

Effect of fluoride-containing gel on the roughness of a titanium surface and the promotion of bacterial growth

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Purpose: The aim of this study was to evaluate whether fluorides at various pH cause changes in the surface roughness of titanium implants that alter the adherence of bacterial biofilms. **Materials and Methods:** The titanium disks were assigned randomly to the following seven groups according to the fluoride agents and application time (1 minute or 30 minute) used: control (no treatment); group 1 (1.23% acidulated phosphate fluoride [APF] at pH 3.5 for 1 minute); group 2 (1.23% APF at pH 3.5 for 30 minute); group 3 (1.23% APF at pH 4.0 for 1 minute); group 4 (1.23% APF at pH 4.0 for 30 minute); group 5 (2% NaF gel at pH 7.0 for 1 minute); group 6 (2% NaF gel at pH 7.0 for 30 minute). The surface roughness of the titanium disks and the amount of adherent bacteria were measured. **Results:** Group 2 showed a significantly greater surface roughness than the control group ($P < 0.0001$). No significant differences in the amount of surface bacteria were observed between the treated samples and the controls. In addition, there were no significant differences in bacterial adherence relative to the incubation period between the treated samples and the controls. **Conclusion:** The surface roughness of the titanium disks was significantly greater after treatment with APF at pH 3.5 for 30 min compared with that of the controls. In addition, we found that the amount of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* was similar among all groups (*J Dent Rehabil Appl Sci* 2016;32(1):16-23)

Key words: fluorides; titanium; biofilms; bacterial adhesion

Introduction

The replacement of missing teeth with implant-borne restorations has become a valid and predictable treatment option for the rehabilitation of fully and partially edentulous patients. Several longitudinal studies of implant restorations have been published, with promising results.¹⁻³

Peri-implant disease is a biological complication of

implant treatment. Peri-implant diseases may affect the peri-implant mucosa only (peri-implant mucositis) or these diseases may also involve the supporting bone (peri-implantitis).^{4,5} Biofilms accumulate on all surfaces in the oral cavity, including artificial or dental materials and the accumulation of bacterial biofilms has been identified in numerous studies as the primary etiological factor for the development and progression of peri-implant diseases.⁶⁻⁹ Peri-implant

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diseases following successful integration of endosseous implants are the result of an imbalance of bacterial load and host defense, and may lead to the loss of osseointegration.⁵

Bacterial adhesion to intra-oral, hard surfaces is influenced by surface roughness. A positive correlation between surface roughness and the rate of plaque accumulation has been observed.^{10,11} In a literature review of the impact of surface characteristics on biofilm formation, the authors concluded that rougher surfaces accumulate and retain more plaque, and, after several days of undisturbed plaque formation, rough surfaces harbor a more mature plaque characterized by a greater proportion of rods, motile organisms, and spirochetes.¹² On a rough surface, attached bacteria can survive longer because they are protected against mechanical shear forces and oral hygiene methods.¹³

The roughness of intra-oral hard surfaces, such as natural teeth, restorations, and prosthetic materials, can be modified by routine dental procedures including mechanical debridement, polishing, finishing, and use of fluoride for caries prevention.¹⁴⁻¹⁹ Of these procedures, fluoride prophylactic agents can cause corrosion and change in the surface roughness of titanium and titanium-based alloys, even though titanium has high corrosion resistance due to a thin, stable oxide layer.¹⁸⁻²¹ As a supportive periodontal therapy (SPT), topical fluoride is applied to prevent root caries, especially in areas with gingival recession. Since the fluoride is commonly applied using disposable trays, it may contact not only natural teeth, but also the titanium surface of implant abutments. As mentioned above, the contact with fluoride may lead to an increase in the roughness of the titanium surface, which may be followed by an increase in bacterial biofilm accumulation. However, few studies have evaluated the effect of fluoride therapy on titanium surface topography and the adherence of bacterial biofilm to the titanium.

In this study, we treated titanium surfaces with fluorides at various pH, and evaluated changes in the surface roughness and the adherence and colonization of bacterial biofilm caused by fluoride treatment.

Materials and Methods

Preparation of titanium disks

Titanium disks measuring 5 mm in diameter and 1 mm in thickness were used. The specimens were ground wet with 300- and 800-grit silicon carbide papers, and then were cleaned ultrasonically twice in ethanol for 15 minutes and washed with distilled water.

