Surface & Coatings Technology 330 (2017) 260–269

Contents lists available at ScienceDirect

Surface & Coatings Technology

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

Evaluation of cell activation promoted by tantalum and tantalum oxide coatings deposited by reactive DC magnetron sputtering

Hugo Moreira[a,*], Augusto Costa-Barbosa[b], Sandra Mariana Marques[a], Paula Sampaio[b], Sandra Carvalho[a,c,*]

[a] GRF-CFUM, Physics Department, University of Minho, 4800-058 Guimarães, Portugal
[b] CBMA, Department of Biology, University of Minho, 4710-335 Braga, Portugal
[c] SEG-CEMUC, Mechanical Engineering Department, University of Coimbra, 3030-788 Coimbra, Portugal

ARTICLE INFO

Keywords:
Cell activation
Dental implant
Inflammatory response
Macrophage cell
Tantalum
Tantalum oxide

ABSTRACT

Previous studies have shown both Ta and Ta—O to be bioactive, rapidly forming a strongly-bonded surface-adherent layer of bone-like hydroxyapatite (HAp) when immersed in simulated body fluid (SBF). Consequently, Ta and Ta—O coatings are promising for the surface-modification of Ti or stainless steel endodontic endosseous implants, being conducive to a reduction in the risk of developing post-operatory infection and/or peri-implantitis disease. That said, few studies have investigated the effect of Ta or Ta—O coatings on such phenomena as cell activation, adhesion, and proliferation.

To that effect, Ta, and Ta—O films were deposited onto type 316L stainless steel (SS 316L) substrates by reactive DC magnetron sputtering, after which their biological response was evaluated following co-incubation with the murine macrophage-like cell line RAW 264.7. Cell morphology after adhesion was observed by SEM, whereas cell viability and proliferation were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Lastly, inflammatory response was assessed by quantification of the cytokines interleukin-6 (IL-6) and interleukin-10 (IL-10).

In terms of phase composition, Ta showed a mixture of the α-Ta and β-Ta phases, whereas Ta—O showed a nanocrystalline structure. Moreover, a decrease in average roughness (R_a) from 21 nm to 7 nm was observed between Ta and Ta—O, accompanied by a decrease in water contact angle (θ_W) from 106° to 83°.

In vitro studies showed that cells exhibited significantly better adhesion to Ta, in comparison with both Ta—O and SS 316L. Furthermore, both Ta and Ta—O were shown to be non-cytotoxic, with Ta outperforming Ta—O in terms of relative cell viability, both at 24 h and 48 h. Lastly, both Ta and Ta—O showed vastly inferior IL-6 and IL-10 levels to those obtained for cells treated with bacterial lipopolysaccharides (LPS)—prompting the conclusion that the coatings do not in any way induce an inflammatory response from macrophage cells.

1. Introduction

The oral mucosa hosts a variety of microbial species ranging from bacteria to fungi [1], the proliferation of which is controlled by physiological barriers such as antimicrobial factors and the mucosa-associated lymphoid tissue (MALT)—a diffuse system of small concentrations of lymphoid tissue populated by plasma cells, macrophage cells, and lymphocytes [2]. However, the insertion of endodontic endosseous implants disrupts the continuity of these barriers, inducing a local inflammation with a huge potential for degenerating into severe infection due to the ingress and unhinged proliferation of potentially harmful opportunistic microorganisms [3].

Monocyte and macrophage cells play a key role in the early stages of tissue healing after implant insertion, acting not only to regulate inflammation and dispel infection, but also to promote bone healing and osseointegration [4]. In short, macrophage attachment and activation to the implanted materials is crucial in determining the extent of acute/chronic inflammation [5].

