Surface & Coatings Technology 259 (2014) 193–198

Contents lists available at ScienceDirect

Surface & Coatings Technology

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

# Antibacterial properties and cytocompatibility of tantalum oxide coatings

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ARTICLE INFO

Available online 5 April 2014

*Keywords:*
Tantalum oxide
Biocompatibility
Antibacterial
Human skin fibroblast cells
*S. aureus*
*A. actinomycetemcomitans*

ABSTRACT

The surface characteristics of biomaterials play an important role in their interaction with biological systems. Tantalum (Ta) oxides and their coatings have been proved to increase their applications in the biomedical fields such as medical devices and dental implants by improving their osseointegration and corrosion resistance. In this study, Ta and amorphous tantalum oxide coatings were deposited by using magnetron sputtering. A hydrophilic crystalline β-$Ta_2O_5$ coating was obtained by rapid thermal annealing of the deposited tantalum oxide coating at 700 °C. In this study, *Staphylococcus aureus* and *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) are Gram-positive and Gram-negative bacteria, respectively, that exhibit physiological commensalism on the human skin and oral areas. Both bacteria were chosen as the model for in vitro anti-bacterial analyses by a fluorescence staining method employing Syto9. The cytocompatibility of human skin fibroblast cells (CCD-966SK) on the coatings was also determined using an MTT assay. It showed that the amorphous tantalum oxide coatings possessed good antibacterial performance, while the hydrophilic crystalline tantalum oxide coatings exhibited outperformed human skin fibroblast cellular biocompatibility.



## 1. Introduction

Biodegradation and infection become increasingly important in longer term applications such as central venous catheters and permanently implanted devices. Titanium (Ti) and its alloys have been used widely as metallic biomaterials in the fields of artificial orthopedic and dental implants. The favorable biomechanical and biocompatible properties of these materials have made them popular among presently available biomaterials [1,2]. The excellent biocompatibility of Ti has been attributed to the presence of a natural or artificial surface layer of titanium dioxide ($TiO_2$) [3,4]. Even though Ti has excellent biomechanical properties, the high incidence of long-term failures of Ti-based hip implants (i.e., after several years) remains unresolved. This has been attributed to insufficient early osseointegration and the presence of corrosion wear particles at later stages. Various techniques of Ti surface modification have been employed over the past decades for fabricating implant surfaces that promote osseointegration, a faster healing time, a higher bone-to-implant contact ratio, and the longevity of Ti implants. Therefore, the modification of the Ti surface by coatings with antibacterial and biocompatible elements is necessary to improve clinical treatments.

Tantalum (Ta) has become a promising metal for biomedical implants or implant coatings for the orthopedic and dental applications in recent years due to its excellent corrosion resistance, fracture toughness, and biocompatibility, and has been applied to different orthopedic implants [5–8]. Previous studies [9,10] show that Ta metal is good for the osteogenesis in animal implantation tests, and suitable for cell adhesion, proliferation and differentiation in-vitro studies. Besides, Ta has been used to make stents and artificial heart valves for cardiac and vascular devices due to its higher corrosion resistance and radio-opacity property. The biocompatibility and hemocompatibility of Ta have been identified and widely applied to various biomedical materials and devices [11,12]. Similar to titanium, Ta is highly unreactive and biocompatible in the body. Ta does not exhibit toxicity to surrounding cells, nor does it inhibit local cell growth of surrounding bone. Ta composites showed different material characteristics depending on the surface modification techniques and the chemical bonding structures. A natural thin layer of tantalum oxide, which existed on the surface of Ta metal, might played a key role on its biocompatibilities. [13–15].

