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Fabrication of tantalum oxide layers onto titanium substrates for improved corrosion resistance and cytocompatibility

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ABSTRACT

Due to its good biocompatibility and chemical stability, tantalum attracts much attention for biomedical applications, however, limited by its relatively high cost. In this study, tantalum oxide layers were deposited onto titanium (Ti) substrates with polymer-assisted deposition (PAD) technique to improve their corrosion resistance and cytocompatibility. Scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), scratch tests and water contact angle measurement proved the successful formation of tantalum oxide layers onto the surfaces of Ti substrates. The potentiodynamic polarization curves revealed that the formed tantalum oxide layers improved the corrosion resistance of Ti substrates. Moreover, the *in vitro* tests displayed that Ti substrates coated with 6 layers of tantalum oxide promoted proliferation, alkaline phosphatase activity, mineralization and osteogenic gene expressions (ALP, Col I, OC and OPN) of osteoblasts, respectively. The study affords an approach for the fabrication of Ti-based implant with improved corrosion resistance and cytocompatibility.

1. Introduction

Biomaterials are widely used in biomedical fields, which could be divided into three categories from their material properties: polymeric materials, ceramic biomaterials, and metallic materials. Polymeric materials (such as hydrogel) were widely used for soft tissue applications mainly due to their degradability and relatively low mechanical strength [1–3]. Ceramic materials (including hydroxyapatite) were generally used as filling materials for bone tissue repair, due to their good bioactivity and mechanical property [4]. Nevertheless, the inherent fragility limited their specific applications, such as long bone replacement. In contrast, metallic materials, such as titanium and its alloys, had good biocompatibility and bulk mechanical properties, which were widely used in orthopedic field for manufacturing load-bearing devices (*e.g.*, hip joint and spine) [5,6]. As for a titanium-based implant, potential corrosion would occur during its long-term implantation, resulting from mechanical damage or chemical etching under physiological environment. Moreover, such corrosion would lead to ion release and wear debris, in turn inducing inflammatory reactions at the surrounding tissues, even ultimately resulting in implant loosening and failure [7]. To resolve the issue above, it is urgent to improve the corrosion resistance of Ti-based implants. Previous studies confirmed that deposition a corrosion resistant layer onto the surfaces of Ti-based implants was efficient for improving their corrosion resistance [8,9]. Moreover, considering the weak interactions between bone and Ti implants owing to their bio-inertness, the deposited film is expected to be biocompatible for inducing desired biological responses.

Tantalum (Ta) is well known for its excellent corrosion resistance and biocompatibility [10–12], which is a promising material to be used in orthopedics field. Although tantalum was used to fabricate plates, suture wires and scaffolds [13,14], the high density and cost consuming of tantalum implants limit their applications in a form of bulk material, in particular of large-sized medical devices, such as a hip joint. Moreover, the high melting temperature of bulk tantalum material is another issue to be considered for the fabrication of a medical device [15,16]. Previous studies proved that porous tantalum implants had many advantages for orthopedic applications, such as low modulus, high surface friction, and desired osseointegration [17,18]. Thus, to create tantalum oxide layers onto the surfaces of Ti substrates is reasonable to produce a high quality Ti based implant.

Previously, various strategies had been developed for the fabrication of tantalum oxide layer onto a substrate, including radio frequency sputtering [19], liquid phase deposition [20], electrochemical deposition [21], laser processing [22] and sol–gel technique [23]. Although sol–gel technique is easily to be handled in practice, the organometallic precursors (*e.g.*, tantalum butoxide and titanium ethoxide) used in sol–gel process are expensive and unstable [24,25]. As for other methods, they all require specific instruments. Thus, we selected polymer assisted deposition (PAD) technique in this study to generate tantalum oxide layers onto Ti substrates with a relatively cheap precursor of tantalum chloride. To the best our knowledge, it is the first study to apply PAD for the fabrication of tantalum oxide layers on Ti substrates. Generally,

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the PAD technique is based on deposition of a mixture solution composed of soluble polymer and metal precursor onto a substrate, and then annealing at high temperature under oxygen environment to get rid of organics [26]. It thus forms a metal oxide layer onto the substrate. The polymer serves as a capsule of metal ions, protecting metal ions from un-desired chemical reactions. Previous studies confirmed that such mixture solution could be kept stable for several months [27,28].

The objective of this study was to deposit tantalum oxide layers onto titanium substrates with PAD technique and to investigate on the effect of the coating on the proliferation and differentiation of osteoblast both on cellular and molecular levels.

2. Materials and methods

2.1. Materials

Titanium (Ti) disks (diameter: 15 mm; thickness: 3 mm) were obtained from Northwest Institute for Non-ferrous Metal Research, China. $TaCl_5$ (99.9%) polyethylenimine (PEI, MW 10 kD, 99%) and Hoechst 33258 were supplied by Sigma Chemical Co. (MO, USA). BCA kit, cell counting kit-8 (CCK-8) and alkaline phosphatase (ALP) assay kit were purchased from Beyotime Co. (Beijing, China). Rhodamine-phalloidin was obtained from Invitrogen Co. (USA). Other chemicals were purchased from Oriental Chemical Co. (Chongqing, China).

2.2. Precursor solution preparation

The precursor solution was prepared by dissolving 0.25 g of $TaCl_5$ into 5 mL water containing 20% (v/v) hydrogen peroxide. The mixture solution was stirred for 5 min, resulting in a turbid solution. Next, 0.6 g of citrate was added and stirred for another 30 min to obtain an apparent solution. Subsequently, 0.6 g of PEI was added to the mixture solution, resulting in a brown solution. The final concentration of Ta was adjusted to be 0.12 mmol/mL.

