Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 63–75
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Nanocomposite hydroxyapatite formation on a Ti–13Nb–13Zr alloy exposed in a MEM cell culture medium and the effect of H_2O_2 addition

M.A. Baker [a,*], S.L. Assis [b], O.Z. Higa [b], I. Costa [b]

[a] *The Surface Analysis Laboratory, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK*
[b] *IPEN/CNEN-SP, Av. Prof. Lineu Prestes 2242, CEP 05508-900, São Paulo, Brazil*

Received 19 March 2008; received in revised form 23 July 2008; accepted 12 August 2008
Available online 29 August 2008

Abstract

Titanium alloys are known to nucleate an apatite layer when in contact with simulated body fluid. This improves the bioactivity of titanium implants and accelerates osseointegration. Promoting the formation of hydroxyapatite on biocompatible metals is, therefore, a very important topic of biomaterials research. In this paper, the formation of hydroxyapatite (HA) on the near-β Ti–13Nb–13Zr alloy by immersion in minimal essential medium (MEM), with and without H_2O_2 addition, has been studied using electrochemicals methods, scanning electron microscopy and X-ray photoelectron spectroscopy. The in vitro biocompatibility of this alloy was evaluated by cytotoxicity tests. The Ti–13Nb–13Zr alloy exhibits passive behaviour over a wide potential range in MEM and the passive film is composed of an inner barrier layer and an outer porous layer. The addition of H_2O_2 leads to a thickening of the outer porous layer and strongly reduced current density. With regard to the surface composition, immersion in MEM solution results in the formation of an island-like distribution of HA + amino acids. Addition of H_2O_2 to the MEM solution strongly promotes the formation of a thicker, continuous but porous nanocomposite layer of HA + amino acids. The Ti–13Nb–13Zr alloy is non-toxic and the nanocomposite HA + amino acid layer formed in the MEM solution favours the growth of osteoblast cells. For Ti alloys, the release of H_2O_2 in the anti-inflammatory response appears to be an important beneficial process as it accelerates osseointegration.

Keywords: Ti–13Nb–13Zr; Hydroxyapatite; XPS; MEM; H_2O_2

1. Introduction

The excellent corrosion resistance of Ti alloys is due to a thin and adherent oxide film on their metallic surface. This film is spontaneously formed by exposure of the Ti alloy to the atmosphere or to aerated environments owing to the high affinity of Ti for oxygen. Due to this fact, when the oxide film is damaged it can be easily repaired even when oxygen is present at low partial pressures (ppm). Although the oxide film on Ti and Ti alloys is highly stable, when these materials are used as implants, electrochemical reactions with physiological fluids can occur which are intensified by the combined interaction of corrosion and mechanical stresses and/or wear. Two general reviews on the surface modification and studies of surface interactions of Ti and Ti alloys in biomedical applications have been given by Jones [1] and Liu et al. [2].

The electrochemical reactions occurring at the surface of Ti implants are affected by the surface characteristics such as composition, structure, roughness, morphology and composition of the physiological body fluids [3]. The composition, thickness and nature of the oxide film on Ti alloys depend on the environment to which it is exposed. McCafferty and Wightman [4] have undertaken X-ray photoelectron spectroscopy (XPS) of the native oxide on pure Ti and shown it to be comprised solely of TiO_2, together with a strong outer hydroxide component and adsorbed water. However, importantly, they quoted the oxide thickness to be approximately 8 nm and accordingly did not

* Corresponding author. Tel.: +44 1483 686294; fax: +44 1483 686291.
E-mail address: m.baker@surrey.ac.uk (M.A. Baker).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.08.008

observe a Ti metal peak in the Ti 2p spectrum. In studies of the native oxide formed on Ti–6Al–4V, lower oxidation states of Ti were also observed [5,6]. The oxide thickness in many studies is found to vary between 2 and 8 nm, and considering that the analytical depth of XPS is of approximately 5 nm, the absence of intermediate oxidation states in the recorded data is probably a result of the intermediate oxides occurring at the interface beyond the probed analysis depth. In aqueous and simulated body fluid environments, it is generally accepted that the oxide film is predominantly TiO_2, but small amounts of intermediate oxides, Ti_2O_3 and TiO have also been reported [6–8].

