Applied Surface Science 346 (2015) 50–56

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Surface modification of blood-contacting biomaterials by plasma-polymerized superhydrophobic films using hexamethyldisiloxane and tetrafluoromethane as precursors

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ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 16 March 2015
Accepted 29 March 2015
Available online 6 April 2015

Keywords:
Blood-contacting biomaterials
Nanoparticles
Plasma polymerization
Superhydrophobic
Surface modification

ABSTRACT

This paper proposes a plasma polymerization system that can be used to modify the surface of the widely used biomaterial, polyurethane (PU), by employing low-cost hexamethyldisiloxane (HMDSO) and tetrafluoromethane (CF_4) as precursors; this system features a pulsed-dc power supply. Plasma-polymerized HMDSO/CF_4 (pp-HC) with coexisting micro- and nanoscale morphology was obtained as a superhydrophobic coating material by controlling the HMDSO/CF_4 (f_H) monomer flow ratio. The developed surface modification technology can be applied to medical devices, because it is non-cytotoxic and has favorable hemocompatibility, and no blood clots form when the device surface direct contacts.

Experimental results reveal that the obtained pp-HC films contained SiO_x nanoparticles randomly dispersed on the micron-scale three-dimensional network film surface. The —CF functional group, —CF_2 bonding, and SiO_x were detected on the film surface. The maximal water contact angle of the pp-HC coating was 161.2°, apparently attributable to the synergistic effect of the coexisting micro- and nanoscale surface morphology featuring a low surface-energy layer. The superhydrophobic and antifouling characteristics of the coating were retained even after it was rubbed 20 times with a steel wool tester. Results of in vitro cytotoxicity, fibrinogen adsorption, and platelet adhesion tests revealed favorable myoblast cell proliferation and the virtual absence of fibrinogen adsorption and platelet adhesion on the pp-HC coated specimens. These quantitative findings imply that the pp-HC coating can potentially prevent the formation of thrombi and provide an alternative means of modifying the surfaces of blood-contacting biomaterials.

1. Introduction

During the past century, the use of cardiovascular and other blood-contacting biomedical devices, including heart valves, vascular grafts, and stents, has evolved from a mere dream to a widely adopted practice. However, one of the major problems encountered in using these devices is thrombi or blood clot formation (i.e. poor blood compatibility) on their surfaces [1]. Blood–material interactions affect the blood–material compatibility, which involves a complicated blood clotting process (including protein adsorption, platelet adhesion, and activation) and blood coagulation cascades (intrinsic and extrinsic pathways). Consequently, these interactions strongly affect the short- and long-term thrombotic responses of the materials [2]. Protein adsorption is the first event in blood–material interactions, and the clotting enzymes and fibrinogen play a major role in blood clotting. Previous studies have revealed that fibrinogen is involved in blood clotting through the activation of platelets, which results in thrombosis [3,4]. Therefore, low fibrinogen adsorption and low platelet adhesion are essential for achieving favorable blood compatibility [5]. Hence, surfaces that are "antifouling" to fibrinogen adsorption should be modified to prevent platelet adhesion and activation, by adjusting the surface wettability and surface roughness [6]. Related studies have indicated that platelet adhesion and activation could be influenced by surface topographic features at the submicron level, causing

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http://dx.doi.org/10.1016/j.apsusc.2015.03.208
0169-4332/

substantial reduction in the platelet response compared with that of controls. By contrast, topographic features with dimensions on the micron scale did not reduce the platelet response compared with that of controls, suggesting that a platelet size ranging from 2 to 3 μm in diameter or less can reduce the platelet response [7,8].

A superhydrophobic surface, which is characterized by a water contact angle (WCA) exceeding 150° and a slide angle of less than 10°, has unique surface properties such as self-cleaning and antifouling abilities, and has been intensively researched. Moreover, it has wide applications in both industry and daily life [9]. Two crucial criteria must be satisfied to realize a superhydrophobic surface: low surface energy and hierarchical surface topography [10]. The superhydrophobic approach was inspired by the natural combination of surface chemistry and surface morphology initially discovered by Neinhaus and Barthlott on the surface of lotus leaves in 1998 [11]. Artificial superhydrophobic surfaces can be produced using numerous methods, including template synthesis, sol-gel processing, lithography, layer-by-layer deposition, and plasma polymerization, for creating hierarchical-scale surface roughness [12].

Plasma polymerization, a promising technique for preparing superhydrophobic surfaces that is widely used for the surface modification of medical devices [13,14], yields ultrathin films consisting of cross-linked structures. This technique can be used to modify the physicochemical properties of biomaterial surface and is simple, easy to use, and environment friendly; can be used with a variety of raw materials; has a low deposition temperature and a low cost; and affords favorable substrate adhesion and high material biocompatibility [14,15]. A plasma polymerization system featuring a pulsed-dc power supply can enable uniform film deposition and control the chemical composition of the deposited films when process parameters such as the duty cycle, pulse frequency, and output voltage are adjusted; such control is useful for improving the film properties [16]. In addition to reducing powder formation and facilitating the accurate control of reactive substances, the use of pulsed-dc power has enabled the possibility of large-scale plasma deposition [17].