Three commercially available fluoride agents were used: a) 1.23% acidulated phosphate fluoride (APF) gel at pH 3.5 (Topex[®], Sultan, York, USA); b) 1.23% APF gel at pH 4.0 (60 second[®], Germiphene, Brantford, Canada); and c) 2% sodium fluoride (NaF) gel at pH 7.0 (GEL7[®], Germiphene). The titanium disks were assigned randomly to seven groups (Table 1): control group (no treatment); group 1 (the disks were treated with 1.23% APF at pH 3.5 for 1 minute); group 2 (the disks were treated with 1.23% APF at pH 3.5 for 30 minutes); group 3 (the disks were treated with 1.23% APF at pH 4.0 for 1 minute); group 4 (the disks were treated with 1.23% APF at pH 4.0 for 30 minutes); group 5 (the disks were treated with 2% NaF gel at pH 7.0 for 1 minute); group 6 (the disks were treated with 2% NaF gel at pH 7.0 for 30 minutes). For each group, 20 titanium disks were used. After fluoride gel treatment, samples in the experimental groups were removed from the fluoride-containing gel, washed thoroughly with distilled water, and dried.

Table 1. Distribution of the groups according to the fluoride-containing gels and immersion times

Group (n = 20)	Fluoride-containing gel	Immersion time
Control	-	-
1	APF (pH 3.5)	1 minute
2	APF (pH 3.5)	30 minutes
3	APF (pH 4.0)	1 minute
4	APF (pH 4.0)	30 minutes
5	NaF (pH 7.0)	1 minute
6	NaF (pH 7.0)	30 minutes

APF, acidulated phosphate fluoride; NaF, sodium fluoride.

Bacterial adherence assay

Porphyromonas gingivalis, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* were used to examine bacterial adherence to the disks. *A. actinomycetemcomitans* ATCC 33384, *F. nucleatum* ATCC 23726, and *P. gingivalis* ATCC 33277 obtained from Korean Collection for Oral Microbiology (Chosun University, Gwangju, Korea) were used. Brucella blood agar plate (KOMED, Sungnam, Korea) was used for the culturing of *P. gingivalis*, *F. nucleatum* and *A. actinomycetemcomitans*. Colonies of *P. gingivalis*, *F. nucleatum* and *A. actinomycetemcomitans* incubated in an anaerobic environment (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc., Cornelius, USA) with an atmosphere of 90% N₂, 5% CO₂, and 5% H₂ were suspended in reduced phosphate buffered saline (tPBS), pH 7.2 to a turbidity equivalent to a McFarland 1.0 index. Fifty microliters of this suspension was added each well of 24-well plates containing reduced Brain Heart Infusion broth (2 mL) and the titanium disks. After 1, 3, and 5 days of anaerobic incubation, the quantity of bacterial protein was determined using the Bradford protein assay (Sigma-Aldrich, St. Louis, USA) as follows. After anaerobic incubation, the specimens were washed gently twice with 1 mL of PBS in fresh 24-well plates. To remove adhering bacteria, the disks were incubated with 1 mL of lysis buffer (20 mM Tris-HCL, pH 7.5, 150 mM NaCl, 1 mM Na₂EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, and 1 μ g/mL leupeptin) for 10 minutes in 48-well plate.¹ Five microliters of this lysis buffer solution was transferred to the 96-well plates and 250 μ L of the Bradford reagent was added to each well. The Bradford reagent and protein were mixed on a shaker for 30 minutes and the absorbance was measured at 595 nm (ELx 800™ Absorbance microplate reader, BioTek, Winooski, USA).

Surface roughness measurements and scanning electron microscopy

The surface roughness of each titanium disk was evaluated using a SufTest SJ-400 (Mitutoyo, Kawa-

saki, Japan).

Scanning electron microscopy (SEM) was performed to visualize the bacterial colonization. The disks were incubated at 37°C in bacterial culture medium inoculated with *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans* for 5 days in anaerobic conditions in 24-well culture plates, then washed twice with PBS. Disks with attached bacteria were fixed in 2.5% glutaraldehyde in PBS for 1 hour at room temperature. The fixed samples were then washed three times with PBS for 10 minutes, and dehydrated for 30 minutes in a graded series of ethanol. After critical point drying, the samples were mounted on stubs, coated with gold, and observed by SEM. The surface of each disk was examined by variable pressure field emission scanning electron microscopy (VP-FE-SEM) SUPRA55VP (Carl Zeiss, Oberkochen, Germany) ($\times 10,000$, $\times 30,000$).

