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Oxygen plasma modified P(3HB-4HB) used as anticoagulant materials

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ABSTRACT

In this article, we report the modification of P(3HB-4HB) film with oxygen plasma. The results showed that both the chemical components and topography of the P(3HB-4HB) film changed after oxygen plasma modification. In addition, the decrease of contact angle and the increase of the surface oxygen content were observed. The results of BSA adsorption onto those films studied by QCM-D showed that the plasma treatment could improve the protein-resistant activity of the film. After 10-minute plasma treatment, the BSA-resistant activity of the film improved 27% in PBS buffer solution and 57.5% in aqueous solution. Platelet adhesion test showed that the platelet-resistant activity of the film improved 68.6%, 82.3%, 96.8% after treated for 2, 5 and 10 min, respectively. Also, the cck8 assay of L929 cells showed that there was no cytotoxicity for the sample treated with oxygen plasma. This film has the potential to be used as anticoagulant materials, which required high protein-resistant activity.

1. Introduction

As an important branch of the biological materials, anticoagulant materials has been widely used in tissues and organs contacted with blood, such as blood dialysis system, extracorporeal circulation system, artificial heart valve, heart pacemakers, artificial blood vessels, stents, operation suture, catheter and so on [1–5]. It should not only be biocompatible to avoid inflammation, but also have the ability to prevent the formation of blood clots.

Blood coagulation process is very complex and it involves the protein and platelet participation [6–10]. For anticoagulation film, platelet adhesion is an important symbol of the blood compatibility because that the adhesion of platelet onto biomaterials promotes surface-induced thrombosis. In addition, it is widely accepted that the interactions with the plasma proteins will occur when a biomaterial comes in contact with blood. The protein adsorption layer will induce the platelet adhesion onto the surface of the biomaterials. Thus, anticoagulation film is expected to be protein resistant. As reported, there are many factors affecting the protein adsorption and platelet adhesion, such as wettability, charge, morphology and so on [11,12], while hydrophilic and negative electricity surface is not benefit for protein adsorption and platelet adsorption.

PHB is biopolyester produced by microorganisms under unbalanced growth conditions [13]. It has been exploited various applications [14,15], and it could be suitable as targeting implant for tissue engineering as its excellent biodegradability, biocompatibility and adjustable mechanical properties [16–18]. But it was limited to be used as anticoagulation materials because it activate the coagulation system in contact with blood [19].

As mentioned before, blood coagulation process is very complex and it involves the protein and platelet participation. One way to prevent blood coagulation is to improve protein-resistant and platelet-resistant activity of the material. Recently, many methods have been used to improve the protein-resistant and platelet-resistant activity, such as blending, surface grafting and endothelialization [1,3–5,12]. These methods could improve anticoagulation, but they need a complicated process and a long treatment time. So how to improve the anticoagulation activity of the PHB with a simple method is of great interesting.

Low temperature plasma treatment is one of the favorable surface-modification methods. It was widely used as an intermediate step to graft functional molecular onto polymer material to meet particular demand without changing the substrate [20–23]. In this study, we only use this simple method to modify the surface of P(3HB-4HB) because it could not only improve the surface hydrophilicity, but also change the charge of film surface, as the carboxyl group could be introduced to the film surface [24]. The results show that after treatment, the adsorption of model protein BSA onto the film could reduce evidently by 27.0% in PBS and 57.5% in aqueous. The platelet resistant activity of the film improved 31.4%, 17.7%, 3.2% for 2, 5, 10 min treated sample, respectively. The cck8

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assay also showed that there was no cytotoxicity for the film treated with oxygen plasma. It could be a simple method to modify the P(3HB-4HB) as anticoagulant materials.

2. Materials and methods

2.1. Preparation of P(3HB-4HB) substrates

P(3HB-4HB) powder (Shenzhen Ecomann Biological Technology Co. Ltd., China) had been purified by solvent re-precipitated with chloroform. The polymer film was obtained by spin-coating of 0.5% (w/v) solutions in the solvent (chloroform:tetrahydrofuran = 90:10) on silicon wafers and Quartz Crystals(QSX301, Q-Sense, Sweden).

2.2. Plasma modification

Oxygen plasma treatment was carried out in the DL-01 model plasma generator (Suzhou Omega Machinery Electronic Technology Co. Ltd., China) with power of 50 W, and duration of 1, 2, 5, 10 min.

2.3. Contact angle and surface free energy

Static contact angles were characterized with a contact angle goniometer (OCA15, DADAPHYSICS, England) at 25 °C using distilled water and diodomethane as reference liquid. A total of 1.00 μL of reference liquid was pumped onto the surface of the P(3HB-4HB) through a stainless steel needle at a rate of 1.0 μL/s. Results are mean values of five independent measurements on different points of the films studied. The surface energy was calculated by the Owens–Wendt–Rabel–Kaelble (WORK) method.

$$\gamma_{ls} = \gamma_{gs} + \gamma_{gl} - 2 \cdot \left(\sqrt{\gamma_{gl}^{d} \cdot \gamma_{gs}^{d}} + \sqrt{\gamma_{gl}^{p} \cdot \gamma_{gs}^{p}} \right) \quad (1)$$

where γ^d represents the dispersive components; γ^p is the polar components. γ_{gs} represents the solid surface energy; γ_{gl} represents the liquid surface tension; γ_{ls} represents the solid and liquid interfacial tensions.

