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Fabrication and biocompatibility investigation of TiO_2 films on the polymer substrates obtained via a novel and versatile route

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ABSTRACT

Titanium oxide (TiO_2) films were successfully deposited onto the polymer substrates of polytetrafluoroethylene (PTFE), polyethylene (PE), and polyethylene terephthalate (PET), which were pre-modified with polydopamine coating (polydopamine and its coating are coded as PDA and PDAc, respectively), by a simple liquid phase deposition (LPD) process. The morphology and chemical state of the obtained TiO_2 films were characterized by field emission scanning electron microscope (FE-SEM) and X-ray photoelectron spectroscopy (XPS), respectively. Subsequently, the biocompatibility of the samples was investigated by 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay and acridine orange staining of MC-3T3 osteoblast cells, and the results demonstrated that the fabricated TiO_2 films could markedly improve the in vitro cytocompatibility. So, the presented route is anticipated to be a promising surface modification methodology to improve the practical outcome of the implanted materials for its versatility and validity.

1. Introduction

Recently, some inactive polymers with different physicochemical properties such as chemical composition, surface hydrophobicity and reactivity, typically, polytetrafluoroethylene (PTFE), polyethylene (PE), and polyethylene terephthalate (PET), are widely employed as the clinical implanted materials [1–3] for their desirable mechanical and thermodynamic characteristics [4–6]. However, the practical outcome of these materials is greatly restricted by the poor tissue-material interactions after implantation owing to their inert and hydrophobic surfaces. So, the surface modification for improving the biocompatibility of the polymer substrates is quite necessary [6,7]. One of the most promising modification procedures is to construct bioceramic coatings, especially, titanium oxide (TiO_2) films on the implanted materials since surface TiO_2 films can tailor the biological reaction and interaction between artificial and living matter [8,9]. However, it was very difficult to construct bioceramic coatings on the inactive polymers (e.g. PTFE and PE, etc.) surfaces unless special large-scale apparatus [10] or plasma pretreatment [11] was applied.

In this work, a novel and versatile route of fabricating adherent and uniform TiO_2 films on different polymer substrates have been realized by a simple liquid phase deposition (LPD) process for the first time. Our inspiration profits from a magic compound of dopamine, a functional molecule containing catechol and amine groups, and both of which may be crucial for the high adhesive property of *Mytilus edulis* foot protein 5 (Mefp-5) [12]. It has been demonstrated that this kind of simple structure is a powerful building block for spontaneous deposition of thin polymer coatings on almost all material surfaces. Moreover, the active groups of $-NH_2$ and –OH on the formed coating surfaces can facilitate the further modification [12]. Here, three polymer substrates of PTFE, PE, and PET were respectively immersed into a dilute dopamine aqueous solution and then the spontaneously formed thin polymer film was anticipated to provide a versatile platform for fabricating TiO_2 films through a relative simple route, as schematically shown in Fig. 1(a).

2. Materials and methods

2.1. Materials

3-Hydroxytyramine hydrochloride (dopamine hydrochloride) and tris(hydroxymethyl) aminomethane (Tris) were purchased from Acros Organics. Ammonium fluotitanate ($(NH_4)_2TiF_6$) was purchased from Shanghai SSS Reagent Co., Ltd. Boric acid

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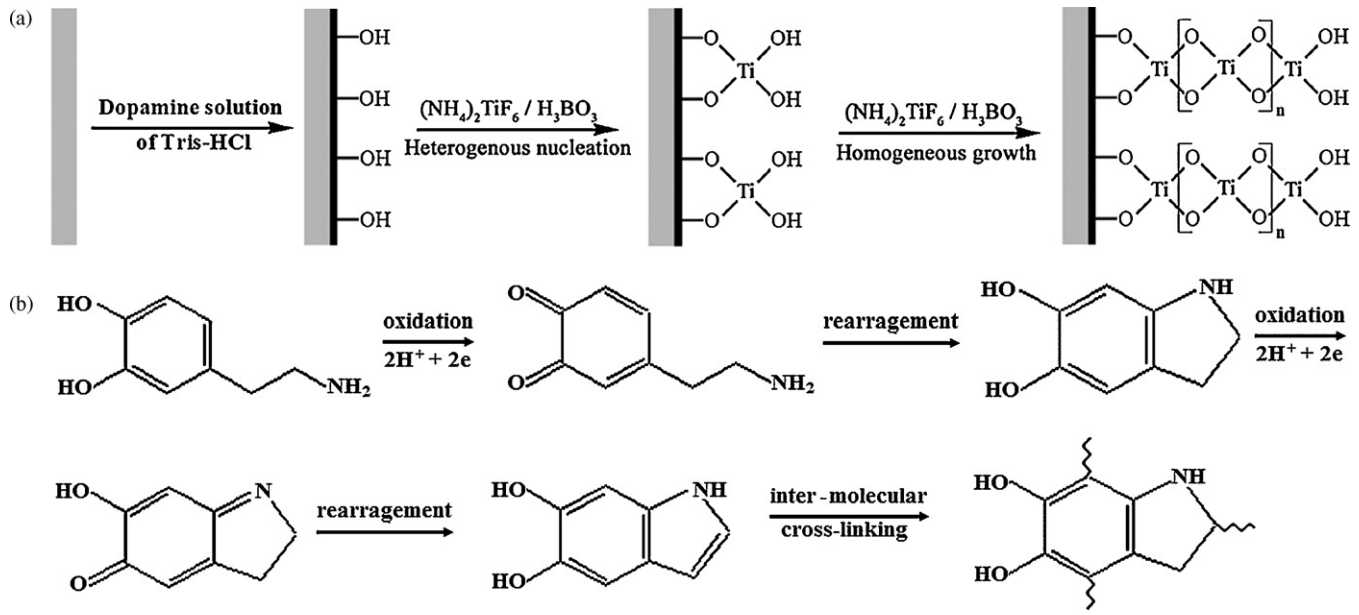

