Available online at www.sciencedirect.com
ScienceDirect
Surface & Coatings Technology 201 (2006) 2807–2812
SURFACE & COATINGS TECHNOLOGY
www.elsevier.com/locate/surfcoat

Influence of gas nitriding of Ti6Al4V alloy at high temperature on the adhesion of *Staphylococcus aureus*

M. Isabel Sarró [a], Diego A. Moreno [a,*], Carlos Ranninger [a], Eleanor King [b], José Ruiz [c]

[a] *Universidad Politécnica de Madrid, Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, José Gutiérrez Abascal, 2, E-28006 Madrid, Spain*
[b] *Department of Materials, Imperial Collage of Science, Technology and Medicine, Prince Consort Road, London SW7 2BP, United Kingdom*
[c] *Centro Nacional de Investigaciones Metalúrgicas (CSIC), Avda. de Gregorio del Amo, 8, E-28040 Madrid, Spain*

Received 5 October 2004; accepted in revised form 22 May 2006
Available online 7 July 2006

Abstract

In this in vitro study, we investigated the influence of titanium nitride (TiN) treatments on the *Staphylococcus aureus* colonisation and biofilm formation on Ti6Al4V alloy at different times. The development of biofilm in static tests was carried out in a TSB culture medium at 37 °C, for 12, 24, 48 and 72 h. Bacterial adhesion on nitrided titanium alloy was analysed by both epifluorescence microscopy and scanning electron microscopy. The results revealed that the number of attached bacteria, their viability, and distribution were different for nitrided alloys than for the control group (non-nitrided alloys).
© 2006 Elsevier B.V. All rights reserved.

Keywords: Titanium alloy; Surface treatment; TiN; Bacterial adhesion; Biofilm

1. Introduction

Titanium and titanium alloys are widely used in a variety of medical applications, where the combination of mechanical and chemical properties is of crucial importance. The increased use of titanium alloys, especially Ti6Al4V, is due to its low density, its excellent mechanical and anti-corrosive properties, and its biocompatibility [1–5], compared to stainless steel or cobalt-chrome alloys [6,7].

However Ti6Al4V have very poor tribological properties, for this reason, many techniques have been developed to improve their wear resistance properties. For the last decades, numerous artificial prosthesis with titanium and alloys as Ti6Al4V with different types of coatings or surface treatments has been developed [8–13]. One of these surface modifications is made up of a titanium nitride (TiN) layer favouring nitrogen diffusion into the surface of the material. This method modifies the surface of the alloy and improve the wear resistance, and the tribological properties of titanium alloys [14–18].

The artificial prosthesis are intend for long-term or permanent implantation, but the influence of these coatings in bacterial colonisation is poorly documented, and in many cases these prosthesis have to be removed for different reasons, most of them related to infections [19,20].

The basis of the pathogenic mechanism of these infections in biomaterials used for the implants is related to the ability of some microorganisms to attach to the surfaces and form biofilms [21–24] in which the bacterial cells exhibit increased resistance to antimicrobial agents and the host immune system [25,26].

Biofilm infections typically showed recurring symptoms, after cycles of antibiotic therapy [27], until the sessile population with the prosthesis is removed from the body [28].

Normally, microorganisms of the *Staphylococcus* group [29] are the most common etiological agent in nosocomial infections of prostheses. *Staphylococcus aureus* has a marked preference for metallic prostheses, such as titanium alloys (Ti6Al4V) [20,23] whereas *Staphylococcus epidermidis* mostly affects non-metallic implants, such as plastic endovascular catheters [20,30]. These microorganisms are classified as opportunists due to their low pathogenic capacity, but when they are associated to biomaterials, they can cause serious infections [31]. For these reasons,

* Corresponding author. Tel.: +34 91 336 3164; fax: +34 91 336 3007.
E-mail address: diego.moreno@upm.es (D.A. Moreno).

0257-8972/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.surfcoat.2006.05.023

an important part of recent biomaterials research should be directed to study the physical and chemical properties of the material that could be relevant in bacterial adhesion [22]. In this study, the behaviour of bacterial adhesion to Ti6Al4V nitrided at two different temperatures is under comparison, in order to study the effect of the TiN film on bacterial adhesion.

2. Experimental

2.1. Material

The chemical composition of the titanium alloy used (Ti6Al4V) is presented in Table 1 and their mechanical properties in Table 2. Coupons were made up of 10 mm × 30 mm × 2 mm from a 2-mm thick plate, laminated, according to ASTM B-265-94 GR 5 standardization. As a control group non-nitrided coupons were used (C coupons). The nitriding treatment was performed in a 99.999% nitrogen-pure atmosphere in the electric oven of a horizontal tubular chamber following these steps: i) preliminary purge of the oven with N_2 (gas), with a caudal of $0.3 \times dm^3 \cdot min^{-1}$ for 1 h, ii) second purge of with N_2 (gas) with the same caudal at 300 °C for 15 min, iii) nitriding treatment of the coupons with a caudal of $0.10\ dm^3 \cdot min^{-1}$ into the oven at 1000 °C for 2 h (A coupons) and 1100 °C for 4 h (B coupons), iv) the coupons were cooled down to 200 °C with a caudal of N_2 (gas) of $0.20\ dm^3 \cdot min^{-1}$.

