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Corrosive effects of fluoride on titanium under artificial biofilm

Azusa Fukushima[a,b], Gen Mayanagi[c], Keiichi Sasaki[a], Nobuhiro Takahashi[b,*]

[a] *Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, Sendai, Japan*
[b] *Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, Sendai, Japan*
[c] *Liaison Center for Innovative Dentistry, Tohoku University Graduate School of Dentistry, Sendai, Japan*

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ABSTRACT

Purpose: This study aimed to investigate the effect of sodium fluoride (NaF) on titanium corrosion using a biofilm model, taking environmental pH into account.
Methods: Streptococcus mutans cells were used as the artificial biofilm, and pH at the bacteria–titanium interface was monitored after the addition of 1% glucose with NaF (0, 225 or 900 ppm F) at 37 °C for 90 min. In an immersion test, the titanium samples were immersed in the NaF solution (0, 225 or 900 ppm F; pH 4.2 or 6.5) for 30 or 90 min. Before and after pH monitoring or immersion test, the electrochemical properties of the titanium surface were measured using a potentiostat. The amount of titanium eluted into the biofilm or the immersion solution was measured using inductively coupled plasma mass spectrometry. The color difference ($\Delta E^{*}ab$) and gloss of the titanium surface were determined using a spectrophotometer.
Results: After incubation with biofilm, pH was maintained at around 6.5 in the presence of NaF. There was no significant change in titanium surface and elution, regardless of the concentration of NaF. After immersion in 900 ppm NaF solution at pH 4.2, corrosive electrochemical change was induced on the surface, titanium elution and $\Delta E^{*}ab$ were increased, and gloss was decreased.
Conclusions: NaF induces titanium corrosion in acidic environment *in vitro*, while NaF does not induce titanium corrosion under the biofilm because fluoride inhibits bacterial acid production. Neutral pH fluoridated agents may still be used to protect the remaining teeth, even when titanium-based prostheses are worn.
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1. Introduction

Fluoride has been shown to help prevent dental caries by enhancing the remineralization of the tooth surface and inhibiting bacterial acid production [1,2], while fluoride may also deteriorate titanium and its alloys. Previous investigations have revealed that immersion in fluoride solutions caused discoloration, dissolution and corrosion of titanium [3,4]. These corrosive changes result from the incorporation of hydrogen fluoride into the oxide layer of the titanium surface, considerably reducing its protective properties [3]. The corrosion of the titanium surface is enhanced not only by high concentrations of fluoride, but also by acidic pH [5]. The oral care products contain fluoride ions at different concentration levels and their pH can range from neutral to acidic values (22,500 ppm F in fluoride varnish, 12,300 ppm F in 1.23% acidulated phosphate fluoride, 1000–1500 ppm F in toothpaste, 900 ppm F in mouth rinses [6,7]). These products have been shown to cause marked discoloration and dissolution of titanium in acidic environment in test tubes [8–10], suggesting not to use fluoride-containing oral care products for patients who have titanium-based dental implants in their oral cavity.

However, these above mentioned studies do not seem to simulate the oral cavity. In the oral cavity, the acidification can be induced mainly in oral biofilm by the acid production through sugar metabolism by microorganisms living in oral biofilm. This microorganism-induced acidification is intermittent, especially occurs when dietary sugars are supplied as meals and snacks, and not continuous as in test tubes. Microorganisms, especially early colonizers of oral biofilm such as *Streptococcus* and *Actinomyces*, inevitably attach to the titanium surface in the oral cavity and induce acidification on the surface through their sugar metabolism, while fluoride can inhibit the bacterial sugar metabolism, thus reducing acidification [11,12]. These contradictory situations in vivo suggest that fluoride solution, which is frequently used as a mouth rinse, could be used in the oral cavity without corrosive

* Corresponding author at: Division of Oral Ecology and Biochemistry, Department of Oral Biology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. Fax: +81 22 717 8297.
E-mail address: nobu-t@dent.tohoku.ac.jp (N. Takahashi).

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reactions of titanium dental implants. To date, however, there has been no study investigating the corrosive effects of fluoride on titanium under conditions mimicking this physiological environment where the bacteria is present as biofilm.

The working hypothesis of the present study was that short-term exposure to sodium fluoride (NaF) does not induce titanium corrosion, because fluoride inhibits bacterial acid production, thus preventing the formation of an acidic environment in which fluoride is known to accelerate titanium corrosion.

Therefore, we aimed to investigate the effect of NaF (900 ppm F or less) on titanium corrosion using a biofilm model of *Streptococcus mutans* by monitoring pH at the bacteria–titanium interface, measuring the amount of eluted titanium and evaluating the electrochemical corrosive properties, color and gloss of the titanium surface.

2. Materials and methods

2.1. Materials

Square samples ($1 \times 10 \times 10$ mm) of commercially pure titanium (ASTM Grade 2, Nishimura Kinzoku, Fukui, Japan) were ground to a mirror polish on one side. These samples were ultrasonically washed in acetone for 30 min and rinsed with deionized water.