Statistical analysis

The data were analyzed using SPSS™ Version 19.0 (IBM Inc., Armonk, USA). One-way analysis of variances (ANOVA) test and Tukey's post-hoc tests were applied to assess both the effect of the fluoride-containing gel on the surface roughness of the titanium disks and difference in the amount of bacteria on the treated titanium disks. The level of significance was $P < 0.05$.

Results

Surface roughness measurements

Surface roughness values (mean \pm SD) for all groups are listed in Table 2. Only group 2 (1.23% APF at pH 3.5 for 30 minutes) showed significantly greater surface roughness compared to the control group ($P < 0.0001$).

Protein concentration measurements

Bacterial adherence assay was performed on disks from the control group, group 2 (1.23% APF at pH 3.5 for 30 minutes), group 4 (1.23% APF at pH 4.0

Table 2. Surface roughness of titanium disk

Group	Control	APF (pH 3.5)		APF (pH 4.0)		NaF (pH 7.0)	
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Ra (μm)	0.19 \pm 0.08	0.21 \pm 0.08	0.31 \pm 0.03*	0.23 \pm 0.07	0.22 \pm 0.09	0.22 \pm 0.08	0.21 \pm 0.09

Values are presented as mean \pm standard deviation.

* $P < 0.05$.

APF, acidulated phosphate fluoride; NaF, sodium fluoride.

for 30 minutes), and group 6 (2% NaF gel at pH 7.0 for 30 minutes). For each experiment period (1, 3, and 5 days), four titanium disks per group were used. The results of the adherence assay are shown in Fig. 1. At 1, 3, and 5 days of incubation, the protein content on the surface of the disks showed no significant difference between any experimental group and controls. Also, there were no significant differences in bacterial adherence between incubation periods.

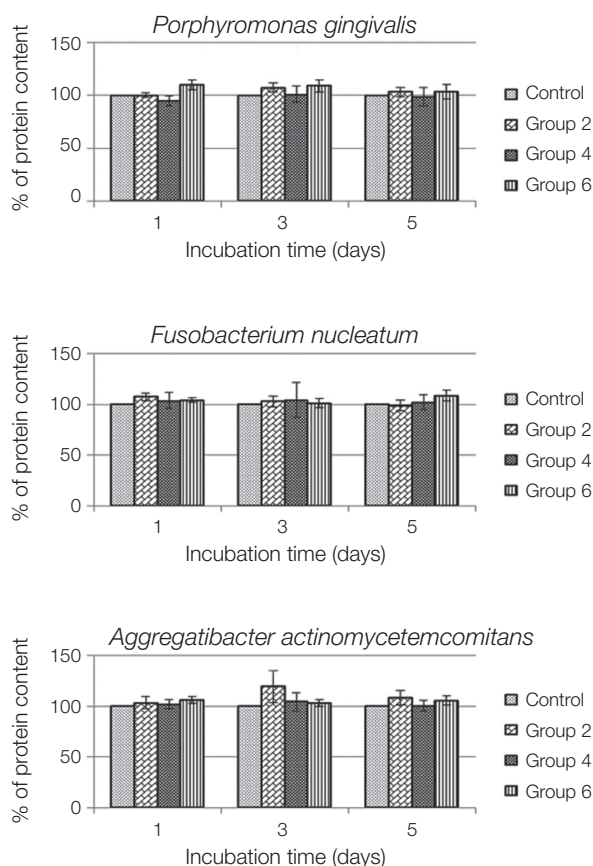


Fig. 1. Percentage of bacterial protein content on differently treated titanium samples relative to the amount measured on the control samples. The protein content measurements showed no significant differences in the amount of bacteria on the treated samples compared with that of the controls.

Scanning electron microscopy

Disks were incubated with bacteria for 5 days and used for surface evaluation under a SEM. SEM images of the specimens are shown in Fig. 2 - 4. Most of bacteria appeared as one, discontinuous layer on the surface of the titanium disks. However, some of bacteria are arranged in multiple layers. The attached bacteria were distributed evenly on the surface of the titanium disks, with no particular orientation with respect to the surface. All bacterial strains examined were attached to the titanium surface by their outer membrane. SEM images of the experimental groups showed no substantial difference compared with that of controls.