2.2. Characterization test

The TiN thickness was measured in transverse cross-section, using a Nikon MM-11 microscopy equipped with X–Y table, digital lecture (1 μm sensitive) and exit RS232.

The roughness of coupons was measured using a Taylor–Hobson (Suntronic 3+) roughmeter. The measurements were obtained in longitudinal and transversal direction obtaining the surface roughness (Ra) values, which define the arithmetic mean of departure of a surface profile from a mean line.

X-ray diffraction (XRD) technique was used for surface phase identification of nitrided and control samples. The diffractometer used was a Philips model PW-1830 with an automatic slit, Cu Kα radiation ($\lambda = 1.5405$ Å), 3 to 60° 2θ explored area, and 0.05° 2θ s1 goniometer speed.

The coupons were observed using a SEM (Zeiss DSM 960) at 15 kV equipped with energy dispersive X-ray (EDX) in order to determine the elemental composition of the TiN layer.

2.3. Bacterial adhesion

Immediately before the bioadhesion tests, the coupons were cleaned with acetone and sterilised under a burner with ethanol. The tests were carried out in 20 mL universal bottles with a liquid TSB (Triptone Soya Broth, OXOID) culture medium.

Table 1
Chemical composition (wt.%) for Ti6Al4V alloy

N	C	H	Fe	O	Al	V	Ti
0.01	0.025	0.002	0.17	0.17	6.20	4	Balanced

Table 2
Mechanical properties of the Ti6Al4V alloy

Properties	σ_R MPa	σ_e (0.2%) MPa	Elongation %	Reduction of area %	E, GPa
Ti6Al4V	1050	900	11	25	110

Under sterile conditions, the coupons were introduced in the bottles and incubated in the presence of *S. aureus*, Rosenbach 1884 VP, ssp. *aureus* (CECT-435) at 37 °C under static conditions. The initial concentrations of the inoculants were 10^3 cells·mL^{-1}, which was determined by epifluorescence microscopy (see Section 2.4). This technique was validated by a direct count on Petri dishes [31].

Coupons were taken out after 12, 24, 48 and 72 h, and washed with Milli-Q sterile water to remove the planktonic bacteria that had not really adhered to the surface of the material. Then sample coupons were observed by both epifluorescence microscopy and scanning electronic microscopy.

2.4. Epifluorescence microscopy

To enumerate the bacteria in the culture medium and to study the biofilm formation along time the epifluorescence microscopy was used.

To determine initial bacterial concentration, a 1 mL aliquot from the fresh *S. aureus*-culture medium (pre-incubated for 24 h at 37 °C in TSB) was stained with a 10 μg mL^{-1} concentration of DAPI (4′,6′-diamidino-2-phenylindole, dihydrochloride) for 10 min in the dark with gently stirring. The stained microorganisms were diluted 1/10 and 10 mL volume was filtered using filters with 0.22 μm pore size (Isopore GTBP 02500™, Millipore). The filters were washed with Milli-Q sterile water and air-dried. Finally, the filters were mounted with oil mounting (Molecular Probes, Inc. Eugene, Oregon, $\eta^{25} = 1.517 \pm 0.003$) and observed under the microscope.

To study the biofilms, the coupons were washed and stained with the LIVE/DEAD® BacLight™ Bacterial Viability Kit, L-7012 (Molecular Probes, Inc. Eugene, Oregon), composed of two fluorochromes: SYTO 9 and propidium iodide. The green fluorescent SYTO 9 labelled both, live and dead bacteria. In contrast, propidium iodide penetrates only in bacteria with damaged membranes and fluoresces in red. Thus, live bacteria with intact membranes fluoresce green, while dead bacteria with damaged membranes fluoresce red.

Both fluorochromes were mixed in a 1:1 proportion and were simultaneously added at final concentration of 3.41 μM of SYTO 9 and 6.01 μM of propidium iodide. Staining was applied at room temperature in the dark and gently stirred for 15 min. Afterwards, the coupons were washed with Milli-Q sterile water and air-dried. Then, they were observed using a Zeiss Axioskop 2 epifluoresence microscope with a 50-W Hg lamp. The epifluorescence filter set Zeiss 487901 (BP 365; FT 395; LP 397) was used for filters stained with DAPI. The Zeiss 487709 (BP 450–490; FT 510; LP 520) epifluorescence filter set was used to simultaneously observe the two fluorochromes, and the Zeiss 487714 (BP 510–560; FT 580; LP 590) filter set

Download English Version:

https://daneshyari.com/en/article/1663396

Download Persian Version:

https://daneshyari.com/article/1663396

Daneshyari.com