Fig. 1. (a) A schematic view of the TiO_2 deposition on the polymer substrates mediated by PDAc. (b) The "oxidation–polymerization" mechanism for the formation of PDA proposed by Messersmith and co-workers [12].

was obtained from Beijing Xinguang Chemical Reagent Factory. Dimethyl sulfoxide (DMSO) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco. Trypsin–EDTA solution and 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were supplied by Sigma. Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. MC-3T3 osteoblast cells were supplied by the Fourth Military Medical University.

2.2. Preparation of TiO_2 films on the polymer substrates

PET, PE, and PTFE plates were used as the substrates and cleaned thoroughly in acetone and water by ultrasonication (59 kHz, 45 W) in succession, followed by drying in ambient conditions. Then, the pretreated substrates were dipped into a 9 mM dopamine Tris–HCl solution (pH 8.5) for 12 h, followed by ultrasonication in ultra-pure water and blowing dry with N_2 [10]. The PDA coated substrates were immersed into a fresh prepared aqueous solution containing 0.1 M $(NH_4)_2TiF_6$ and 0.3 M H_3BO_3 with a pH value of 3.88 at room temperature for 12 h, and then ultrasonically cleaned in water and blown dry with N_2. The prepared samples were coded as PET (or PE, PTFE)–PDAc–TiO_2.

2.3. Characterizations

Contact angle meter (DSA100, Krüss, Germany) was used to measure the static water contact angle of the prepared samples. An ellipsometer (L116-E, Gaertner, USA) equipped with a He–Ne laser (632.8 nm) set was adopted to measure the film thickness. X-ray photoelectron spectroscopy (XPS, PHI-5702, Physical Electronics, USA) was performed using a monochromated Al–Kα irradiation to determine the chemical state of certain elements with the C 1s binding energy of 284.8 eV as the reference. The morphologies of the pristine and TiO_2 film coated polymer plates were observed by a field emission scanning electron microscope (FE-SEM, JEOL, JSM-6701F, Japan).

2.4. Cell culture

MC-3T3 osteoblast cells were employed to evaluate the biocompatibility of the pristine and TiO_2 film coated polymer plates. The cells were routinely cultured in DMEM containing 10% FBS and incubated at 37 °C in a humidified chamber with 10% CO_2. The culture medium was refreshed every 2 days. When the cells became almost confluent, they were released by treating with 0.25% trypsin–EDTA solution for 3 min at 37 °C and resuspended in the DMEM with a final concentration of 10^4 cells/ml.

2.5. In vitro cytocompatibility assay

In vitro cytocompatibility was investigated by MTT colorimetric assay. Briefly, the sterilized samples were placed into a 24-well culture plate and cells were seeded for 2 h, 4 h, 2 days, and 4 days, respectively. At the prescribed time point, 100 μl of MTT phosphate buffer solution (PBS) (5 mg/ml, pH 7.4) was injected into each well containing the test sample and culture medium. The cultures were then kept at 37 °C for 4 h. At the end of incubation, the supernatant was removed and 1 ml of DMSO was added into each well. The 24-well plate was then shaken to dissolve the purple formazan crystals and the optical density (OD) value of the resulting solution was recorded using a micro-plate reader (Model 550, BIO-RAD, Japan) at the wavelength of 490 nm. The assay was conducted in triplicate for each sample and three parallel experiments were performed.

To calibrate the cellular survival rate, blank and control groups were set. In the blank group, only culture media was added into the well. While, in the control group, cells and culture media without polymer substrates were added. The blank and control groups were treated with the same procedures and incubated for the same time as those in the experimental group. The measured OD values of the blank, control, and experimental groups were coded as OD_{bla}, OD_{con}, and OD_{exp}, respectively. Finally, the cellular survival rate was calculated by the following equation:

$$\text{survival rate} = \frac{OD_{exp} - OD_{bla}}{OD_{con} - OD_{bla}}.$$

2.6. Acridine orange staining

MC-3T3 osteoblast cells cultured on the pristine and TiO_2 film coated polymer plates were visualized by fluorescent acridine orange staining. After being seeded for 2 days, all samples were