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Sputtered Gum metal thin films showing bacterial inactivation and biocompatibility

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ABSTRACT

Super-elastic Titanium based thin films Ti-23Nb-0.7Ta-2Zr-(O) (TNTZ-O) and Ti-24Nb-(N) (TN-N) (at.%) were deposited by direct current magnetron sputtering (DCMS) in different reactive atmospheres. The effects of oxygen doping (TNTZ-O) and/or nitrogen doping (TN-N) on the microstructure, mechanical properties and biocompatibility of the as-deposited coatings were investigated. Nano-indentation measurements show that, in both cases, 1 sccm of reactive gas in the mixture is necessary to reach acceptable values of hardness and Young's modulus. Mechanical properties are considered in relation to the films compactness, the compressive stress and the changes in the grain size. Data on Bacterial inactivation and biocompatibility are reported in this study. The biocompatibility tests showed that O-containing samples led to higher cells proliferation. Bacterial inactivation was concomitant with the observed pH and surface potential changes under light and in the dark. The increased cell fluidity leading to bacterial lysis was followed during the bacterial inactivation time. The increasing cell wall fluidity was attributed to the damage of the bacterial outer cell which losing its capacity to regulate the ions exchange in and out of the bacteria.

1. Introduction

Titanium alloys are widely used in biomedical applications due to their light weight, low modulus, high strength, excellent biocompatibility and corrosion resistance [1,2]. These properties depend on the chemical composition and phase stabilization [3]. β-type titanium alloys present the lowest Young's modulus due to the low density of the body-centered cubic (bcc) structure. In addition, they are known for their resistance to stress corrosion cracking and to hydrogen embrittlement [4].

Research on the β Ti-based alloys with non-toxic and non-allergizing elements such as Zr, Nb and Ta have increased rapidly during the past decade, especially with the aim to enhance their mechanical properties and super-elastic performance [2–5]. Recently, several quaternary alloys Ti-Nb-Zr-Ta have been developed to be used in the biomedical field [6,7]. Among these alloys, the multifunctional Ti-23Nb-0.7Ta-2Zr-1.2O alloy composition (at.%), called "Gum metal" firstly elaborated by Saito et al. in 2003 [8], is privileged presenting potential applications. It has super-elastoplastic behavior at room temperature: high strength and low Young's modulus.

Super-elastic thin films can be used for medical applications and/or micro-actuation. For example, the fabrication of stents for neurovascular blood vessels as well as the production of membrane-based micro-pumps can be based on thin film techniques [9].

In the present work, Ti-23Nb-0.7Ta-2Zr and Ti-24Nb (at.%) thin films doped with oxygen and/or nitrogen (TNTZ-O and TN-N) were deposited by magnetron sputtering of composite targets. Achache et al. [10] have recently reported that dopant interstitial elements like oxygen and nitrogen improve mechanical properties. In both cases, the most suitable properties were reached for 1 sccm of reactive gas in the mixture. The influence of the gas mixture composition on structure, morphology and mechanical properties of the

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as-deposited films is reported. Biocompatibility and the bactericidal activity of the prepared films are also studied. The interfacial potential changes between the bacterial cells and the sputtered films during the disinfection process are also addressed in this study.

2. Experimental details

2.1. Deposition

Gum metal films were deposited on glass substrates by magnetron sputtering of the composite targets: Ti-23Nb-0.7Ta-2Zr and Ti-24Nb (at.%). The chamber was evacuated to a pressure of 4×10^{-4} Pa. The target discharge current was kept at a fixed value of 1 A. The targets-substrates distance was kept constant and equal to 8 cm and deposition was carried out for 1 h (film thickness is about 3 μm). The working pressure was kept at 0.2 Pa and the substrates were placed at a floating potential of zero (0 V).

2.2. Characterizations

Young's modulus and hardness were obtained by means of a Nano Indenter XP, MTS Systems Corporation; with continuous stiffness measurement (CSM) option. A three-sided pyramidal diamond tip (Berkovitch) was used. The average of ten indents was taken to determine the values of hardness and Young's modulus. The penetration depth was kept as low as possible (less than 10% of the film thickness) to minimize the influence of the substrate stiffness [11].