Previous studies [16,17] show that bioactive Ta coatings improve the in vitro biocompatibility of the Co–Cr and Ti–Ni alloys, which is assessed in terms of the attachment, proliferation and differentiation of pre-osteoblasts (MC3T3-E1) and platelets. Our previous study [18] show that a tantalum-nitrogen (TaN) coating with a hydrophobic surface (contact angle = 92.0 ± 1.2°, mean ± SD) exerts antibacterial effects against *Staphylococcus aureus*. Inorganic compound tantalum oxide is radio-opaque, chemically stable, thermally stable, and possesses good cytocompatibility, with better corrosion resistance than untreated Ti surfaces [19,20]. Modifying the tantalum oxide

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http://dx.doi.org/10.1016/j.surfcoat.2014.03.061
0257-8972/

film to form a bioactive surface is critical for clinical applications. *S. aureus* and *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) are Gram-positive and Gram-negative bacteria, respectively, that exhibit physiological commensalism on the human skin, nares, and mucosal surfaces. Both bacteria are chosen as the model for the present in vitro study [21]. To verify the cytocompability of the tantalum oxide coatings, the cell viability of human skin fibroblast cells (CCD-966SK) cultured on the deposited samples is also investigated.

## 2. Experimental details

Pure-Ti plate samples [15 mm × 15 mm × 1 mm, surface roughness (Ra) = 0.2 μm, biograde 2; Uniti Titanium, Moon Township, PA, USA] were used as control samples. Tantalum and tantalum oxide coatings were deposited by using pulsed unbalanced magnetron sputtering with high-purity Ta targets (99.99 at.%). The target and substrate were separated by 70 mm, and the samples were placed on a rotational substrate holder for the deposition. A base pressure prior to deposition was less than $1 \times 10^{-3}$ Pa. Before deposition, the substrates were etched for 20 min at a substrate bias potential of −800 V in argon (Ar) plasma. To enhance film adhesion, Ta ion bombardment (Ta cathode power = 50 W and bias voltage = −600 V) was applied before the deposition. After etching, the Ta coating was deposited with the Ta cathode power = 200 W, and a bias voltage of −80 V was used. With the flow rate of Ar fixed at 96 sccm, the chamber maintained a deposition pressure of 0.7 Pa. Due to low electrical conductivities of the tantalum oxide coatings, they were deposited by driving the cathodes in pulsed DC mode at a frequency of 20 kHz. Oxygen ($O_2$) was introduced through a separate mass flow controller for the reactive sputtering deposition. The tantalum oxide was deposited with the cathode power of the Ta target set to 100 W. With the flow rate of Ar fixed at 75 sccm, $O_2$ was introduced into the chamber to maintain a deposition pressure of 0.7 Pa. A substrate bias voltage of −80 V was used. The total thickness of the coatings was controlled to 0.5 ~ 0.7 μm by using a deposition time of 20 min. The temperature of the sample during the deposition was measured by a thermocouple located near the sample to be within the range of 110 ± 20 °C. The deposited tantalum oxide coatings were annealed at 700 °C for 1 min by using a rapid thermal annealing (RTA) system at a ramping rate of 100 °C/s to obtain a crystalline structure of $Ta_2O_5$.

The composition of the deposited and annealed films was determined using X-ray photoelectron spectroscopy (XPS; PHI1600) with MgKα radiation. Survey spectra in the range from 0 to 1100 eV were recorded for each sample, followed by high-resolution spectra over different elemental peaks, from which the composition was calculated. The energy was calibrated by reference to the Au $4f_{7/2}$ peak from a clean gold surface at 83.8 eV. To evaluate the adhesion strength of the deposited coatings, a Rockwell indentation test was conducted according to VDI 3198 standard. The surface morphology of the deposited coatings was examined using the high-resolution field-emission scanning electron microscopy (SEM; JSM-7000 F, Joel). Glancing-angle X-ray diffractometry (XRD; PANalytical X'pert Pro) at a glancing angle of 1° and Cu radiation were employed for phase identification. The diffractometer was operated at 40 kV and 30 mA. The static contact angle was measured (FTA-125, First Ten Angstroms, USA), and the obtained images were analyzed to calculate the contact angle of deionized water for each sample at room temperature. Each reported contact angle is the mean and SD value for at least three independent measurements.

