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Surface properties and cell response of low metal ion release Ti-6Al-7Nb alloy after multi-step chemical and thermal treatments

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Abstract

Ti-6Al-7Nb samples treated by innovative multi-step chemical and thermal processes were characterized in order to evaluate their surface properties and cell interaction. The main object was to asses if the treatments were effective in order to obtain a surface presenting at the same time bone-like apatite induction ability, low metal ion release, good cell response and high protein binding. The morphology, crystallographic structure, porosity and wettability of the treated materials were investigated, as well as their interaction with simulated body fluid during soaking for different times. Cytotoxicity, protein adsorption tests and in vitro fibroblast and osteoblast-like cell cultures were also performed.

Keywords: Titanium alloys; Surface modification; Osteointegration; Ion release; Protein adsorption; Cell culture

1. Introduction

Titanium and its alloys are widely used in dentistry and orthopedics because of their outstanding biocompatibility and mechanical properties. Their interaction with cells depends most on surface properties (crystallographic structure, morphology and composition of surface layers, wettability) and on metal ion release. They spontaneously form in air an oxide layer containing dense and stable TiO_2, with a thickness of a few nanometers [1]. When implanted, they form a morphological connection with living tissues (bioinert behavior), and also show some osteointegration ability with direct bone–implant contact. Problems arise when a low-quality bone is present or when faster healing is required [1].

Macroscopical modifications in morphology are often used to obtain a better connection: surface is made porous by plasma-spray coating or simply rougher by blasting, or covered with titanium beads [2,3]. Plasma spray is an expensive technique and many authors have reported that plasma-sprayed hydroxyapatite (HA) coatings enhanced implant performance at an early stage after implantation, but caused poorer long-term performances because of low adhesion of the coating to the metal and low crystallinity of the HA [2,3]. Furthermore, it is difficult to obtain uniform and thin coating on implants with a complex geometry. On the other hand, some simple chemical and thermal treatments have been developed and tested in order to modify composition and morphology of surface layers, leading to a moderate bioactive behavior [4–8]. These surfaces are characterized by a sub-micrometric or nanometric texture.

In the present study, new multi-step chemical and thermal treatments were tested and compared with single-step treatments applied on a Ti-6Al-7Nb alloy used for prosthetic implants. Morphology, crystallographic structure, porosity and wettability of the treated surface were investigated, together with some compositional analyses. Ion release tests in simulated body fluid (SBF), cytotoxicity and protein adsorption tests were performed, followed by a wide in vitro characterization based on fibroblast and osteoblast-like cell cultures.

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2. Materials and methods

2.1. Materials

A Ti-6Al-7Nb titanium alloy for prosthetic implants supplied by Centerpulse Orthopedics and commercialized as *Protasul100* (composition given in Table 1) was employed, and all experiments were carried out on disk-shaped samples (18 mm diameter, 2 mm thickness). Their surface was grit blasted with corundum, giving a roughness of $R_a = 4 \times 6\,\mu m$. This will be indicated by letter B (blasted) in every acronym for samples.

Two kinds of chemical treatment were used: a passivation in nitric acid, 30 min in 35 wt% HNO_3 aqueous solution at room temperature (this treatment will be indicated by letter P, standing for passivation) and/or an alkali treatment, 24 h in 5 M NaOH aqueous solution at 60°C (this treatment will be indicated by S, standing for [caustic] soda).

Two kinds of thermal treatments were performed: a heat treatment in air, 1 h at 600°C in a standard electric furnace, with a heating rate of 300°C/h as well as the initial cooling rate (this treatment will be indicated by A), or a heat treatment in vacuum, 1 h at 600°C under rotative pump dynamic vacuum conditions (5×10^{-1} bar), same heating/cooling rates as above (this treatment will be indicated by V).

After P and S treatments, the samples were washed with distilled water and dried at room temperature. After A and V treatments, they were washed with acetone and dried at room temperature.

The innovative multi-step treatments consist of passivation followed by alkali treatment, and a subsequent heat treatment. Therefore, the acronyms will be BPSA or BPSV depending on the atmosphere used for the heat treatment. If the samples underwent only an alkali treatment and a heat treatment in air, they will be named bovine serum albumin (BSA) and so on.

2.2. Surface characterization

Surface morphology and composition were assessed by scanning electron microscopy (SEM Philips 525 M) and energy dispersion spectrometry (EDS Philips-EDAX 9100).

Surface crystalline structure was investigated by X-ray diffraction analysis (X'Pert Philips diffractometer), using a parallel-beam configuration (PB-XRD) and Cu-Kα incident radiation. The incident angle was set at 1.2°.

Surface porosity of the modified samples was evaluated by mercury intrusion porosimetry (FISONS Porosimeter 2000), using a cylindrical geometry as a model for pore shape.

Table 1
Composition of the Ti-6Al-7Nb alloy (wt% values)

	Ti	Al	Nb	Ta	Fe	H	N	O	C
Min	Bal.	5.5	6.5						
Max		6.5	7.5	0.5	0.25	0.009	0.05	0.20	0.08

2.3. Wettability tests

Contact angle measurements were carried out in order to evaluate the wettability of the surface-modified alloy as resulting from every treatment. An equal volume of distilled water was placed on every sample by means of a micropipette, forming a drop or spreading on the surface. Photos were taken through lenses (LEITZ IIA optical stage microscope equipped with LEICA camera) to record the shape of the drops and measure the contact angle.

2.4. Metal ion release

Metal ion release tests were performed by immersing the samples in a standard SBF solution kept at 37°C in a BICASA *Alpha* incubator. The SBF used in this work was proposed by Kokubo and co-workers [9–11] to mimic the inorganic salt composition of human physiological fluids. Small amounts of solution were withdrawn at different times, ranging from 3 h to 60 days, and replaced with fresh solution. The replacement of the withdrawn solution with fresh one leads to dilution effects; knowing exactly the amount of soaking solution and the withdrawn (and replaced) quantity it was possible to process the data taking into account the experimental procedure. An atomic absorption spectrophotometer (ICP-GFAA technique) was used to evaluate metal ions concentration in the withdrawn solution, focusing on titanium and aluminum content. Every different treatment tests were carried out on at least three samples.

2.5. Soaking in simulated body fluids

Samples immersed in SBF for up to 30 days were carefully dried and observed at SEM to investigate the possible precipitation of HA on their surface. Such phenomenon is often associated to bioactivity, and represents a simple in vitro test [9–11].

2.6. In vitro biocompatibility tests

At least 20 samples per treatment were prepared for in vitro tests involving cell cultures. Cytotoxicity tests consisted of the quantification of the activity of lactate dehydrogenase (LDH) in culture medium of cells in contact with samples. The activity of the LDH enzyme rises when cells are damaged: the LDH activity induced