In this study, plasma-polymerized hexamethyldisiloxane (HMDSO)/tetrafluoromethane (CF_4), hereafter abbreviated as pp-HC, films were deposited on polyurethane (PU) substrates by adjusting the monomer flow rate (f_H). The microstructures, mechanical properties, WCA, and in vitro myoblast cell culture were examined. Moreover, fibrinogen adsorption and platelets adhesion tests were performed for the deposited films.

2. Material and methods

In this study, the pp-HC films were prepared using a pulsed-dc plasma-enhanced chemical vapor deposition (PECVD) technique. The plasma polymerization system comprises a deposition chamber (PU substrates were placed on the lower plate (cathode)), an ENI bipolar pulsed-dc power supply connected to the upper plate (anode) for generating glow discharge plasma, a pumping system for maintaining the deposition chamber at a stable working pressure, and a saturated bottle for controlling the monomer flow rate (Fig. 1(a)). Before film deposition, the PU substrates were cleaned using oxygen plasma bombardment for 3 min at 50 W. A mixture of HMDSO/CF_4 and Ar was used as the carrier gas, and the mixture was passed into the chamber to deposit pp-HC films for 30 min at 50 W. Table 1 shows the process parameters of the plasma polymerization system, and Fig. 1(b) depicts a schematic drawing of the pp-HC films.

A field-emission scanning electron microscope (FESEM; accelerating voltage: 3.0 kV) was used to observe the cross-sectional morphology of the deposited films. A Fourier transform infrared spectrometer (FTIR; resolution: 8 cm^{-1}) operating in an attenuated total reflection mode (accumulated scanning of 16 times) was used to confirm the functional groups present in the deposited films. X-ray photoelectron spectroscopy (XPS) was used to confirm the chemical composition of the pp-HC films. A First Ten Ångstroms 1000 WCA measurement system was employed to measure the WCA of the pp-HC films deposited on the PU substrate, and the surface roughness of the deposited films was observed using an atomic force microscope. An Elcometer 107 Cross Hatch Cutter was used to determine tape adhesion according to the specifications of the standard test method ASTM D3359-02 [18]. The standard test method ASTM G171-03 [19] was applied to evaluate the adhesion of the pp-HC films. A steel wool scratch tester (Fu Chien abrasion tester) was used to evaluate the wear resistance.

Table 1
Process parameters for plasma polymerization of the pp-HC films.

Deposition parameters	Values		
Substrate temperature	Near room temperature		
Working pressure (Pa)	13.32 ± 2.67		
Pulsed frequency (Hz)	50		
T_{on}/T_{off}	1/40		
HMDSO/CF_4 flow rate (sccm)	pp-HC 30/100	50/100	80/100
Deposition time (min)	30		

A cytotoxicity test was performed according to the Biocompatibility Safety Assessment of Medical Devices (ISO-10993-5 [20]) to evaluate the cell proliferation results. A cell line, c2c12 myoblast, was obtained from American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% 0.5 mM L-glutamine, and 1% penicillin/streptomycin/neomycin and incubated in a 95% air/5% CO_2 humidified incubator at 37 °C. Myoblast cells were incubated on the specimens after 1, 3, 5, 12, and 24 h and washed with phosphate buffered saline (PBS). Fresh medium was then added to uniformly mix the WST-1 cell proliferation reagent (1:10 dilution) (WST-1 reagent from Roche) in the dark, and the solution was placed in a 37 °C incubator for 4 h. When the reaction was complete, 200 μL of the solution was moved to a 96-well plate. An automated enzyme-linked immunosorbent assay reader (λ = 450 nm) was used to measure the absorbance (optic density), which indicates cell viability.

In the human-fibrinogen adsorption test, a blank PU substrate and pp-HC films were soaked in a saturated solution of the antigen (in a fibrinogen solution with a PBS concentration of 3 μg/mL) for 2 h at 37 °C [21]. The specimen and films were then washed three times with PBS to remove the unadsorbed fibrinogen and subsequently soaked in 3.7% methanol for 1 h to fix the fibrinogen adsorbed on the specimens. Finally, after rinsing, fixation, dehydration, and ambient drying, the specimens were freeze-dried, and Au–Pd alloy was deposited before FESEM observation.

A platelet adhesion test was performed by using platelet-rich plasma (PRP). The specimens were placed on 12-well culture dishes after being soaked in PBS. The PRP was then added and the specimens were incubated at 37 °C for 90 min and 180 min. The resulting samples were washed three times with PBS to remove unattached platelets and subsequently soaked in 3.7% methanol for 30 min to fix the platelets attached to the specimens. Finally, after rinsing, fixation, dehydration, and ambient drying, the specimens were freeze-dried, and Au–Pd alloy was deposited before FESEM observation [21].

3. Results and discussion

A superhydrophobic pp-HC film was developed in this study. The surface morphology and WCA of a blank PU substrate and the deposited films obtained at monomer (HMDSO) flow rate (f_H) of