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Influence of fluoride, hydrogen peroxide and lactic acid on the corrosion resistance of commercially pure titanium

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Abstract

Titanium is widely used in dental implantology and orthopaedics due to its excellent corrosion resistance and mechanical properties. However, it has been reported that Ti is sensitive to F^-, H_2O_2 and lactic acid. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) were used to investigate the corrosion resistance of CP-Ti disks after 9 days immersion in different test solutions, based on artificial saliva containing F^- (0.5% and 2.5%), H_2O_2 (0.1% and 10%) and/or lactic acid. Because activated macrophages and bacteria can also release locally some of these oxidative compounds, we investigated the role of these cells when plated onto titanium disks. The surface roughness (R_a) was highly increased when titanium disks were immersed in artificial saliva containing F^-, H_2O_2 and lactic acid. After 21 days of cell culture, R_a was significantly increased on disks incubated with activated-J774.2 cells or *Streptococcus mitis*. AFM appeared to be more sensitive than SEM in evaluating the corrosion of the titanium. Chemical species, either environmental or those released by macrophages and bacteria, can provoke a marked attack of the titanium surface.

Keywords: Titanium; Corrosion; Fluoride; Hydrogen peroxide; Lactic acid

1. Introduction

Nowadays, titanium (Ti) is widely used in biomedicine owing to its excellent corrosion resistance, mechanical properties, and biocompatibility. Titanium alloys (and especially TiAl6V4 syn. TA6V4, containing aluminum and vanadium) are preferred in orthopaedics due to their high toughness, whereas commercially pure titanium (CP-Ti) is favoured in dental implantology. Moreover CP-Ti has been proposed to prepare wires or lingual arches in orthodontic patients with an allergy to the nickel contained in, for example, nitinol (NiTi) [1].

Titanium exhibits a superior corrosion resistance due to the presence of stable and dense titanium oxides, mainly TiO_2, formed on the surface of the metal (passivation) [2]. In a comparative study of 12 different metals and their alloys in contact with corrosive media, it was found that Ti exhibited the lower corrosion tendency in media representing conditions normally found in the human mouth [3]. In the mouth, Ti can be in contact with fluoride ions, F^- (coming from toothpastes, food, drinking water or mouthrinses) [4]. F^- is known to be corrosive for Ti. The F^- content of commercially fluoridated acidic toothpastes, mouthrinses or cariostatic gels is between 0.1% (1000 ppm) to 1% (10,000 ppm) and is most often added as the sodium salt, NaF [5]. It is questionable whether such a high F^- content may be deleterious to implanted Ti devices. In vitro studies have reported that corrosion of CP-Ti in F^--containing prophylactic agents can occur [6,7]. Moreover, in the oral cavity, Ti can be in contact with other compounds able to initiate metal corrosion. Hydrogen peroxide (H_2O_2) is produced by bacteria and leukocytes during inflammatory response and has been shown to attack Ti surfaces [8]. Furthermore, the oral environment is a very complex polymicrobial system [9]. The bacterial strain *Streptococcus*

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mitis can produce lactic acid; it is encountered in dental plaque and is known to be responsible for subgingival diseases [10,11]. Organic acids, and especially lactic and formic acids, have also been reported to cause Ti corrosion [12].

The aim of this study was to investigate by atomic force microscopy (AFM) the effects of F^-, H_2O_2 and lactic acid on the surface roughness of CP-Ti disks. Large surface areas were imaged by scanning electronic microscopy (SEM) and surface analysis was performed by energy dispersive X-ray (EDX). Living cells known to release potentially corrosive products (macrophages and bacteria) were seeded on Ti disks and cultured for 3 weeks. The J774.2 macrophagic cell line and *S. mitis* were chosen in this study.

2. Material and methods

2.1. Surface preparation

Thirty disks (8-mm diameter, 3-mm in thickness) of CP-Ti grade 2 were machined from ASTM B 348-97 bars (Laboratoire Lemouel, Angers, France). The Ti disks were wet-ground with 220 to 4000 grit silicon carbide paper, deburred, and further polished with OP-Chem polishing clothes using the OP-S colloidal silica suspension (all reagents purchased from Struers, Champigny sur Marne, France). The disks were ultrasonically cleaned in deionized water. Disks were washed successively in acetone and deionized water, and sterilized in an autoclave at 121 °C for 20 min. Polished titanium disks were randomized in 10 groups (containing three disks) and incubated in one of the following test solutions or seeded with cells (macrophages and bacteria) and placed in the cell culture oven.