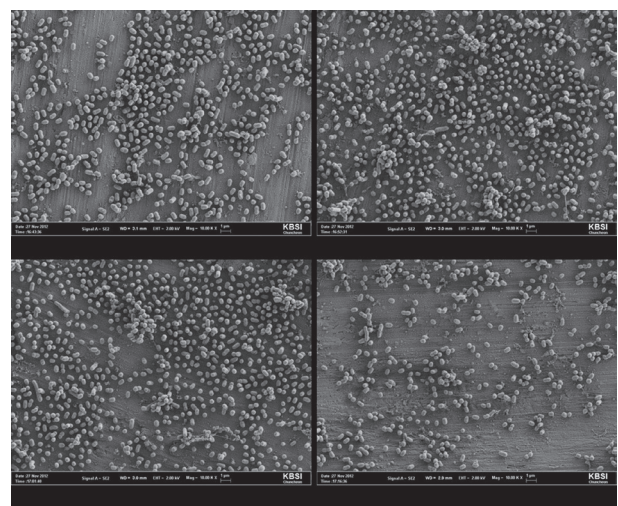


Fig. 2. SEM images of *P. gingivalis* on the titanium surface (Magnification: $\times 10,000$). SEM images showed no substantial difference among the 4 groups.

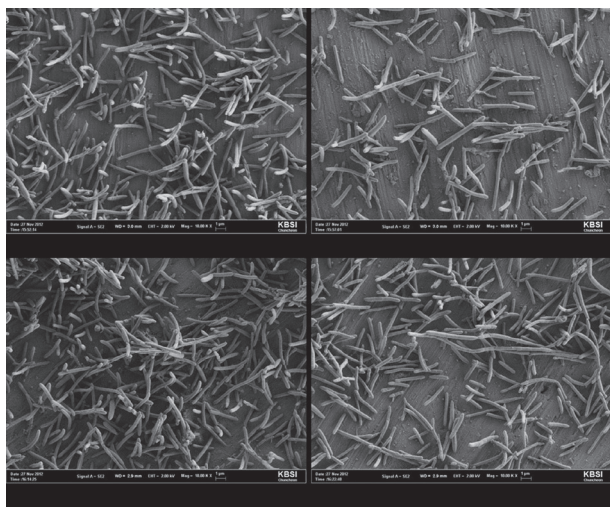


Fig. 3. SEM images of *F. nucleatum* on the titanium surface (Magnification: $\times 10,000$). SEM images showed no substantial difference among the 4 groups.

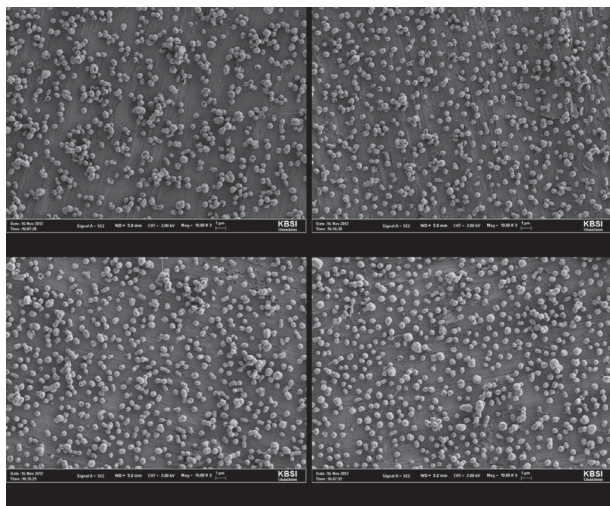


Fig. 4. SEM images of *A. actinomycetemcomitans* on the titanium surface (Magnification: $\times 10,000$). SEM images showed no substantial difference among the 4 groups.

Discussion

The recession of soft tissue in the oral cavity due to age, traumatic tooth brushing habits, and periodontal disease or treatment results in a greater area of tooth surfaces that are at risk for the development of root caries. According to epidemiologic studies, root caries is prevalent among patients with treated and untreated periodontal disease.^{22,23} Therefore, professional fluoride application is recommended to prevent root caries. Topical fluoride agents such

as stannous fluoride (SnF_2), NaF, and APF are used in the professional fluoride application procedure. APF is produced by acidulation of a NaF solution with phosphoric acid. The acidic pH of APF etches the tooth surface to allow for more uptake of fluoride compared with the neutral pH of NaF. In some studies, the topical application of APF agents has been shown to be an effective method to reduce the incidence of dental caries in test populations.^{24,25} Currently, the use of these agents, particularly as gels, is popular in dental clinics. The pH of the rinses and gels used for caries prevention in dentistry are usually between 3.5 and 7.0, and the fluoride concentration is between 1,000 and 10,000 ppm.²⁰

In this study, titanium disks were treated with fluoride gel for 1 or 30 minutes. The application time of the fluoride gel was determined to simulate a clinical situation. In SPT visits, patients were usually treated with fluoride gel for 1 minute. And 30 minutes of application time represent delayed application of fluoride without cleaning. We ordered to patients in SPT without gargling for 30 minutes after application of fluoride.

