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Biocompatibility of a-C:H film coating for synthetic vascular graft

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ABSTRACT

Amorphous hydrogenated carbon (a-C:H) films have many excellent properties such as biocompatibility, anti-corrosion, and chemical stability. Therefore, there are many reports on application of a-C:H film as surface modification technique for biomaterials. However, it is difficult to deposit a-C:H film on complex structures such as artificial heart blood pump and synthetic vascular grafts. In our previous work, we have developed an electrode which is adapted to such irregular structures for plasma CVD technique.
In this study, a-C:H film was deposited on a synthetic vascular graft inner-wall by r.f. plasma CVD technique with cylindrical electrode. The purpose of such coating is improvement of biocompatibility of the vascular graft. The biocompatibility of the a-C:H film was evaluated by cytocompatibility and plasma protein adhesion. For the a-C:H film deposition, cytocompatibility and protein adsorbent of the vascular grafts were improved for biological response under cell culture with mouth fibroblasts and plasma proteins (albumin, fibrinogen, and globulin), respectively. This study indicates that the a-C:H films coatings is expected to surface modification for medical appliances.

1. Introduction

Amorphous hydrogenated carbon (a-C:H) films have many excellent properties such as biocompatibility, anti-corrosion and chemical stability [1–4]. Additionally, radio frequency (r.f.) plasma chemical vapor deposition (CVD) technique, which is one of the most popular a-C:H film deposition technique, is possible to deposit a-C:H films on a most polymeric materials used for the medical appliance. Therefore, a-C:H films have been expected a surface modification coating to medical equipment such as artificial heart blood pump and synthetic vascular graft. However, achieving uniform deposition of thin films is impossible since it is difficult to generate the uniform plasma around complicated (three-dimensional) structure surfaces [5–8]. In our previous work, we have developed a special electrode which is possible to adjust to complex objects flexibility, and proposed a new technique of film deposition for three dimensional structures by using the special electrode [8,9].

In this study, we focus on the a-C:H film deposition in order to improve biocompatibility of the synthetic vascular graft. It is well known that a-C:H film has been expected to improvement of blood compatibility, and cytocompatibility. However, evaluation of biocompatibility for the a-C:H film, which is deposited on a synthetic vascular graft inner-wall, has not been reported enough. The biocompatibility of the a-C:H film coating on synthetic vascular graft (ePTFE and polyester) inner-wall was investigated for biological response under cell cultures and protein adhesion for biological response.

2. Experimental

2.1. a-C:H film deposition onto inner-wall of synthetic vascular grafts (ePTFE and polyester)

Fig. 1 shows the schematic diagram of the cylindrical electrode with r.f. plasma CVD method. This system consists of an aluminum cylindrical electrode that is put on a cathode side electrode, an anode side electrode, an r.f. generator (model SS-301AAE, Fuji Electronic Industrial Co., Ltd.), a matching box (model HC-2000, Tokyo Hy-power Labs., Inc.), and a vacuum pump (model 1397, Sargent-Welch Scientific Co.). In this experiment, we deposited a-C:H film on two kinds of synthetic vascular, which are made of expanded polytetra-fluoroethylene (ePTFE) fiber (ePTFE vascular: $\varphi = 16$ mm L = 60 mm) and polyester fiber (Polyester vascular: $\varphi = 24$ mm L = 60 mm), respectively. The frequency of plasma source and the electrical power was kept at 13.56 MHz and 100 W, and decomposed the hydrocarbon gas (CH_4) at 10 Pa for a deposition time of 30 min. At the cylindrical electrode, a negative bias voltage was −260 V.

The surface morphology of the a-C:H film deposited on the ePTFE and polyester vascular graft inner-wall was observed using scanning electron microscope (SEM: model JSM-5310LVB, Jeol Ltd., Japan). Additionally, the structure of the a-C:H film was investigated using an Ar-laser Raman spectrophotometer (Raman: model NRS-2100, Jasco

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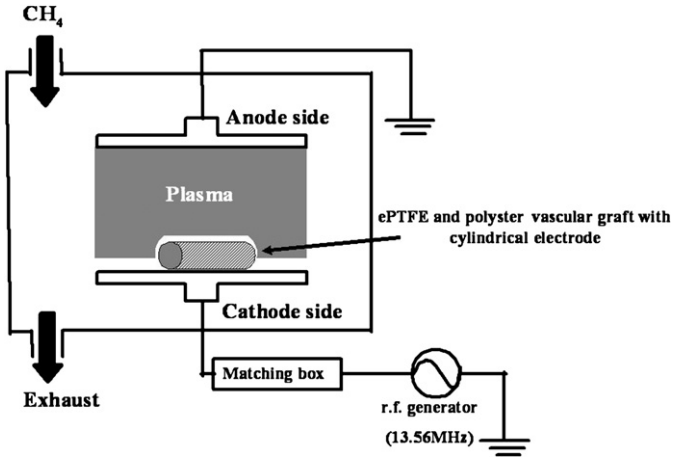

Fig. 1. The schematic diagram of the cylindrical electrode with r.f. plasma CVD technique.

Co., Japan), and chemical compositions and bonding states of the a-C:H film surface were measured by a X-ray photoelectron spectrometer (XPS: model JPS-9000MC, Jeol Ltd., Japan). The X-ray source was Mg/Kα. Moreover, contact angle of the film surface was measured by $\theta/2$ method with 2 μL deionized water.

