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Corrosion behavior of titanium in response to sulfides produced by *Porphyromonas gingivalis*

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ABSTRACT

Objective. To investigate the effects of sulfides produced by *Porphyromonas gingivalis* (*P. gingivalis*) on the corrosion behavior of titanium.

Methods. Commercially pure titanium disks were mirror-polished and immersed in culture medium (BHI), spent medium after culturing *P. gingivalis* (BHI-S), and culture medium with *P. gingivalis* (BHI-P), and incubated aerobically at 37 °C for 3–14 days. Titanium corrosion was evaluated through surface observation (using scanning electron microscope: SEM), color change (ΔE*ab), glossiness (Gs(20°)), chemical composition and state (using X-ray photoelectron spectroscopy: XPS), and titanium release.

Results. ΔE*ab and Gs(20°) did not significantly differ among specimens placed in test mediums for the study duration ($p > 0.05$). SEM images of specimens showed no signs of localized or overall corrosion. XPS analysis indicated showed clear titanium metal state peaks on all specimens in addition to sulfide and sulfate on BHI-S and BHI-P specimens. Valency fraction of titanium decomposed from Ti2p spectrum of BHI-S and BHI-P specimens indicated no progression of oxidation. No significant levels of titanium release were found regardless of the mediums' sulfide content. Results suggested that sulfides produced by *P. gingivalis* attached on the surface of titanium specimens but did not cause titanium corrosion over the immersion period of 14 days.

Significance. It is imperative for dental practitioners to be aware of any elements which may influence the clinical success of titanium implants.

1. Introduction

Dental implants have become a common option to restore the function of lost teeth as modifications and technological advances in its development have contributed significantly to its survival rates [1]. The clinical success of dental implants is dependent on biomechanical factors that determine the integrity of the bone/implant [2–4]. Since the discovery of

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osseointegration by Brånemark, titanum and titanium alloys have been used as implant materials, for they possess exceptional mechanical properties such as high strength to weight ratio, superior fatigue strength, tensile strength, and fracture resistance [5–7]. These properties make them very attractive and suitable for the fabrication of dental prosthesis, such as denture frame.

Despite possessing remarkable properties, biologically failed titanium implants which were retrieved from the oral cavity displayed cracks or crack-like defects where implant width and length did not correlate with the observed damage; this suggested that cracks were caused by factors other than mechanical stress [8]. When considering other factors which may contribute to implant failure, periodontitis may be a possible cause since some studies have shown that implant failures are higher in patients with a history of periodontitis [9,10]. Rodrigues et al. studied implants which were retrieved due to peri-impantitis and showed that titanium dental implants were highly susceptible to pitting attack, indicating corrosion occurred in the oral environment [11]. Implant failures caused by titanium corrosion raises the possibility of oral bacteria having an involvement. *Porphyromonas gingivalis* (*P. gingivalis*), among some other microorganisms, is a major pathogen of human periodontitis [12]. *P. gingivalis* are also present in large numbers during biofilm formation on titanium implants, and have shown to be a part of the distinctive bacterial profile in peri-implantitis [12–14]. The microorganism has several virulence factors and release volatile sulfur compounds (VSCs), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), dimethyl sulfide ($(CH_3)_2S$), as a result of their metabolism [15].

In vitro investigations on the effects of sulfide on titanium has been previously performed [16,17]. Sulfide in Na_2S solution induced titanium corrosion depending on its concentration and pH [17]. Corrosion was indicated through the formation of a thick oxide layer on titanium immersed in sulfide alkaline solutions (pH 11.6–12.1), however no corrosion was indicated for sulfide neutral solutions of pH 7.5 [17]. This indicates that even though titanium alloys were corrosion-resistant because of the stability of the titanium oxide layer, they are not inert to corrosive attack. The pourbaix diagram for the Ti aqueous system demonstrates that corrosion resistance of titanium alloys vary with pH with lower corrosion resistance occurring in higher acidity/basicity due to the breakdown of the passive oxide film [18]. This means that when the stable oxide layer is broken down or removed due to environmental conditions, titanium can be as corrosive as many other base metals. Mechanical and biological complications of titanium implants has been attributed to corrosion [19]. Corrosion caused by sulfide could possibly cause implant surface defects or peri-implantitis due to the dissolution of metal followed by induction of metal ions and debris into surrounding tissues.

Studies have reported that the combination of corrosion, stress, and bacteria can trigger peri-implantitis and contribute to implant failure [20]. Corrosion can also compromise the fracture resistance of implants by altering its surface structure increasing their susceptibility to mechanical fatigue [4,21]. The adherence of bacteria and its sub-products has shown to disrupt the passivity with titanium surfaces [20]. Oral bacteria such as *Streptococcus mutans* (*S. mutans*) and *Actinomyces naeslundii* (*A. naeslundii*) significantly reduce the pH of their environment by producing organic acids which break down the oxide layer and compromise the corrosion resistance of titanium [22–24]. *P. gingivalis* are found at higher counts from implants with peri-implantitis [13], therefore its effects on titanium should be investigated.

P. gingivalis has shown to attach on titanium surfaces in vitro [25,26]; however the effect of *P. gingivalis* and its metabolic products on the corrosion of titanium has not yet been investigated. The purpose of this study was to determine the influence of sulfides produced by *P. gingivalis* on the corrosion behavior of titanium.

2. Materials and methods

2.1. Specimen preparation

Commercially available pure titanium disks 1.3 mm thick and 13.0 mm in diameter were prepared from wrought titanium rods (Grade 2, Tokyo Titanium, Tokyo, Japan). The disks were mirror polished (MP) with a polishing machine (Automet 250 and Ecomet 250, Buehler, Tokyo, Japan) using 320–1200 grit silicon carbide paper, 3 μm diamond suspension, and 0.02 μm colloidal silica suspension according to metallographic procedures. Titanium specimens were ultrasonically washed with acetone and distilled water for 5 min then dried. The samples were sterilized with an autoclave prior to immersion in test media.

2.2. Bacterial strains and growth conditions

P. gingivalis ATCC 33277 was maintained in brain heart infusion (BHI) broth supplemented with hemin (5 μg/mL; Sigma Chemical Co., St. Louis, MO, USA), menadione (0.5 μm/mL; WakoPure Chemical Industries, Osaka, Japan), L-cysteine hydrochloride monohydrate (WakoPure Chemical Industries, Osaka Japan), and yeast (10 mg/mL, Becton Dickenson) in an anaerobic chamber (80% N_2, 10% H_2, 10% CO_2).

2.3. Test conditions

BHI was used as a representative immersion medium and each solution was modified accordingly. BHI-S medium was prepared by culturing *P. gingivalis* then separating the bacteria in stationary phase from the culture medium via centrifugation for 5 min (10,000 rpm) at 4 °C. The supernatant layer containing no bacteria was transferred to wells and used as BHI-S. BHI-P medium was prepared by diluting the bacterial suspension with BHI to an optical density of approximately 0.5–0.6 using a spectrophotometer (Ultraspec 2100 pro Amersham Biosciences, New Jersey, USA) at 660 nm, which corresponds to a concentration of approximately 1×10^7 colony forming units (CFU)/mL of viable bacteria by serial dilution and plate counts (Fig. S1).

Titanium specimens were immersed in 3 mL of each test medium, BHI, BHI-S (VSCs only), and BHI-P (bacteria and VSCs), and placed in separate 12-well plates (BD, Basel, Switzerland) (n = 5). Each plate was placed inside sealable plas-

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