2.4. X-ray photoelectron spectroscopy (XPS)

The Quartz Crystals coated polymer surface atomic ratio of oxygen/carbon was measured on a Kratos AXis Ultra (DLD) (England) operated using Al Kα (1486.4 eV) monochromatic X-ray source at pressure of 2×10^{-9} Torr and a scan area of 0.7 mm × 0.3 mm. Analyses consisted of a survey scan performed at a pass energy of 160 eV to identify all the species present, followed by high resolutions scans (40 eV) of the species of interest. Sample charging effects on the measured bounding energy (BE) positions were corrected by setting the lowest BE component of the C 1s spectral envelope to 284.6 eV, corresponding to the C—C/C—H species. The generated data were converted to TXT format and processed using XPSPEAK 41 software.

2.5. Atomic force microscope (AFM) analysis

The MFP-3D-S atomic force microscope (Asylum Research, America) was used to examine the changes in surface topography due to the oxygen plasma treatment. AFM imaging was performed under dry conditions at room temperature (24 ± 2 °C). Samples were analyzed over a 2.0 × 2.0 μm region at a resolution of 512 × 512 pixels.

Root mean square roughness (Rq or RMS) values were determined from height retrace image of each sample.

2.6. Electro kinetic measurements

The zeta potential ξ as a function of the pH of PHA polymers was obtained using an electro kinetic analyzer (Anton Paar Surpass, Austria). For the determination of ξ, streaming current measurements were performed using the Adjustable Gap Cell (Anton Paar Surpass, Austria). A rectangular streaming channel (length 20 mm, width 10 mm, height 100 μm) formed by a pair of P(3HB-4HB) films coated on silicon plate at room temperature, and the surface of polymer is facing each other. 1.0 mM potassium chloride (KCl) solution was used as the background electrolyte, 0.1 M potassium hydroxide and 0.1 M hydrochloric acid solutions were used to adjust pH in the range pH 3–10. The zeta potential ξ is calculated according to:

$$\varsigma = \frac{dI}{dP} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times \frac{L}{A} \quad (2)$$

where dI/dP represents the slope of streaming current versus differential pressure, η is electrolyte viscosity, ε represents dielectric coefficient of electrolyte, ε_0 is permittivity, and L/A is the ratio of length L and cross section A of the streaming channel.

2.7. Bovine serum albumin adsorption

The adsorption of bovine serum albumin (BSA) on P(3HB-4HB) film was measured by Q-Sense E4 system (Q-Sense AB, Sweden). Quartz crystals (QSX 301, Q-Sense AB, Sweden) were spin coated with P(3HB-4HB). BSA was purchased from Sigma–Aldrich company (Germany). The BSA was dissolved in PBS buffer (pH 7.2) at a concentration of 50 μg/ml. In this measurement, Milli-Q water was injected into the QCM chamber where the P(3HB-4HB) coated resonator was mounted to obtain a baseline before injection of BSA solutions after PBS buffer. PBS buffer was introduced in the QCM cell again to wash off the non-adsorbed protein molecules. At last, Mili-Q water was injected into the QCM chamber after PBS buffer injecting 10 min. The measurements were performed in continuous flow mode (50 μl/min) under a stable temperature (26 ± 0.1 °C), the total cell volume is 100 μl. The shifts both of frequency (ΔF) and energy dissipation (ΔD) were monitored at the same time. ΔF related to changes in mass on the sensor, this mass is the total mass, including hydrodynamic ally coupled water, water associated with the hydration layer of proteins and water trapped in cavities in the film [25]. ΔD related to changes in the structure and viscoelastic property of adsorbed layer.

The adsorbed layer mass (Δm) and viscoelastic properties were calculated using Q-Tools (Q-Sense AB, Sweden) software. It is recommended to use Sauerbrey relation to calculate the adsorbed mass per area, especially for the rigid adsorbed layer:

$$\Delta m = -C \frac{\Delta f}{n}, \quad (3)$$

where C is a constant of the crystal sensor, describes the sensitivity of the device to changes in mass, and n is overtone number of F (in the present case, $n = 1, 3, \ldots$). For most protein and hydrophilic polymer layer, great amount of water was contained in those layers, and the film is soft. In this situation, Voight–Kelvin model was recommended to describe the mass and viscoelastic properties of adsorbed layer. The viscoelastic properties were inferred from D, which is defined as the ratio of the dissipated and stored energy during crystal oscillation:

$$D = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}}, \quad (4)$$

In this paper, the mass results calculated by voigt model, only the modeling mass were shown.