The influence of fluoride agents on the titanium surface has been examined previously. High fluoride concentrations and an acidic pH destroy the corrosion resistance of titanium, as crevice and pitting corrosion can also occur.^{26,27} Pröbster et al.²¹ described that acidic fluoride agents cause severe surface damage similar to that of hydrofluoric acid. The authors suggested that acidic fluoride agents should not be used in patients with titanium implants or restorations. Stájer et al.²⁰ also suggested that agents with high fluoride concentrations at an acidic pH increase the roughness of the titanium surface. On the other hand, Toniollo et al.²⁸ demonstrated that the use of a fluoride-containing solution at neutral pH as a mouthwash does not damage the surface of cast CP titanium and can be used by patients with titanium-based restorations. In the present study, the surface roughness of the disks in the group treated with APF at pH 3.5 for 30 minutes was significantly greater than that observed in controls. However, the disks treated with APF at pH 4.0 and with NaF at pH 7.0 did not show a significant difference from

controls regardless of the application times.

The increase in surface roughness of the titanium disks is a clinically important finding because it influences the possibility of adhesion of residues and microorganisms to the surface. In many studies, a positive correlation between surface roughness and the rate of plaque accumulation has been reported.^{10,11,29} Kim et al.³⁰ reported that a 1.23% APF gel caused a significant increase in the adherence of *Streptococcus mutans* and *Streptococcus sobrinus* to resin composites. However, Ståjer et al.²⁰ reported no significant difference in the amount of bacteria (*P. gingivalis*) on fluoride agents-treated titanium disks compared with controls, although the surface roughness of the titanium disks was significantly different.

Our results showed no difference in the amount of *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans* on the treated titanium disks compared with controls, despite the observation that the surface roughness of group 2 titanium disks was significantly greater than the other groups and controls.

While fluoride-containing agents can cause corrosion and change in the surface structure of titanium, the effect seems to be dependent on pH and the concentration of fluoride ion. The surface roughness of titanium may increase when treated with a high concentration of fluoride at an acidic pH. Although some studies have reported that an increased roughness of a titanium surface increases the adherence of bacteria to the surface, our results showed no significant difference in the amount of bacteria between the treated samples and the controls. Together, the results suggest that, the professional fluoride application during SPT visits for preventing root caries is reasonable, even in patients with titanium-based restorations.

Conclusion

The surface roughness of titanium disks is increased by treatment with APF at pH 3.5 for 30 minutes, but not by treatment using other fluoride agents or more basic pH solution. In addition, we found that the amount of *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans* was similar among all groups.

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불소함유 겔이 티타늄 표면의 세균성 바이오필름 성장에 미치는 영향

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목적: 이번 연구는 다양한 pH의 불소 제제들이 티타늄 표면 거칠기에 미치는 영향을 평가하는 것이었다.

연구 재료 및 방법: 기계절삭형 티타늄 디스크를 시중에서 유통되는 세 가지 불소겔로 처리하였다. 불소겔의 종류와 처리 시간에 따라, 대조군, 1군(pH 3.5의 APF로 1분간 처리), 2군(pH 3.5의 APF로 30분간 처리), 3군(pH 4.0의 APF로 1분간 처리), 4군(pH 4.0의 APF로 30분간 처리), 5군(pH 7.0의 NaF로 1분간 처리), 6군(pH 7.0의 NaF로 30분간 처리)의 7군으로 분류하였다. 디스크의 표면 거칠기를 측정한 후, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*을 배양하여 디스크에 부착하는 세균의 양을 측정하였다.

결과: 표면 거칠기는 그룹2에서만 유의하게 증가하였다($P < 0.0001$). 세균의 부착량은 실험군과 대조군 사이에 유의한 차이를 보이지 않았다.

결론: pH 3.5의 APF를 30분간 처리한 그룹에서 표면 거칠기가 유의하게 증가하였지만, 세균의 부착에 대해서는 유의한 차이를 보이지 않았다.

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주요어: 불소; 티타늄; 표면 거칠기; 바이오필름

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