



A facile novel fluorocarbon copolymer solution coating process for improving platelet compatibility of titanium



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ARTICLE INFO

Article history:

Received 1 September 2016

Received in revised form 31 May 2017

Accepted 8 July 2017

Available online 10 July 2017

Keywords:

Titanium

Phosphonic acid

Fluorocarbon

Surface modification

Hemocompatibility

Cytotoxicity

ABSTRACT

Titanium (Ti) and its alloys possess mechanical properties that are desirable in many biomedical applications compared to other metals. Furthermore, the native metal oxide layer that prevents further oxidation is also known to be biocompatible. However, clinical findings have shown that titanium and its alloys are prone to adverse bioreactions such as platelet adhesion and activation which could lead to thrombotic complications. It has been found that surfaces modified with fluorocarbons could reduce the degree of both platelet adhesion and activation. Nevertheless, direct fluorocarbon deposition onto titanium substrates would require significant technical efforts. Instead, this research utilized a facile coating process with novel copolymers containing 2,2,2-trifluoroethyl methacrylate (TFEMA) and vinylphosphonic acid (VPA) to modify the titanium surface, giving the surface lower surface energy and higher hydrophobicity, significantly reducing the thrombus formation while exhibiting good cytocompatibility. The anchorage group, phosphonic acid provided by VPA, can be covalently bound to the oxide surface of titanium metal. *Via* free radical polymerization, VPA and TFEMA formed copolymers with different hydrophobicity were then used to modify titanium substrates, on which a series of surface characterization, *in vitro* platelet adhesion tests, and cytotoxicity assays were performed. Nuclear magnetic resonance (NMR) and X-ray photoelectron spectroscopy (XPS) confirmed the synthesis of the copolymers and the modification of Ti substrates. The platelet adhesion tests showed significantly reduced amount of adherent platelets on certain copolymer-modified Ti substrates with low degrees of activation. The *in vitro* cytotoxicity assays further highlighted that the modifications conducted on Ti does not induce cytotoxicity.

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1. Introduction

Despite the vast varieties of metals used in biomedicine, the species that can withstand the test of long-term implants are still limited. Taking properties such as low weight to strength ratio, excellent corrosion resistance, and low modulus into consideration, titanium metal and its alloys seem to be the most promising material, based on clinical applications to date. Titanium has shown favorable tissue compatibility in biomedical applications, meaning that the material does not perturb the host tissue and *vice versa*, in such a way that the implant loses its intended function. It has been demonstrated that under certain circumstances, a close apposition can be established between Ti implants and bone tissue, which enables a strong anchorage of the implant in bone

[1,2]. Upon contact with tissues, the good tissue compatibility of Ti manifests itself in the absence of a chronic inflammation because the surface oxide layer can form peroxide compounds with surrounding tissues and protect the implant from the host immune responses, and henceforth, promote osseointegration [3]. Due to these excellent characteristics, titanium is found in a number of biomedical applications, including hard tissue replacements (*i.e.* arthroplasty) in plastic and reconstructive surgeries, the screw-like cylindrical roots of dental implants, and the artificial heart valves or intravascular stents in cardiovascular surgery [4].

Even though titanium possesses good biocompatibility, it cannot perform an active role in thromboresistance as comparable to that of the endothelium. The native oxide surface on titanium is beneficial to the adhesion of many proteins, which is essential to subsequent cellular adhesion and tissue growth, but in the case of applications in blood-contacting devices, protein adsorption could induce thrombotic responses, form blood clots, and when dislodged, may give rise to complications such as heart attacks or strokes. Therefore, long-term

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implantation of blood-contacting applications often requires the prescription of antithrombotic drugs, which may cause other issues such as the impediment of the healing process of surgical wounds. In order to improve the hemocompatibility of native titanium surfaces, various surface modification techniques had been developed, such as silanized titanium oxide and self-assemble monolayers on titanium [4]. The objective in this investigation is to improve the surface characteristics of titanium while retaining the desirable bulk properties of the metal.

Due to the chemical inertness of the native titanium oxide layer on the surface, only a few organic species, such as organosilanes and organophosphates are able to form covalent bonds and are thus addressed as anchoring functional groups on titanium. These anchoring groups allow the chemical incorporation of hemocompatible and biocompatible compounds onto the Ti metal or alloy surface. However, the coverage of silanization on Ti metals is restricted by the low percentage of hydroxyl groups in the native oxide layer of Ti, and may further result in hydrolytically unstable modification films [4,5]. In addition, since the physiological environment is usually aqueous in nature, silanization may not be the optimal technique to implement. On the contrary, phosphonic acids can give strongly surface bound metal organic interfaces and can deliver surface coverages far greater than can be achieved by direct silanization methods [6–8]. The mechanism of the formation of the titanium-phosphonate bond involves the cleavage of oxide bonds Ti—O—Ti by phosphonic acid. Since the Ti—O—Ti bonds possess excellent chemical stability, even under acidic conditions, the coordination of the phosphoryl groups most probably assists the cleavage of the titanium oxide bonds [9]. Afterwards, the basic Ti—OH or the acidic Ti—O⁻ can attack the bonded phosphonate group to form bidentate or tridentate structures [10,11].

