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Antibacterial and bioactive nanostructured titanium surfaces for bone integration

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ABSTRACT

An effective and physiological bone integration and absence of bacterial infection are essential for a successful orthopaedic or dental implant. A titanium surface able to actively promote bone bonding and avoid microbial colonization represents an extremely interesting challenge for these purposes. An innovative and patented surface treatment focused on these issues is described in the present paper. It is based on acid etching and subsequent controlled oxidation in hydrogen peroxide, enriched with silver ions. It has been applied to commercially pure titanium (Ti-cp) and alloy Ti6Al4V. The chemistry and morphology of the surfaces are modified by the treatment on a nanoscale: they show a thin oxide layer with porosity on the nanoscale and silver particles (few nanometers in diameter), embedded in it. These features are effective in order to obtain antibacterial and bioactive titanium surfaces.

1. Introduction

Biomaterials for prosthetic implants must replace the missing tissue and return the natural functionality. In order to reach these objectives, artificial materials need to interact, in a proper way, with the biological environment and to physiologically bond to the surrounding tissues. A sort of "race for the surface" has been described, as consequence of the implantation of a biomaterial [1,2]. When the surface comes into contact with biological fluids, the absorption of water molecules and proteins immediately occurs [3]. Then tissue cells match against bacteria, eventually present, for material colonization.

An ideal biomaterial for prosthetic implants will promote cellular adhesion and it will avoid the bacterial one. The main surface features that affect and guide cellular and bacterial adhesion are topography, roughness, chemical composition and energy. On the basis of this knowledge, the scientific community has investigated various strategies for obtaining surfaces able to promote cellular adhesion, as well as reduce the risk of bacterial contamination. The current main challenge is to obtain both actions on the same surface.

Titanium and its alloys are the biomaterials of choice for dental and orthopaedic implants, thanks to their good mechanical and corrosion resistance properties, as well as biocompatibility. However, they are neither bioactive nor antibacterial, even though a reduced bacterial colonization has been observed on titanium, if compared to stainless steel, due to its better bone integration ability [2].

A lot of solutions have been proposed, in order to improve the bone bonding ability of titanium implants [4]. An improved osteoblast adhesion and differentiation has been documented on micro-roughed titanium surfaces, leading to the development of numerous surface modifications able to introduce this feature [5–10]. Recently, an increasing attention has been devoted to surface nanotextures in order to stimulate cell behaviour [11–14]. The combination of micro and nano roughness has been also proposed in order to promote osteoblast adhesion, proliferation and differentiation [15,16]. While an increased bacterial adhesion has been observed for micro-rough surfaces [17,18], a constant or even reduced bacterial attachment has been documented for nanotextured ones [19–22]. Bioactive coatings (mainly of hydroxyapatite and calcium phosphates) [23–25] and thermo-chemical treatments based on alkaline solutions (NaOH) [26,27] or hydrogen peroxide [28–30] have been proposed in order to modify the surface topography and chemistry of titanium surfaces for obtaining a bioactive behaviour. It must be underlined that none of them present an antibacterial activity.

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Moreover, many techniques suitable for obtaining antibacterial titanium based surfaces can be found in the scientific literature. In order to overcome the increasing problem of bacterial resistance to antibiotic therapies the use of inorganic antibacterial agents (and among them especially of silver) has achieved growing attention. The introduction of silver onto metallic surfaces seems a promising strategy for obtaining a local antibacterial activity, reducing the drawbacks of both systemic therapies and antibiotic resistance. Silver containing titanium-based surfaces can be obtained by Ag-doping of the metallic surface or Ag introduction of a surface oxide layer or coating silver doping the metallic surfaces can be obtained by means of the preparation of silver containing alloys, by ion implantation/Plasma Immersion Ion Implantation (PIII) and in situ reduction of silver nanoparticles. Binary Ti–Ag alloys, sand-blasted and acid etched, have been proposed, in order to obtain silver release and bone integration [31]. Ti–Ni–Ag ternary alloys, obtained by arc melting in vacuum, result in Ag-nanoparticles embedded in a Ni–Ti matrix [32]. Ion implantation has been employed for the introduction of Ag and Cu onto the titanium surfaces [33,34], while PIII to induce the precipitation of silver nanoparticles onto titanium substrates [35,36]. In situ reduction of silver nanoparticles (from $AgNO_3$ containing media) has been employed for their loading on various titanium substrates: polished titanium [37], TiO_2 nanotubes obtained by anodization [38] and TiO_2 coating prepared by sol–gel [39]. Silver doped titanium oxide layers have been obtained by means of Ion Beam Assisted Deposition (IBAD) with titanium and silver targets [40], Plasma Electrolytic Oxidation (PEO) [41,42] and anodic Spark Deposition [43] in electrolytes containing silver nanoparticles. Moreover, some coating techniques have been used for the preparation of antibacterial titanium-based surfaces. Sputtering has been used for the preparation of various antibacterial thin films: titanium oxide–silver by using a composite target [44,45], multilayers of Ti and Ag by magnetron sputtering [46], titanium oxide and titanium oxide containing Ag by reactive magnetron sputtering [47], TaN–Ag and titanium oxide enriched with Ag nanoparticles by co-sputtering [48,49]. Silver doped titanium oxide coatings have been prepared also by sol–gel technique, using $AgNO_3$ and titanium isopropoxide (or tetrabutyl titanate or titanium sulphate) as precursors [50–53]. Other techniques employed for the realization of TiO_2–Ag coatings are plasma spray [54] and electrophoresis [55]. Silver doped hydroxyapatite has been deposited on titanium-based substrates by Ion Beam Assisted Deposition (IBAD) [56], Plasma Electrolytic Process (PEP) [57], sol–gel [58], sputtering [59] and precipitation in calcification solutions [60]. Plasma Electrolytic oxidation (PEO) in an electrolyte containing Ca, P and Ag-nanoparticles has also been considered for obtaining antibacterial and bioactive layers onto titanium substrates [61]. Ion exchange, between Ag and Na, has also been employed in order to introduce silver onto a sodium titanate layer obtained by a chemical/hydrothermal treatment in NaOH [62] or micro-arc oxidation [63], and even onto bioactive glass coatings on Ti6Al4V substrates [64,65]. A composite coating obtained by a first growth of TiO_2-nanotubes by anodization, followed by silver nanoparticles deposition by sputtering and finally hydroxyapatite precipitation in Hanks' solution has also been proposed for combining antibacterial and bioactive properties on titanium substrates [66]. The combination of TiO_2 nanotubes loaded with silver nanoparticles and calcium-phosphate coating has also been considered in a recent patent for obtaining antibacterial and bioactive metallic surface for medical implants [67].