The microstructural analysis was carried out by using X-ray diffraction (XRD) with a 4 circles Seifert PTS-3000 X-ray diffractometer using CuKα radiation. To minimize the texture effect on the intensity of the diffraction peaks, a two axis summation technique was used. It consists in rotating the specimen around the normal axis (Φ) from 0° to 360° while the declination angle (ψ) oscillates between −70° and +70°. A complete oscillation in ψ is performed at each acquisition step in 2θ but the Φ rotation is only partial. As the integration is not performed on the whole pole figure, the peaks intensities are still affected by texture effects. The instrumental broadening was obtained on a reference silicon powder.

The surface and cross-sectional morphology of the thin films were investigated by scanning electron microscope (SEM, Hitachi SU8030). The chemical composition was obtained by Energy Dispersive Spectroscopy (EDS). The light elements content in the films (e.g. oxygen and nitrogen) could not be quantified by this technique.

2.3. MTT assay

L929 and HeLa cells were used to test the biocompatibility of the sputtered films (ISO 10993/USP 32 NF 27). The L929 cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% v/v fetal bovine serum, kanamycin (0.1 mg mL^{-1}), penicillin G (100 U mL^{-1}), and sodium bicarbonate (1.5 mg mL^{-1}) at 37 °C in a CO_2 atmosphere of 5%. HeLa cells were grown in MEM + 10% FBS + antibiotics at 37 °C in a CO_2 atmosphere (5%). L929 and HeLa cells were incubated at a concentration of 3.9×10^5 cells mL^{-1} for 24 h in a Corning® CellBind® cell culture plates (Sigma-Aldrich, CLS3337) in the respective medium. After 24 h, the reaction media was replaced by fresh media and contacted with sterile samples. The plates containing the cells and the samples were incubated at 37 °C in 5% CO_2 atmosphere for 24 and 48 h. After incubation, the 10 μL MTT solution was added into each well including control wells. To enhance the metabolism of MTT, the plates were incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The medium was then removed and only anchored cells remained in the wells. The cells were then washed with PBS and extracted in 200 μL acidic isopropanol. Absorbance was read at 570 nm to determine the cell viability. The experiments were carried out in triplicate and the data is reported as mean of standard deviation (±SD, n = 5%). The relative cell viability percentage was compared with control cells contacted with uncoated glass.

2.4. Bacterial inactivation and micro-oxidation on the sputtered films

Escherichia coli (*E. coli* K12 ATCC23716) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig (Germany). Sputtered surfaces were sterilized by keeping them in an oven at 80 °C overnight. 100 μL culture aliquots with an initial concentration of ~10^5 CFU mL^{-1} in NaCl/KCl (8/0.8 g L^{-1}, pH 7) were placed on the coated and uncoated (control) samples. Experiments were run at room temperature and the samples were placed on glass Petri dishes provided with a lid to prevent evaporation during illumination. Then, the samples were transferred into a sterile tube containing NaCl/KCl saline solution and subsequently mixed thoroughly using a Vortex for 2 min. Serial dilutions were made in NaCl/KCl solution. A 100 μL sample of each dilution was pipetted onto a nutrient agar plate and spread over the surface of the plate using the standard plate method. Agar plates were incubated lid down, at 37 °C for 24 h before the CFU counting. Three independent assays were done for each sample. The statistical analysis of the results was performed for the CFU values calculating the standard deviation values (n = 5%). The average values were compared by one-way analysis of variance with the values of statistical significance.

The irradiation was carried out in a cavity provided with tubular actinic Osram Lumilux 18 W/827 emitting in the range 360–700 nm with total output of 5 mW cm^{-2}. These lamps are generally used in hospitals and schools indoor illumination.

The interfacial potential and pH were followed on a Jenco 6230N (pH/mV/Temp meter) provided for with a microprocessor and a RS-232-C IBM interface.

3. Results and discussions

3.1. Chemical composition

The chemical compositions of TNTZ and TN films, measured by the EDS, are presented in Table 1. The compositions of metallic

Table 1
Chemical composition and mechanical properties of TNTZ and TN thin films.

		Chemical composition (at.%)				Mechanical properties (GPa)	
		Ti	Nb	Zr	Ta	Young's modulus	Hardness
Oxygen flow rate (sccm)	0	74.7	23.6	0.9	0.8	104.2	8.1
	0.5	74.3	23.1	2.1	0.5	112	12.7
	1	75.1	22.1	1.7	1.1	122	14
Nitrogen flow rate (sccm)	0	76	24	0	0	98	6.7
	1	76.2	23.8	0	0	116	8.2