) and the base (−) components. The "acid" component is also called the acceptor because it is positively charged, thus is ready to accept electrons; the "base" component is also called donor because it is negatively charged, thus has extra electrons to give away. The total surface tension is given by

$$\gamma_{\text{tot.}} = \gamma^{\text{LW}} + 2\sqrt{\gamma^{+}\gamma^{-}}, \quad (1)$$

where $\gamma^{+}$ is the acceptor and $\gamma^{-}$ the donor part of the surface energy. Taking Young's equation: $\gamma_{\text{lv}} \cos\theta = \gamma_{\text{sv}} - \gamma_{\text{sl}}$ [14] into consideration (where $\theta$ is the measured contact angle, $\gamma_{\text{lv}}$, $\gamma_{\text{sv}}$ and $\gamma_{\text{sl}}$ are the liquid–vapor, solid–vapor and solid–liquid interfacial tension, respectively), the LW/acid–base equation can be written as follows:

$$\gamma_{\text{l}}(1+\cos\theta) = 2\sqrt{\gamma_{\text{l}}^{\text{LW}}\gamma_{\text{s}}^{\text{LW}}} + 2\sqrt{\gamma_{\text{l}}^{+}\gamma_{\text{s}}^{-}} + 2\sqrt{\gamma_{\text{l}}^{-}\gamma_{\text{s}}^{+}}. \quad (2)$$

Eq. (2) can be used to determine the three surface tension components ($\gamma_{\text{s}}^{\text{LW}}$, $\gamma_{\text{s}}^{-}$ and $\gamma_{\text{s}}^{+}$) by measuring contact angles and solving three simultaneous equations. To do this, three liquids of known surface tension are required. We used deionized water, formamide and diiodomethane. The surface tension values of these liquids can be found in Ref. [15]. Contact angle measurements were conducted using the sessile drop technique with the First Ten Angstroms 200 Goniometer.

### 2.4. Platelet-rich-plasma preparation, incubation and fixation

A total of 450 ml of human whole blood from a drug-free donor was drawn and mixed with tri-sodium citrate. The ratio of blood to tri-sodium citrate was 9:1. To prepare the platelet-rich plasma (PRP), the blood was centrifuged at 3200 rpm for 10 min to separate the blood corpuscles. The plasma was extracted and centrifuged again at 3200 rpm for 15 min. Platelet clumps were obtained, and were allowed to redistribute back into the plasma, which was then 30 ml after draining away the excess. The platelet count in the PRP was determined by flow cytometry to be $1.6 \times 10^6$ cells/μL. The plasma was then held at 37 °C water bath for 30 min to allow the platelets to return to their original shape. After cleaning and sterilization processes, the PRP was seeded onto the surfaces of the a-C(Si) films placed on a petri dish. The incubation was carried out at 37 °C at 5% $CO_2$ for 30 min. Afterwards, the supernatant was discarded, and the samples were rinsed twice with phosphate-buffered saline (PBS) to remove the proteins and non-adherent platelets. The cells on the specimens were fixed with 2.5% gluteraldehyde for 30 min, followed by 1% osmium tetraoxide on ice for 45 min. After the fixation, the samples were washed and dehydrated in a graded ethanol series of 50%, 70%, 80%, 95% and 100% (twice) for 10 min each.

### 2.5. Platelet count and morphology (scanning electron microscopy)

Leica S360 scanning electron microscope was used to observe the platelets on the a-C(Si) films. The surfaces were coated with ~15 nm of gold using the Polaron SC 7640 coating unit to prevent surface charging. An average platelet count using the mean of six non-repeating areas of size 125 μm × 90 μm was conducted on each sample. Results are expressed as counts/unit area and standard error. Statistical analysis was carried out using one-way analysis of variance (ANOVA) at an average of six replicates. Results with $p<0.05$ were considered to be statistically significant. The morphological shape changes were categorized according to Goodman et al. [16]. There are five types of platelet morphologies. Unactivated platelets of Type I are ~2 μm in diameter showing no pseudopodial. With increasing activation, the platelets develop pseudopodial, which eventually becomes hyaloplasm at the highest state of activation (Type V). The platelets then can have a size up to ~5 μm.

## 3. Results and analysis

### 3.1. Bonding and surface roughness

Fig. 1 shows the Raman spectra of the a-C(Si) films. The Raman spectra were deconvoluted into G (graphitic) and D (disorder) peaks, where the D-peak is the shoulder of the G-peak at lower wavenumbers. Fig. 2 clearly shows the decrease in the relative intensity of the D-peak. The breaking of the $sp^2$ sixfold aromatic rings bonding structure will cause a decrease in the intensity of the D-peak, since the present of the D-peak is due to the present of the $sp^2$ aromatic rings [17]. This increases the overall disordering of the C-network, and enhances the chance of $sp^3$ formation. The decrease in $I_D/I_G$ intensity ratio with increasing Si corresponds to the decrease in the average crystallite size of $sp^2$-bonded clusters [17], as well as the increase in $sp^3$ fraction [18]. The disordering and loss of aromatic bonding cause the amorphous-carbon signature peak to downshift [17,19], and this phenomenon is

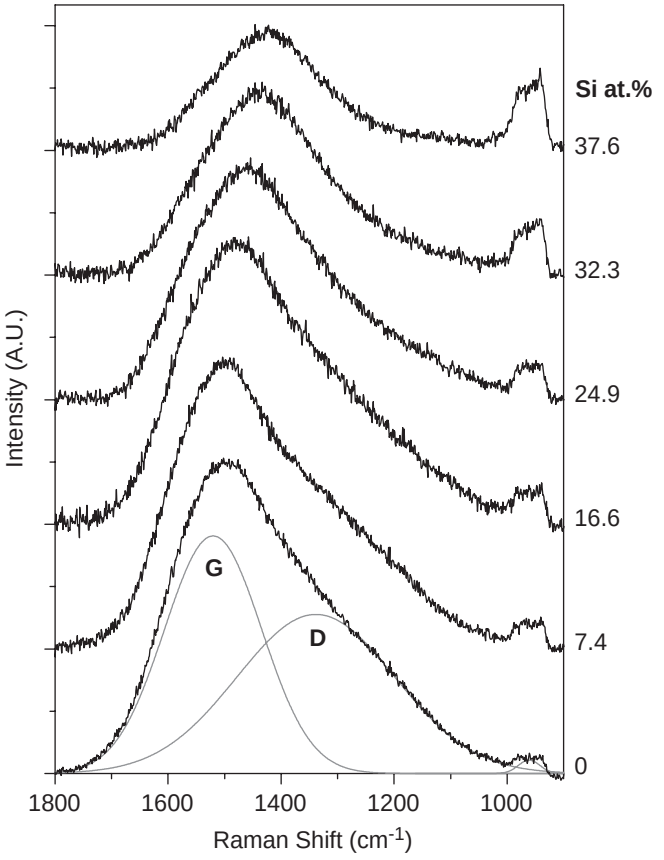


Fig. 1. Raman spectra of a-C(Si) films and disordered graphite, peak at ~960 $cm^{-1}$ is the 2nd order Raman band of Si substrate.