2.3. Fabrication of tantalum oxide coating

Titanium disks were mechanically polished with a series of silicon carbide papers. The polished Ti substrates were then rinsed with acetone, absolute ethanol and distilled water each for 10 min under ultrasonication, respectively. A single layer was fabricated as follows. Briefly, 50 μL precursor solution was dropped onto a Ti substrate and kept at room temperature for 30 s. The Ta chelating polymer layer was formed onto Ti substrate with spin-coating at 3000 rpm for 40 s. Next, the sample was calcinated with a muffle oven under oxygen environment at 600 °C for 2 h. To obtain thick tantalum oxide film, a multi-layered coating was fabricated by repeating the process of a single layer coating above. In this study, the Ti substrates coated with 2 and 6 layers of tantalum oxide were denoted as Ta-2L and Ta-6L, respectively.

2.4. Surface characterization

Surface morphologies and roughness of native Ti and Ta coated ones were observed with scanning electron microscopy (SEM; FEI Nova Nano SEM, Phillips Co., Holland) and atomic force microscopy (AFM; Dimension, Bruker, Germany), respectively. A scratch test was performed to evaluate the adhesion strength of tantalum oxide layer using a scratch tester (CSM Instruments, Switzerland). The progressive load was applied from 1 N to 100 N along 5 mm length on a Ta-6L substrate. The surface chemistry of different samples was characterized with XPS (Model PHI 5400, Perkin Elmer, USA). The crystal phases of specimens were characterized by X-ray diffraction (XRD; D/Max 2500PC, Rigaku, Japan). The water contact angles of different samples were determined by a video based contact angle goniometer (model 200, Future Scientific Tai Wan, China). The potentiodynamic polarization curves were measured with an electrochemical workstation (PGSTAT30, Eco CHEMIE BV, Holland) in a simulated body fluid (SBF) at 37 °C. A 1000 mL volume of SBF was composed of following components: 0.293 g $CaCl_2$, 5.403 g NaCl, 0.225 g KCl, 0.504 g $NaHCO_3$, 0.426 g Na_2CO_3, 0.23 g $K_2HPO_4 \cdot 3H_2O$, 0.311 g $MgCl_2 \cdot 6H_2O$, 0.072 g Na_2SO_4 and 17.892 g HEPES ($C_8H_{18}N_2O_4S$). A saturated calomel electrode (SCE) and a platinum foil were employed as a reference electrode and a counter electrode. The treated and untreated Ti samples were used as working electrodes, respectively.

2.5. Protein adsorption

Bovine serum albumin (BSA) was used for the protein adsorption assay in this study. Briefly, 200 μL of BSA (1 mg/mL in PBS) was pipetted onto the native titanium (Ti) and tantalum oxide-coated titanium samples in a 24-well tissue culture polystyrene (TCPS) plate. The plate was then placed in a sterile humidified incubator at 37 °C for 15 and 60 min, respectively. Empty wells of the TCPS plate were used as background reference. The nonadherent protein was removed by using PBS washing for 3 times. Then, 200 μL of 2% sodium dodecyl sulfate (SDS, Sigma) was added to each well and the plate was subsequently incubated at 37 °C with shaking for 1 h to extract adhered protein. Protein concentration was determined with a Bicinchoninic Acid (BCA, Sigma) assay kit using a microplate reader (Bio-Rad 680) at a wavelength of 570 nm. The adsorbed protein amount was determined by subtracting the amount of proteins adsorbed onto corresponding wells (native titanium, tantalum oxide-coated titanium samples and TCPS, respectively).

2.6. Cell culture

Osteoblasts obtained from neonatal rat's calvaria were cultured in high-glucose Dulbecco's Modified Eagle's Medium supplement with 10% bovine serum (FBS, Gibco) [29]. Cells were incubated at 37 °C under 5% CO_2 atmosphere. The medium was refreshed every 48 h. When reaching confluence, cells were detached with 0.25% trypsin in 1 mM tetrasodium EDTA. The 3rd passage of osteoblast was used for the following experiments.

2.7. Cell viability

Cell counting kit-8 (CCK-8) was employed to measure cell viability. Osteoblasts were cultured onto tissue culture polystyrene (TCPS) plates, native Ti, Ta-2L and Ta-6L substrates at an initial seeding density of 2×10^4 cells/well in a 24-well plate. After culture for 4 and 7 days, 200 μL of fresh medium and 20 μL of CCK-8 solution were added to each well. The plates were then incubated at 37 °C for another 1.5 h. The optical density of the solution was measured with a spectrophotometric microplate reader (Bio-Rad 680, USA) at a wavelength of 450 nm.

2.8. Cell morphology

Osteoblasts were cultured onto native Ti, Ta-2L and Ta-6L substrates at an initial seeding density of 8×10^3 cells/well in a 24-well plate. After culture for 2 days, osteoblasts were washed twice with PBS followed by fixing with 4% glutaraldehyde at 4 °C for 4 h. Cells were then treated with gradient ethanol solution (15%, 30%,45%, 60%, 75%, 90% and 100%, each for 10 min). Finally, ethanol was substituted with isoamylacetate at room temperature for 5 min. After freeze drying, the samples were coated with Au for SEM imaging.

For fluorescent observation, samples were permeabilized with 0.1% Triton X-100 at 4 °C for 10 min and then washed with PBS for 3 times. Next, to block the non-specific binding sites, the treated samples were incubated with 10% bovine serum albumin (BSA) at 37 °C for 30 min. Subsequently, 200 μL of goat monoclonal antibody against vinculin