Ti alloys with good mechanical and corrosion resistance properties, such as Ti–6Al–4V and Ti–6Al–7Nb, have been quite extensively used in biomedical applications [2]. Sittig et al. [3] have investigated the oxide film composition using XPS for commercially pure Ti (cp-Ti), Ti–6Al–4V and Ti–6Al–7Nb alloys surface treated by mechanical polishing, passivation in HNO_3 solution or acid etching in $HNO_3 + HF$ solution. The oxide film formed on polished cp-Ti was composed mainly of TiO_2, with small concentrations of TiO and Ti_2O_3 at the Ti/oxide interface. The oxide film grown on polished Ti–6Al–7Nb showed the formation of Al_2O_3 and Nb_2O_5 in addition to TiO_2 with the relative concentrations of Al and Nb in the oxide being similar to that of the bulk. For Ti–6Al–4V, the surface concentration of Al was increased and that of V decreased compared to the bulk. There is concern about the potential toxic nature of Al and V cations released into the body as a result of metal corrosion of these alloys. Consequently, a new alloy composition, Ti–13Nb–13Zr, was developed in the 1990s for biomedical applications. This alloy has good corrosion resistance, improved biocompatibility and an elastic modulus similar to that of bone [9–11]. Lopéz et al. [9] have investigated the oxide film naturally grown on a Ti–13Nb–13Zr alloy by XPS. Their results showed that Zr was strongly enriched and Nb depleted in the oxide layer. Yu and Scully [10] found that β-Ti–13Nb–13Zr exhibits improved corrosion resistance when compared to other implant alloys such as cp-Ti or Ti–6Al–4V in both a simulated physiological solution (Ringer's solution) and a simulated occluded cell environment (5 M HCl). Khan et al. [11] studied the corrosion behaviour of Ti–6Al–4V, Ti–6Al–7Nb and Ti–13Nb–13Zr in various protein solutions and found that the corrosion resistance of the three alloys is similar in these environments.

Despite their high corrosion resistance and biocompatibility, the surface properties of Ti alloys can be improved by treatments to yield surfaces with more suitable properties for biomedical applications. Calcium compounds, especially hydroxyapatite (HA), are known to promote osseointegration and surface treatments that favour the growth of these compounds on Ti and Ti alloys and are the subject of much current research on biomaterials [1,2,12–14]. Many workers studying osseointegration of Ti alloys agree that the formation of a rougher gel-like layer with a high surface area and increased concentration of Ti–OH surface species, on the outer surface of the passive film of Ti, is important in promoting the growth of apatite [1,2]. Such surface active groups can be formed by different methods; for instance, NaOH treatments have been employed [12,13]. Wang et al. [12] exposed Ti in a 5 M NaOH solution at 60 °C for 24 h and suggested that this treatment results in the formation of an outer sodium titanate gel layer. Subsequent immersion in simulated body fluid (SBF) (a solution containing inorganic ions in concentrations similar to that of human blood plasma) induced bone-like apatite formation. Takadama et al. [13] investigated the formation of apatite on a Ti–6Al–4V alloy using similar NaOH solution conditions, but followed by thermal treatment. Using X-ray diffraction and XPS methods, these authors suggested that the alkaline treatment induces sodium titanate formation, which upon immersion in the SBF solution leads to the formation of surface Ti–OH groups. These groups then induce the growth of calcium titanate and amorphous calcium phosphate.

Another method of promoting the formation of a hydroxide-rich surface is by sol–gel deposition. From dipping Ti substrates into a Ti sol–gel solution and then heating to 500 °C for 10 min, Li et al. [14] deposited a titania gel on Ti. Immersing this surface in SBF solution resulted in formation of a poorly crystallized apatite layer similar to that grown on bone (Ca deficient and with carbonate replacing phosphate and hydroxyl groups in the structure).

Hydrogen peroxide (H_2O_2) can also stimulate the growth of a hydrated Ti oxide surface layer. H_2O_2 is a well-known oxidizing agent, promoting the cathodic reaction:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad [E^\circ = 1.77 - 0.059pH\,(V)]$$

that results in an enhanced corrosion/oxidation rate [15,16]. Exposure to H_2O_2 causes thickening and roughening of the oxide on Ti [17–21].

Implantation of materials into the body causes an inflammatory response and the generation of H_2O_2 by inflammatory cells into the extracellular space is an accepted biochemical mechanism [15]. Tengvall et al. [15,16] proposed that the end-product of Ti implanted into the body is the formation of a duplex oxide with a mostly TiO_2 inner layer and a stable, hydrated TiOOH gel-like oxide outer layer with good ion and protein exchange properties. Proteins, proteoglycans, inorganic ions and other macromolecules will be incorporated into this gel-like outer layer and provide a surface to which fibroblast and osteoblast cells readily attach.

Pan et al. [17,18] studied such processes using surface analytical techniques and their work is of particular relevance to the current study. Pan et al. investigated the effects of exposing commercially pure Ti to a phosphate-buffered saline (PBS) solution, with and without the addition of 100 mM H_2O_2 [17], after exposure to a minimal essential medium (MEM) solution (a solution composed of salts, amino acids, vitamins and other

ID	Title	Pages
2346	Nanocomposite hydroxyapatite formation on a Ti–13Nb–13Zr alloy exposed in a MEM cell culture medium and the effect of H2O2 addition	13