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Time course analysis of antithrombogenic properties of fluorinated diamond-like carbon coating determined via accelerated aging tests: Quality control for medical device commercialization

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ABSTRACT

We have previously reported that fluorine-incorporated amorphous carbon (a-C:H:F) coating dramatically reduced the number and activation of adherent platelets in contact with human blood. In order to convert a-C:H:F coating into a commercial reality, it is necessary to estimate its life span and the sustainability of antithrombogenic properties using accelerated aging tests under various temperature conditions. The purpose of this study was to investigate the effect of different temperature conditions using accelerated aging tests on the antithrombogenic properties of a-C:H:F.

The a-C:H:F film was deposited on silicon substrates from a mixture of acetylene and octafluoropropane using the inductively coupled plasma enhanced chemical vapor deposition method. The a-C:H:F coated substrates were then stored at room temperature, 55 °C, 70 °C and 90 °C, respectively. The surface chemical compositions of a-C:H:F film were examined using X-ray photoelectron spectroscopy (XPS). The antithrombogenic properties were evaluated through incubation with platelet-rich plasma isolated from human whole blood, and the properties of adhesion between film and metallic stents were evaluated by examining before-and-after balloon expansion. The XPS analysis showed that the relative amount of fluorine atoms slightly decreased and the amount of oxygen increased over time on the surface of a-C:H:F samples. Furthermore, the change in chemical composition was the most prominent in the samples stored at 90 °C. However, no significant difference in the number of adherent platelets were observed among a-C:H:F coated sample surfaces after accelerated aging tests, suggesting that changes in chemical composition due to elapsed time and temperature changes do not significantly affect the antithrombogenic properties. Furthermore, the stability of the adhesive properties on a-C:H:F coated stents was revealed, because there were no cracks or instances of delamination on any a-C:H:F coated stent surfaces after expansion.

This work demonstrated that excellent antithrombogenic properties of a-C:H:F were maintained over time at each temperature, and thus a-C:H:F film could be utilized as a coating material for medical device commercialization.

Prime novelty statement: We report that the antithrombogenic and adhesive properties of fluorine-incorporated amorphous carbon (a-C:H:F) are stable even after accelerated aging tests, and thus a-C:H:F film could be utilized as a coating material for medical device commercialization.

1. Introduction

A "stent" is a small mesh metal tube that is used to treat narrow or weak arteries in the body. Stent placement is a widely accepted non-invasive form of treatment for cardiovascular diseases such as angina and heart infarction; however, there remain serious concerns about late-phase complications such as in-stent restenosis and late stent thrombosis. In-stent restenosis is the re-narrowing of opened arteries after stent placement. Thrombosis is caused by accumulation of platelets and fibrin on stent materials or at sites of stent injury. These blood components affect inflammation and smooth muscle cell hyperproliferation/migration of arterial wall, and accordingly result in restenosis after stent placement [1–3]. Although the antithrombogenic properties of stents are indispensable for prevention of such complications, it is known that the

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conventional bare metal stents (BMS) can induce early thrombosis within 30 days, followed by vascular smooth muscle cells proliferation/migration, and result in restenosis [4]. Drug-eluting stents (DES), which have been developed to improve BMS, markedly reduce early restenosis to a greater extent than BMS by eluting drugs from stent surfaces. However, the risk of late stent thrombosis and late restenosis beyond 1 year after stenting still remains due to the delay of endothelialization by the strong side effects of released drugs [5,6]. Thus, to develop a new stage of stents, antithrombogenic properties and endothelium compatibility are essential.

To improve interaction between blood and materials, modification of surface properties is crucially effective, and surface coating is one way to enhance the chemical and physical properties of materials. Diamond-like carbon (DLC) or hydrogenated amorphous carbon (a-C:H) has received much attention as such a coating material. This film has excellent properties such as wear resistance, a high degree of hardness, chemical inertness, corrosion resistance, and biocompatibility [7,8]. Focusing on biocompatibility, including antithrombogenic properties of DLC, Nakatani et al. newly developed a DLC coated coronary artery stent [9]. Previously, we reported that fluorine-incorporated a-C:H (a-C:H:F) coating drastically prevented platelet adhesion and activation to a greater extent than conventional biomaterials or a-C:H film without the suppression of endothelialization [10–13]. Furthermore, by applying a-C:H:F coating to stents, we improved adhesion of a-C:H:F to metallic stents by controlling hydrogenated amorphous silicon carbide (a-SiC:H) and silicon-incorporated a-C:H (a-C:H:Si) interlayers [14].

Preclinical tests and clinical trials are indispensable for verification of the stability of new medical devices implanted into the human body, because their usage environment in the human body is much tougher than that in atmospheric conditions. In addition, deterioration of the product before implantation should be avoided and stability of the biomaterials and medical devices in atmospheric condition should be ensured for commercialization. Regarding a-C:H:F film, Koshel et al. reported that fluorine content and the contact angle of the film decreased over a time course of 4 months [15]. Taking into account the commercial reality of a-C:H:F coated stents for cardiovascular diseases, it is necessary to estimate the sustainability of antithrombogenic properties of a-C:H:F film.

To estimate the long-term effects of expected levels of stress and lifespan of products in shelf life within a shorter time, accelerated aging tests are commonly used to accelerate the aging speed of products under suitable aggravated conditions of heat, humidity, sunlight, high voltage, vibration, etc. Thermal accelerated aging tests under elevated temperature are usually used to establish the lifespans of materials to simulate the long-term aging process of many devices or applications [16–18]. According to the technical information report 17 (TIR17) published by the Association for the Advancement of Medical Instrumentation (AAMI), a thermal accelerated aging test is also used to establish the lifespans of biomaterials.