Nowadays, commercially pure Ti (CP Ti) and Ti alloys (*e.g.* Ti–6Al–4V) are the most extensively used materials in orthodontic reconstructive surgery, due to their excellent corrosion resistance, passivation capacity, and biocompatibility [6]. However, Ti implants are not

Abbreviations: HAp, hydroxyapatite; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IL-6, Interleukin-6; IL-10, Interleukin-10; LPS, Lipopolysaccharides; SBF, simulated body fluid; SS 316L, type 316L stainless steel

* Corresponding authors at: University of Minho, Physics Department, Campus of Azurém, 4800-058 Guimarães, Portugal.
E-mail addresses: hugo.rafael.moreira@sapo.pt (H. Moreira), sandra.carvalho@fisica.uminho.pt (S. Carvalho).

http://dx.doi.org/10.1016/j.surfcoat.2017.10.019
Received 16 May 2017; Received in revised form 19 September 2017; Accepted 6 October 2017
Available online 07 October 2017

entirely without fault: an approximate 14% of inserted implants go on to develop peri-implantitis [7].

Following insertion, Ti implants show an inconvenient propensity for becoming encapsulated in fibrous tissue [8]. At first, this results in a poor bond between implant and bone, and, over time, in implant loosening as a result of poor osseointegration [8]. Implant-associated infection—which results from the reduced immune resistance of the host following surgical trauma, as well as the implant itself acting as a foreign body and thus increasing the risk of infection by prompting the entry and proliferation of microorganisms—is another leading cause for Ti implant failure [8]. Particularly troublesome, however, is the case of peri-implantitis disease, in which the infection of the implant-adjacent bone tissue leads to a receding of the surrounding bone, the subsequent decrease of the biomechanical anchorage of the implant, and, ultimately, implant failure [9,10].

While the mechanical properties of the materials and the loading conditions in the host significantly influence material selection [6], cell and tissue interactions with the implant surface depend dominantly on surface characteristics, with rough, textured, and porous surfaces stimulating cell adhesion, differentiation, and the formation of extracellular matrices [4,11]. In an experiment conducted with surface oxide films formed on Ti plates, for example, it was shown that surface topography, roughness, and energy, as well as the concentration of surface-attached OH groups, significantly influence the initial behaviour of osteoblasts [11]. Thus, one of the most promising approaches in the way of improving implant in-service behaviour has been the surface-modification of pre-existing Ti-based implants as a demand for bioactive surfaces that enhance the implant healing process and promote biomineralisation.

Ta, in particular, has proven to be a promising alternative to Ti, being widely documented as bioactive [12,13] and having been shown to rapidly form a strongly-bonded surface-adherent layer of bone-like hydroxyapatite (HAp) when immersed in simulated body fluid (SBF) due to its high surface energy in comparison with CP Ti [14]. While the high density and cost of Ta implants limit their bulk use [13], a suitable compromise is found in the deposition of Ta thin films onto standard Ti or stainless steel implants. Indeed, studies have shown Ta coatings to improve the *in vitro* biocompatibility of Co−Cr [12] and Ti−Ni alloys [12,15], for example.

On the other hand, the development of implants with oxidised surfaces constitutes another promising approach. In in-service conditions, osteoblasts interact with the oxidised surface, and, due to the oxide layer's ability to bind with Ca, form a diffusion zone, thus promoting a stronger bond between bone and implant [16]. Ta−O layers were shown to improve the cytocompatibility of Ti, for example, with *in vitro* tests showing that the coatings promote the proliferation, alkaline phosphatase (ALP) activity, mineralisation, and osteogenic gene expressions of osteoblasts [13].

Of particular interest, in a study conducted with Ta−O coatings by Almeida Alves et al., it was shown that Ta−O shows higher HAp formation rates than both CP Ti and Ta when immersed in SBF, which could translate to better bioactivity and osseointegration [14]. Moreover, it was shown that the higher the O content of the coatings, the higher the Ca/P ratio of the bone-like HAp layer formed on their surface [14].

However, while the bioactivity of Ta and Ta−O coatings in view of SBF has been successfully verified *in vitro* [14], few studies have investigated the effect of these coatings on phenomena such as cell activation, adhesion, and proliferation. Bioactivity notwithstanding, these coatings would be inapplicable were they to elicit an inflammatory response in the host.