The retention of bacteria on the coated samples was tested by a fluorescence staining method employing SYTO9 nucleic acid stain (Molecular Probes, Eugene, OR, USA). First, 1 ml of *S. aureus* or *A. actinomycetemcomitans* suspension ($1 \times 10^5$ cfu/ml) was added to the surface of specimen. After incubation for 4 h at 37 °C under a relative humidity of 96% and avoiding light exposure, the surface of sample was rinsed three times with phosphate-buffered saline, and then the retained bacteria were fixed with 2% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and stained with 10 μM STYO9 for 30 min at room temperature. The bacteria that had adhered to the samples were quantified by measuring the fluorescence detected at 488 nm by an enzyme-linked immunosorbent assay reader (Synergy HT, BioTek Instruments, Winooski, VT, USA). The results were determined in six independent experiments and quantified in units of relative fluorescence intensity.

The cell viability of human skin fibroblast cells (CCD-966SK) was examined with a microculture tetrazolium (MTT) assay (Sigma-Aldrich, St Louis, MO, USA) after the cells were cultured on the uncoated Ti plates (control) and Ta-, Ta-oxide samples. First, a 1 ml solution containing CCD-966SK cells was seeded at a density of $1 \times 10^5$ cells/ml with 2 ml of medium on the surface of specimen, and then the tested samples were incubated at 37 °C in 5% $CO_2$ for 48 h, which complete proliferation was attributed. Then, the substance used for the MTT test was a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt, which turns into a purple formazan product due to the viable mitochondria in living cells. The MTT (5 mg/ml) was added to the cultured cells and incubated for a further 4 h. The purple formazan was eluted using 100 ml of isopropanol (Sigma-Aldrich). The absorbance (optical density, O.D.) of the purple formazan was quantified by measuring at 570 nm by a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California) with the SoftMax Pro 5.2 241 software (Molecular Devices). The OD of formazan reflects the level of cell metabolic activity, with higher OD values indicating a larger number of living cells on the sample and hence better biocompatibility. Experiments were repeated independently in duplicate.

The statistical correlation of the results of antibacterial activity tests between the coated samples and uncoated pure-Ti plates was determined by Student's *t*-test. Differences were considered significant at the $p < 0.01$ level.

## 3. Results and discussion

### 3.1. Microstructure analyses

Fig. 1 shows the XPS spectra of the deposited Ta and tantalum oxide coatings, which exhibit the major characteristic peaks for oxygen (O1s) and tantalum (Ta4f, Ta4p, Ta4d, and Ta4s). The Ta coating contained a small fraction of oxygen (19.5 ± 2.1 at.%) and indicated the presence of surface oxides on the outermost surface of the Ta coatings due to the easy oxidation of Ta coatings in air. The elemental composition of the deposited $Ta_2O_5$ coating comprised 32.9 ± 2.6 at.% Ta and 67.1 ± 2.2 at.% O, which implied the formation of stoichiometric tantalum pentoxide.

X-ray diffraction spectra from the Ta, deposited $Ta_2O_5$ (denoted as a-$Ta_2O_5$) and annealed $Ta_2O_5$ (denoted as c-$Ta_2O_5$) coatings are shown in Fig. 2. A crystalline β-Ta structure of the Ta coating was obtained by Ta sputtering. For the deposited a-$Ta_2O_5$, a broad diffraction crest appeared in the pattern at approximately 20°–40° 2θ, and it showed an amorphous structure. The XRD pattern of the $Ta_2O_5$ (c-$Ta_2O_5$) annealed by RTA at 700 °C corresponded well to the planes of crystalline structure of β-$Ta_2O_5$ [20,22]. L. A. Aleshina et al. showed that the crystalline β-$Ta_2O_5$ had low electrical conductivity and was a typical dielectric. The surface wettabilities and biocompatibilities were attributed to the coating structures. In this study, amorphous $Ta_2O_5$ coatings were synthesized by using a pulsed magnetron sputtering. In a previous study by S. J. Wu et al. [23], the as-deposited $Ta_2O_5$ coatings by RF sputtering were amorphous, whereas orthorhombic crystalline phase was obtained after post-deposition annealing in pure $O_2$ ambient at 800 °C. Our results showed that crystalline β-$Ta_2O_5$ coatings were produced by annealing the a-$Ta_2O_5$ coatings using RTA at 700 °C. This variation showed that crystallization in the a-$Ta_2O_5$ coatings was governed by the surface mobility of the sputtered atoms. Therefore, post-deposition RTA is favorable to the