According to the research conducted by Tang et al. [12], fluorocarbon-enriched surfaces exert lower surface energies [13] and are more hydrophobic [14], displaying relatively low levels of coagulation activation [15,16] and results in substrates that are less thrombogenic with reduced protein adsorption as well as platelet adhesion and activation [17]. Furthermore, numerous researches had shown that fluorocarbons exhibit excellent cell viabilities and may even promote the proliferation of osteoblast cells [18,19]. In spite of these, to deposit a layer of fluorocarbons onto titanium substrate could require significant technical challenges and instrumentations.

Based upon these findings, in order to improve the hemocompatibility of titanium, this research designed copolymers based upon the 2,2,2-trifluoroethylmethacrylate (TFEMA) and vinylphosphonic acid (VPA) to covalently incorporate hydrophobic fluorocarbons onto the surface of titanium metals by facile solution coating process. Various ratios of TFEMA were mixed with VPA and copolymerized via free radical polymerization, which were then coated onto Ti substrates for modification. Further investigations of the hemocompatibility and cytotoxicity of the modified Ti substrates were conducted with *in vitro* platelet adhesion tests and cell viability assays.

2. Materials and methods

2.1. Materials

All chemicals were used as received unless otherwise noted. Reagent grade of 2,2,2-trifluoroethyl methacrylate (TFEMA; 99%, stabilized with 100 ppm monomethyl ether hydroquinone) and vinylphosphonic acid (VPA; 97%) were purchased from Aldrich, Germany. 2,2'-Azobis(2-methylpropionitrile) (AIBN; 98%) in reagent grade, purchased from Nihon Shiyaku Co., Japan, was purified by recrystallization from methanol prior to use. Dimethylformamide (DMF) (99.8% purity) was purchased from MACRON, USA. The solvents used for further purification of the polymer product were acetic acid ($\geq 99.8\%$), anhydrous methyl alcohol (MeOH; 99.9%), and tetrahydrofuran (THF; 99.9%, stabilized with 250 ppm butylated hydroxytoluene), which were supplied from Sigma-

Aldrich, Germany, MACRON, USA, and Duksan Pure Chemicals Co., Ltd., Korea respectively.

Titanium substrates were prepared from 0.2 mm thick sheets of grade 1 unalloyed Ti bought from AcrUshin Co., Japan. For the pretreatment of Ti, hydrogen peroxide (30%) and sulfuric acid (95.0–98.0%) were purchased from Sigma-Aldrich, Germany and J.T. Baker, USA respectively. Human platelet rich plasma (PRP) was supplied by Tainan Blood Center, Taiwan.

2.2. Synthesis of poly(VPA-co-TFEMA) P_xF_y copolymer

Before synthesis, the monomers VPA and TFEMA and the initiator AIBN (5 mM) were dissolved in dry DMF (25 ml) and purged with nitrogen gas for 1 h. The solution then underwent a free-radical polymerization for 24 h at 65 °C. The copolymerization was terminated by precipitating in methanol-water mixture (4:1 v/v) except for homopolymer of VPA, which is by acetic acid. The precipitates were then collected by centrifugation, and dried overnight under vacuum at room temperature, resulting in white polymer powders. The polymers were characterized by nuclear magnetic resonance (NMR; AV-500, Bruker, Switzerland), Fourier transform infrared spectroscopy (FTIR; 640 IR, Varian, USA), and elemental analysis (EA; Elementar vario EL III, ELEMENTAR Co., German). Overall, a total of two homopolymers (polyVPA and polyTFEMA) and three copolymers were prepared for this study. The molar feed ratios of VPA:TFEMA for these three copolymers were 4:6, 3:7, and 2:8 respectively, with a total monomer concentration of 0.5 M. The copolymers were designated as P_xF_y, with x to y being the stoichiometric ratio of VPA to TFEMA in the feed. For the homopolymer prepared from VPA and TFEMA, the polymer was named as HomoP and HomoF, respectively.

2.3. Preparation of Ti substrate

Commercially pure titanium sheets were tailored into 1 × 1 cm² square pieces. The substrates were sequentially sonicated with ethyl acetate, acetone, neutral cleaner, and water for 15 min each and then dried in a stream of nitrogen. Before coating, the Ti substrates were etched with a piranha solution at room temperature for cleaning. For the coating of HomoP, the Ti samples were etched for 1 h with a 20:80 (v/v) mixture of hydrogen peroxide and concentrated sulfuric acid, and for the coating of all the other polymers, the Ti samples were etched for 5 min using a 30:70 (v/v) mixture of hydrogen peroxide and concentrated sulfuric acid. The oxidized Ti substrates were then rinsed with distilled water and dried in a stream of nitrogen.

2.4. Surface modification of Ti substrate with different polymers

The polymers were made into 30 wt% solutions, with HomoP dissolved in water and the other polymers dissolved in acetone. 40 μL of the polymers solutions were dropped onto the titanium substrates, dried at room temperature, and then gradually heated in an oven to 142 °C for 24 h. The substrates were then rinsed with water and acetone to remove unreacted or physisorbed polymers and afterwards dried in a stream of nitrogen.

2.5. Surface characterization

To determine the hydrophobicity of the modified sample surface, static contact angle measurements (CA; Model 100SB, Sindatek, Taiwan) were performed at room temperature using the sessile drop method with deionized water droplets. The average CA value was obtained from measurements at five different points on the same sample and 3 samples for each polymer species ($n = 3, m = 5$).

For the surface composition analysis of the coated titanium substrates, the samples were examined by X-ray photoelectron spectroscopy (XPS; PHI 5000 VersaProbe II, ULVAC-PHI, USA) for elements C 1s, O

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