As such, the aim of this paper is to produce, characterise, and study the effect of Ta and Ta−O coatings on the activation, adhesion, proliferation, and secretion of paracrine factors of macrophage-like cells.

2. Materials and methods

2.1. Production of coatings

Thin films were sputter-deposited from a high-purity Ta target (99.95% Ta) (200 × 100 mm^2) onto SS 316L (20 × 20 mm^2) and p-type (B-doped) Si (100) by DC magneton sputtering.

In order to determine optimal deposition conditions, hysteresis curves were constructed for target current density (J_{Ta}) of 10 mA/cm^2 and 5 mA/cm^2, under constant Ar flow of 60 sccm and bias voltage of − 75 V. The target (Ta cathode) voltage and working pressure were measured for increments of 5% (0.75 sccm) in the O_2 flow, with time step of 1 min between readings.

Ahead of depositions, substrates were ultrasonically cleaned in distilled water, ethanol, and acetone, for 10 min each, with a Sonica 2400MH S3 ultrasonic cleaner (Soltec, Italy). Substrates were then sputter-etched under Ar flow of 80 sccm and J_{Ta} of 0.5 mA/cm^2 for 15 min. Throughout etchings, a pulsed DC (PDC) was applied to the substrate-holder, with pulse width of 1536 ns, frequency of 200 kHz, and intensity of 250 mA.

Ta depositions were carried out under J_{Ta} of 10 mA/cm^2 for 2 h, whereas Ta−O depositions were carried out under J_{Ta} of 5 mA/cm^2 and O_2 flow of 13 sccm for 4 h. All depositions were carried out under Ar flow of 60 sccm and bias of − 75 V. For the Ta−O depositions, a Ta interlayer (~ 200 nm) was deposited onto the substrates under J_{Ta} of 10 mA/cm^2 for 10 min in order to improve film-substrate adhesion.

For all proceedings, the substrate-holder's distance to the target was kept at 70 mm, its rotation speed at 7 rpm, and its temperature at around 200 °C. Lastly, the sputtering chamber base pressure and the working pressure never exceeded 8×10^{-4} Pa and 8×10^{-1} Pa, respectively.

2.2. Characterisation of coatings

The phase composition of the coatings was determined by X-ray diffraction (XRD) with a D8 Discover diffractometer (Bruker, Germany), operating at 40 kV and 40 mA with Cu Kα radiation ($\lambda_{K\alpha I}$ = 1.540562 Å and $\lambda_{K\alpha II}$ = 1.544390 Å [17]). Tests were carried out with grazing angle of 1°, step size of 0.04°, time step of 1 s, and 2θ range of 10–80°.

The morphology of the coatings was observed by scanning electron microscopy (SEM) with a Nova NanoSEM 200 microscope (FEI, USA), operating at 5 kV in secondary electron (SE) mode. Chemical composition was determined by energy-dispersive X-ray spectrometry (EDS) with a Pegasus X4M spectrometer (EDAX, USA), operating at 20 kV.

The topography of the coatings was observed by atomic-force microscopy (AFM) with a NanoScope III AFM apparatus (Digital Instruments, USA), operating in tapping mode. AFM micrographs were taken over scanning areas of 5 × 5 μm^2. Film roughness was obtained through the roughness subroutine of the AFM apparatus.

Analyses were performed on coatings deposited onto Si, due to its lower surface roughness in comparison with SS 316L, which results in more homogenous coatings—thus improving the reproducibility of the tests.

2.2.1. Analysis of wettability

The wettability of the coatings was evaluated by the sessile drop test. Static contact angles were measured at room temperature (RT) with a OCA20 optical contact angle measuring system (DataPhysics, Germany), in view of Milli-Q ultrapure water, α-bromonaphthalene, and glycerol. Probe liquids were dosed with a Hamilton 500 μL syringe, with dosing volume of 2 μL and dosing rate of 1 μL/s. Contact angles were measured for thin films deposited onto SS 316L, with uncoated SS 316L substrates having been used as commercial controls. Measurements were taken after the probe liquid droplet reached equilibrium, after approximately 1 min.