2.2. Test solutions

The electrolyte reference used was Fusayama and Meyer's artificial saliva [13,14]. The composition of this solution, which closely resembles human natural saliva, is given in Table 1. All ingredients were purchased from Sigma–Aldrich Chemical (Illkirsh, France). The pH was adjusted with a BasiC 20 type pH meter (Crison Instrument SA, Barcelona, Spain) and fixed at 5.3. Disks incubated in this control saliva solution formed the SAL group.

The second medium used had the same content but the pH was lowered by adding lactic acid at a concentration known to occur under dental plaque (pH 4.5) [15]. Disks of this series formed the AC group.

Table 1
Composition of the Fusayama–Meyer's artificial saliva

Compounds	Concentration (g/l)
KCl	0.4
NaCl	0.4
$CaCl_2 \cdot 2H_2O$	0.906
$Na_2HPO_4 \cdot 2H_2O$	0.69
$Na_2S \cdot 9H_2O$	0.005
Urea	1

The third and the fourth media were reference saliva enriched with NaF at concentrations of 0.5% (i.e., 5 g/l or 5000 ppm) and 2.5% (i.e., 25 g/l or 25,000 ppm), respectively. Disks of these series formed the NaF 0.5 and NaF 2.5 groups, respectively.

Two additional media were prepared by enriching artificial saliva with H_2O_2 at concentrations of 0.1% and 10%. Disks of this series constituted the H_2O_2 0.1 and H_2O_2 10 groups, respectively.

The last group of disks was incubated in fluoridated, peroxidized and acidified saliva. It contained NaF 0.5%, H_2O_2 0.1% with the pH value set at 4.5 with lactic acid. This group constituted the most unfavorable situation that can be observed, i.e., in the presence of bacteria, inflammatory response and use of fluoridated toothpastes, gels, or rinses. These disks formed the NaF–H_2O_2–AC group.

In all these groups, the test solution was replaced each day during the incubation period.

2.3. Cell cultures

The murine macrophage cell line J774.2 (European Collection of Cell Culture, Salisbury, UK) was maintained in Dulbecco's modified Eagle's medium (DMEM; Eurobio, Les Ulis, France) containing 7.5% heat-inactivated fetal bovine serum (HIFBS; Seromed Biochrom, Berlin, Deutschland), 100 UI/ml of penicillin and 100 μg/ml of streptomycin sulfate (Eurobio). Cells were cultured at 37 °C in a humidified cell culture oven with 5% CO_2. Ti disks were placed in 6-well cell culture plates. Cells were harvested by scraping, and seeded onto Ti disks at 2.10^4 cells/ml, cultured in DMEM (containing HIFBS) and stimulated by 10 μg/ml of lipopolysaccharide (LPS; Sigma–Aldrich Chemical, Illkirsh, France) for 21 days. Control Ti disks were similarly incubated in DMEM without cells for 21 days. The medium was changed twice a week. Bacteria were isolated from a healthy donor. The strain was identified as *S. mitis* after culture on gelose containing fresh blood, and identification with API 20 strep® kit (bioMerieux Lab, Marcy l'étoile, France). 10^6 CFU/ml were incubated at 37 °C on Ti disks during 21 days. Ten ml of Brain–Heart infusion medium (bioMerieux Lab) were used. The medium was changed twice a week by replacing 9 ml with fresh culture medium.

2.4. Atomic force microscopy

At 3, 6 and 9 days of incubation (respectively D3, D6 and D9), disks of each group were collected and rinsed three times in deionized water. The AFM was used in the contact mode with ULCT-AUMT-AB ultralevers (Veeco, Dourdan, France). Images were recorded using an Autoprobe CP-Research apparatus; surface examination was of an area 20 μm × 20 μm. Roughness average (R_a) measurement was done using the Proscan image processing Software 2.1 (ThermoMicrosopes, Sunnyvale, CA, USA). R_a is the mean height as calculated over the entire measured array; it was determined by taking the mean value of six