2.2. Plasma protein adsorbent test

Investigation of the plasma protein adsorbent was carried out using albumin from human serum (Model: 013-10501, Wako, Tokyo, Japan), fibrinogen from human plasma (Model: 067-03693, Wako, Tokyo, Japan), and γ-globulin from human serum (Model: 075-02291, Wako, Tokyo, Japan). The mixture solution (1 mL/1 mg) of these plasma protein and 0.1 mol/L phosphate-buffered saline solution (PH = 7.4, PBS(−)) were used for the estimation of the protein adsorption on the a-C:H films. The mixture solution was immersed onto the coated (a-C:H/ePTFE and a-C:H/polyester) and non coated samples of the synthetic vascular graft (ePTFE and polyester) inner-wall, which were divided into 100 mm^2, respectively. The plasma protein adsorption were immersion in the samples in the mixture solutions for 3 h. The absorbance measured using a protein adsorption (HITACHI, U-1500) at a wavelength of 450 nm.

2.3. Cell culture

Mouse fibroblasts (NIH 3T3) were grown as a monolayer culture in the Dulbecco's Modified Eagle Medium(D-MEM) that was supplemented with 10% bovine calf serum (FBS, GIBCO 1017, Invitrogen, Co., USA) and antibiotics (penicillin) at 37 °C in an atmosphere of 100% humidity composed of 5% CO_2 and 95% atmosphere. During the growth of the fibroblast, the D-MEM solution was renewed. After the preparation of the D-MEM solution and adjusted to a density of 2×10^4 cells/mL, the fibroblast suspensions were seeded onto the a-C:H film coated (a-C:H/ePTFE and a-C:H/polyester) and non coated samples of the synthetic vascular graft (ePTFE and polyester) inner-wall, which were divided into 100 mm^2, respectively. The fibroblasts were cultured on the samples in each well of a 24-multiwell insert system (Falcon 353047, Becton, Dickinson and Co., USA) for 4 days. The number of cells was determined using a Neubauer hemacytometer (Model A116, Sun Lead Glass Co. Ltd., Tokyo, Japan).

3. Results and discussion

3.1. a-C:H film deposition onto inner-wall of synthetic vascular grafts (ePTFE and polyester)

The a-C:H film depositions on inner-wall of the synthetic vascular grafts (ePTFE and polyester) were carried out by the cylindrical electrode with r.f. plasma CVD technique.

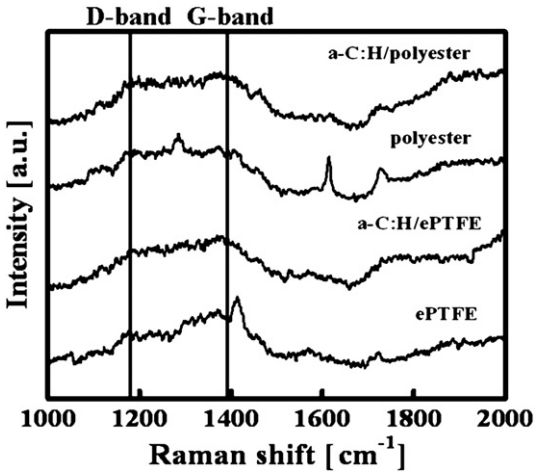

Fig. 2. Raman spectra of the coated and uncoated a-C:H on synthetic vascular graft (ePTFE and polyester).

Fig. 2 shows Raman spectra of the coated and non coated a-C:H film on ePTFE and polyester. The Raman spectra of a-C:H film deposition on the inner-wall of the ePTFE and polyester grafts were fitted to two Gaussian peaks denoted as the D-peak and the G-peak. Generally, G-peak means graphite structure located around 1530 – 1580 cm^{-1} and D-peak means disorder structure located at approximately 1350 cm^{-1} [3,9]. However, G-peak and D-peak downshift depends on textile

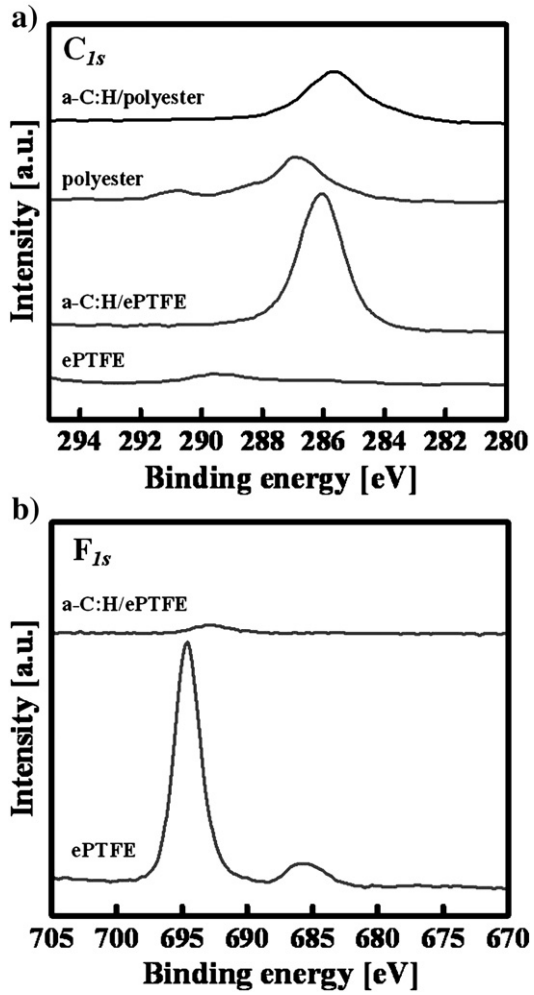

Fig. 3. XPS spectra of carbon (C_{1s}) and fluorine (F_{1s}) for the coated and uncoated a-C:H on synthetic vascular graft (ePTFE and polyester). (a) Carbon (C_{1s}). (b) Fluorine (F_{1s}).