To the best of our knowledge, no thermal aging test using antithrombogenic a-C:H:F film by time course and elevated temperature has yet been analyzed or reported. Thus, in this study, as an aging test for a-C:H:F film under various temperature conditions, the antithrombogenicity and adhesive properties to SUS316L stents were evaluated for commercialization.

2. Materials and methods

2.1. The a-C:H:F film preparation on flat substrates for time course analysis after storage for a month

2.1.1. The a-C:H:F film preparation methods for flat substrates

The a-C:H:F film was deposited on silicon flat substrates (10×10 mm^2) to analyze change in film properties. To improve the adhesion strength between a-C:H:F film and a substrate, interlayers should be introduced. In a previous study, we revealed that two interlayers, hydrogenated amorphous silicon carbide (a-SiC:H) and silicon-incorporated a-C:H (a-C:H:Si) films, dramatically improved adhesion of a-C:H:F film to metallic (SUS316L) stents [14]. We had also proved that adhesion of a-C:H:F to the stents was further strengthened by a-C:H:Si film with gradient Si content. Therefore, in this study, on the basis of the concept of introducing interlayers for better adhesion between a-C:H:F film and a substrate, a three-layered film, a-C:H:F/a-C:H:Si with gradient Si content/a-SiC:H from top to bottom, was prepared on silicon flat substrates (10×10 mm^2). The thickness of a-C:H:F, a-C:H:Si with gradient Si content, and a-SiC:H films was about 200, 100, and 100 nm, respectively. This was performed using radio frequency plasma enhanced chemical vapor deposition (RF-PECVD) equipment (YH-100NX, Onward Giken Co., Ltd., Japan) for aging tests.

The gas used in the a-SiC:H film deposition process was tetramethylsilane (TMS, Shin-Etsu Chemical Co., Ltd., Japan), and the films were deposited at a flow rate of 5.0 sccm at a bias voltage of −2.5 kV for 630 s. The a-C:H:Si films were deposited on the flat silicon substrates from a mixture of TMS and acetylene (C_2H_2, Koatsu Gas Kogyo Co., Ltd., Japan) gas using the RF-PECVD equipment. The TMS flow rate was fixed at 6.0 sccm and the C_2H_2 flow rate was changed from 0.0 sccm to 15.0 sccm gradually at a bias voltage of −2.5 kV for 850 s.

For the a-C:H:F film deposition (outermost surface), a mixture of C_2H_2 and perfluoropropane gas (C_3F_8, Iwatani Corp., Japan) was used, and the flow rates of C_2H_2 and C_3F_8 were 5.0 sccm and 50.0 sccm, respectively. The deposition was carried out at a bias voltage of −1.0 kV for 930 s. After deposition, the samples were stored for a month at various temperatures (room temperature (RT), 55 °C, 70 °C and 90 °C).

2.1.2. X-ray photoelectron spectroscopy (XPS) analysis on a-C:H:F-coated flat substrates before and after storage for a month at various temperatures

Chemical compositions and bonding states of the surfaces of a-C:H:F films on samples were measured by XPS (JPS-9010TR, JEOL Ltd., Japan). The X-ray photoelectron of C1s, F1s and O1s spectra were measured using Mg Kα source (10 mA, 10 kV) to evaluate surface defluorination and oxidation of a-C:H:F films. The carbon, fluorine and oxygen concentration of the film surface was calculated from each peak area and corresponding relative sensitivity factors. Decrease and/or increase of fluorine and oxygen were determined by the comparing F/C and O/C ratios for each concentration.

2.1.3. Contact angle measurements on a-C:H:F-coated flat substrates before and after storage for a month at various temperatures

The wettability of the film was evaluated by measuring the static contact angles of droplets of distilled water (2 μl each) on sample surfaces. The contact angle measurements were conducted using the sessile drop method with a contact angle meter (DM 500, Kyowa Interface Science Co., Ltd., Japan) at five different points on each sample surface.

2.1.4. Human platelet adhesion and activation on a-C:H:F-coated flat substrates before and after storage for a month at various temperatures

Platelet adhesion and activation on foreign surfaces plays a central role in thrombosis. Platelet adhesion and activation must be assessed to determine the blood compatibility of a new material.

Human whole blood (85 ml) was collected from healthy volunteers who had not taken any medication for at least 10 days. After mixing the blood with 15 ml of acid-citrate-dextrose (ACD), platelet-rich plasma (PRP) was isolated by centrifugation at $350 \times g$ and 20 °C for 15 min. Supernatant plasma was collected as PRP, and subsequently remaining blood was centrifuged at $1000 \times g$ and 4 °C for 10 min to obtain platelet poor plasma (PPP). The density of platelets in PRP was adjusted to ~ 3.0×10^5/μl by dilution with PPP. After rinsing with phosphate-buffered saline (PBS; pH 7.4), samples were incubated in 24-well plates containing 1 ml of adjusted PRP at 37 °C for 60 min in an atmosphere containing 5% CO_2 gas. Thereafter, PRP was discarded and the samples were washed with PBS. Adherent platelets on samples were then fixed for 6 h at room temperature in 0.8 ml of freshly prepared 1.0%