2.2. Preparation of bacteria

Streptococcus mutans NCTC10449 were grown as described previously [13]. When the cells reached an exponential growth phase (about 0.5 optical density at 660 nm, corresponding to 1.2×10^8 CFU/mL), they were harvested by centrifugation ($21{,}000 \times g$ for 15 min at 4 °C), washed with 2 mM potassium phosphate buffer (PPB, pH 7.0) and suspended in the same buffer. After incubation at 37 °C for 1 h, cells were washed with 2 mM PPB (pH 7.0). The cell suspension was distributed into 1.5 mL tubes, centrifuged ($16{,}000 \times g$ for 7 min at 4 °C), and kept at 4 °C until use.

2.3. Monitoring of pH

The pH was monitored using an experimental apparatus, as described by Mayanagi et al. [13]. An experimental well (4.0 mm in diameter and 2.0 mm deep) was made of polymethyl methacrylate with a mirror-polished titanium sample at the bottom. An ion-sensitive field-effect transistor (ISFET) pH electrode (H^+ ion-sensitive area, 2.0 mm long, 1.0 mm wide, and 0.2 mm thick; model PH-60T1; Nihon Koden, Tokyo, Japan) was placed on the titanium sample. *S. mutans* cells were packed into the well as an artificial biofilm using a syringe and spatula, and kept at 37 °C for 10 min. Then, 500 μL of 1% glucose or deionized water with or without NaF (Wako Chem., Osaka, Japan; 0, 225, or 900 ppm F) was added to the *S. mutans* cells. The pH was monitored with a pH meter (ISFET mV/pH METER, BAS, Tokyo, Japan) at 37 °C for 90 min. After measuring the pH fall, the remaining glucose or deionized water with or without NaF was absorbed using filter paper, and the contents of the well, including bacterial cells, were collected by pipetting with 500 μL of 2 mM PPB (pH 7.0) and preserved at −80 °C.

2.4. Immersion test

To compare the corrosive effect of fluoride on titanium under an artificial biofilm with that of immersion in a buffer solution, taking environmental pH into consideration, eight immersion test solutions were prepared. The fluoride concentrations of the test solutions were 0, 225, or 900 ppm F (NaF) with 1% glucose, and the pH of the solutions was adjusted to 4.2 or 6.5 by the addition of lactic acid at 37 °C.

The titanium samples were immersed in 5 mL of each test solution in a polystyrene bottle for 30 or 90 min at 37 °C. After immersion, the samples were removed from the solutions, gently rinsed with deionized water. An aliquot of each immersion solution (500 μL) was preserved at −80 °C.

2.5. Electrochemical measurements on the titanium surface

Before and immediately after pH monitoring or immersion, the electrochemical properties of the titanium surface were measured using an electrochemical cell made of polymethyl methacrylate with artificial saliva (NaCl: 6.8 mM, KCl: 5.4 mM, $CaCl_2 \cdot 2H_2O$: 5.4 mM, $Na_2S \cdot 9H_2O$: 0.02 mM, $NaH_2PO_4 \cdot 2H_2O$: 4.4 mM, Urea: 17 mM) [14] as an electrolytic solution at room temperature, which was connected to a computer-controlled potentiostat (VersaSTAT4, Princeton Applied Research, Oak Ridge, TN, USA). All measurements were conducted within 5 min after removing bacterial cells from the titanium surface or after immersion using the standard 3-electrode cell method [Ag/AgCl as the reference electrode, Pt as the counter electrode, and the exposed surface (3.14 mm^2) of the titanium sample as the working electrode]. Initially, open circuit potential (OCP) was monitored for 10 min, according to Japanese Industrial Standards [15]. After the OCP was stabilized, polarization curves [electric potential *vs.* log (absolute current)] were obtained three times from each sample at the potential range from −250 mV *vs.* OCP to +250 mV *vs.* OCP at a scanning rate of 5 mV/5 s. From the polarization curves, the corrosion current was determined by Tafel's method using VersaStudio software (Princeton Applied Research), in which the intersection of the slopes of cathodic and anodic polarization curves was the corrosion current on the logarithmic scale. From the linear potentiodynamic polarization plots (potential *vs.* absolute current), the passive current was determined as the inflection point of the plots, and the polarization resistance was determined as the slope of the plots using VersaStudio software (Princeton Applied Research).

2.6. Measurement of eluted titanium

The amount of titanium eluted into *S. mutans* cells or the immersion solution was measured by inductively coupled plasma mass spectrometry (Agilent 8800, Agilent, Tokyo, Japan; Technical Division, School of Engineering, Tohoku University). The preserved *S. mutans* cells suspension or immersion solution (500 μL) were mixed with 2 mL of 61% nitric acid and 30% H_2O_2 and decomposed by heating (140 °C, 3 h). Samples were diluted to a total volume of 10 mL with ultrapure water. A calibration standard containing nitric acid, H_2O_2, magnesium, potassium, calcium and phosphorus, and an internal standard containing scandium were prepared in the same manner. The detection limit of titanium was 1.65 ppb.

2.7. Discoloration and gloss measurements of the titanium surface

Before and immediately after exposure to bacterial cells or immersion, the color difference (ΔE^*ab) and gloss of the titanium surfaces were determined using a spectrophotometer (CM-700d, Konica Minolta, Tokyo, Japan) with a light source of D65 illuminant, a diameter of 3 mm and an 8° observer [16,17]. The color difference (ΔE^*ab) between before and after exposure to bacterial cells or the immersion test was determined according to the CIE $L^*a^*b^*$ colorimetric system [18].

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