The majority of the solutions proposed for obtaining antibacterial and bioactive surfaces require complex and expensive multi-step processes or coatings techniques, which are not suitable for some applications, such as the dental ones. Moreover, it must be underlined that no commercial antibacterial metal implant satisfies all the orthopaedic/dental requirements and no is on the market nowadays, so this issue is challenging for the research.

The present research work reports an innovative and patented surface treatment able to impart antibacterial and bioactive behaviour to titanium and its alloys in a single step and low cost process, by introducing peculiar surface features.

The present paper is focused on the physical and chemical characterization of the modified Ti-cp and Ti6Al4V surfaces. Some preliminary biological characterizations (antibacterial and biocompatibility tests) have been reported to demonstrate the effectiveness of the proposed technology but a more detailed biological study will be the topic of a future paper.

2. Materials and methods

2.1. Samples preparation

Commercially pure titanium (ASTM B348, Gr2, Titanium Consulting and Trading) and Ti6Al4V alloy (ASTM B348, Gr5, Titanium Consulting and Trading) in the form of cylindrical bars, 10 mm in diameter, were cut in 2 mm thick samples by means of an automatic cutter (Struers Accutom 5), provided with an alumina blade (356 CA). The samples were polished with abrasive SiC papers (up to 4000 grit) and finally washed in acetone and double distilled water, in an ultrasonic bath, in order to remove surface contaminations.

The surface treatment was performed by means of a patented thermo-chemical process [68]. The procedure includes a pretreatment in diluted hydrofluoric acid, aimed to remove the natural titanium oxide layer, and a subsequent controlled oxidation in hydrogen peroxide, enriched with the antibacterial agent. Silver has been selected as antibacterial element, in the present work, and introduced as silver nitrate ($AgNO_3$, Silver Nitrate PA-ACS-ISO 131459,1611, Panreac) in the hydrogen peroxide solution. Different ultrasonic washings were performed at the end of the oxidation treatment in order to remove eventual silver salts and evaluate the adhesion of silver nanoparticles on the surface. Three different concentrations of silver were employed and named C1 (0.001 M), C2 (0.0025 M) and C3 (0.005 M) increasing the $AgNO_3$ concentration in H_2O_2. Control samples, prepared by acid etching and controlled oxidation in H_2O_2, without adding the antibacterial agent, were prepared for comparison purposes and indicated as C0. The complete list of samples names and treatment is reported in Table 1.

Table 1
Samples names and treatments.

SAMPLE NAME	TRATMENT
Ti-cp Ag-C0	HF-etching H_2O_2-oxidation
Ti-cp Ag-C1	HF-etching H_2O_2/Ag conc. C1-oxidation and silver doping
Ti-cp Ag-C2	HF-etching H_2O_2/Ag conc. C2-oxidation and silver doping
Ti-cp Ag-C3	HF-etching H_2O_2/Ag conc. C3-oxidation and silver doping
Ti6Al4V Ag-C0	HF-etching H_2O_2-oxidation
Ti6Al4V Ag-C1	HF-etching H_2O_2/Ag conc. C1-oxidation and silver doping
Ti6Al4V Ag-C2	HF-etching H_2O_2/Ag conc. C2-oxidation and silver doping
Ti6Al4V Ag-C3	HF-etching H_2O_2/Ag conc. C3-oxidation and silver doping

C1 = $AgNO_3$ 0.001 M in H_2O_2.
C2 = $AgNO_3$ 0.0025 M in H_2O_2.
C3 = $AgNO_3$ 0.005 M in H_2O_2.

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