
Corrosive effects of fluoride on titanium: Investigation by X-ray photoelectron spectroscopy, atomic force microscopy, and human epithelial cell culturing

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Received 21 December 2006; revised 1 June 2007; accepted 31 August 2007

Published online 9 January 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.31764

Abstract: High fluoride (F^-) concentrations and acidic pH impair the corrosion resistance of titanium (Ti). Effects of F^- -containing caries-preventive prophylactic rinses, and gels on Ti were investigated by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). Human epithelial cell attachment and proliferation were investigated by dimethylthiazol-diphenyl tetrazolium bromide (MTT) and protein content assays. Aqueous 1% NaF solution (3800 ppm F^- , pH 4.5) or high (12,500 ppm) F^- content gel (pH 4.8) strongly corroded the surface and modified its composition. XPS revealed formation of a strongly bound F^- -containing complex (Na_2TiF_6). AFM indicated an increase in roughness (R_a) of the surfaces: 10-fold for the NaF solution and smaller for the gel or a mouthwash (250

ppm F^- , pH 4.4). MTT revealed that cell attachment was significantly increased by the gel, but was not disturbed by either the mouthwash or the NaF. Cell proliferation determined by MTT decreased significantly only for the NaF-treated samples; protein content assay experiments showed no such effect. This study indicates that epithelial cell culturing results can depend on the method used, and the adverse effects of a high F^- concentration and low pH should be considered when prophylactic gels are applied by patients with Ti implants or other dental devices. © 2008 Wiley Periodicals, Inc. *J Biomed Mater Res* 87A: 450–458, 2008

Key words: dental implant; fluoride; corrosion of titanium; epithelial cell culture; surface analysis

INTRODUCTION

Titanium (Ti) and its alloys are widely used as medical or dental implants in consequence of their good biocompatibility, excellent corrosion resistance, and appropriate mechanical properties.¹ Endosseous dental implants and surgical implants for fixating or replacing hard tissue are made from “commercially

pure” Ti (CP Ti) and the most common Ti alloy, Ti-6Al-4V.^{2,3} Ti is also used in prosthetic dentistry to manufacture crowns and multiple-unit fixed restorations,^{4,5} and in orthodontic dentistry to produce Ti brackets.⁶ Dental arch wires and orthopedic braces are usually made from the special TiNi shape memory alloy.^{2,3}

Ti and its alloys are resistant to corrosion because of the formation of an insoluble titanium oxide layer on the surface.⁷ In air, the oxide, usually TiO_2 , begins to form within nanoseconds (10^{-9} s) and reaches a thickness of 20–100 Å in 1 s. It is very adherent to the parent Ti and impenetrable to oxygen.⁸

Oxidative agents are well known to exert a corrosive effect on the alloys used in dentistry, with the exceptions of Ti and other bioinert materials. Indeed, oxidative processes can thicken and condense the titanium oxide layer on the surface, improving the corrosion stability of the underlying Ti. On the other hand, reductive agents, such as fluoride (F^-), may have the opposite effect and attack this layer. Strietzel et al.⁹

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Contract grant sponsor: SIMI-NAS Project of the 5th FWP of the European Commission (Growth Program); contract grant number: GRD3-2001-61801

Contract grant sponsor: EC and Hungarian Ministry of Economy; contract grant number: GVOP-3.2.1.-2004-04-0408/3.0

Contract grant sponsor: Hungarian Ministry of Health (ETT Project); contract grant number: 434/2006

Contract grant sponsor: Hungarian Scientific Research Fund; contract grant number: OTKA F-68440

demonstrated that Ti ion release was enhanced in the presence of F^- , and this effect was even further accelerated at low pH. High F^- concentrations and an acidic pH are known to impair the corrosion resistance of Ti,¹⁰ and as a result crevice and pitting corrosion occur.^{11,12}

Patients regularly use different oral care products containing F^- , such as toothpastes, rinsing solutions, or prophylactic gels. The Ti alloys applied in the form of orthodontic wire,^{13,14} or as the framework of a prosthesis, therefore come into contact with a wide range of preventive agents and these F^- -containing materials can attack the surface of Ti.^{15,16} SEM investigations have revealed that topical F^- solutions can cause stress corrosion cracking on CP Ti.¹⁷ Galvanic corrosion has been reported to occur between orthodontic wires and brackets (NiTi and CuNiTi) immersed in fluoride mouthwashes.¹⁸ Such corrosion has two undesirable consequences: the mechanical performance of the wire-bracket system deteriorates, and the risk of local Ni^{2+} release is increased.

Moreover, such F^- -containing agents may come into contact with the neck part of dental Ti implants, which may extend into the oral cavity (Fig. 1). The long-term success of dental implants depends to a large extent on the gingival attachment to the neck of an implant. This mucosal seal ensures protection against bacterial attack and other injurious effects exerted by the oral environment. The epithelial attachment (junctional epithelium) may be anchored onto a rough or a smooth surface by hemidesmosomes through a preformed glycoprotein layer. A rough surface is more favorable for the plaque accumulation in the peri-implant crevices of the gingiva, which is an undesired effect in this very sensitive region of the implant. Accordingly, to avoid pathogenic plaque accumulation, the neck of an implant must be polished.^{19,20} From this respect, it is easy to realize the great importance of the maintenance of the continuity of these surfaces.

In 1999, Nakagawa et al.²¹ found a relation between the F^- concentration and the pH at which the corrosion of CP Ti occurred. The results of their anodic polarization and immersion tests indicated that the corrosion of Ti in a F^- -containing solution depends on the concentration of hydrofluoric acid (HF). The passivation film on Ti was destroyed when the HF concentration in the solution was >30 ppm. In 1995, Boere¹⁵ had demonstrated that the corrosion of Ti is enhanced in an acidic environment, because F^- in solution combines with H^+ to form HF, even if the NaF concentration is low.

Nakagawa et al.²² investigated the corrosion behavior of Ti alloys: Ti-6Al-4V, Ti-6Al-7Nb, and the new alloy Ti-0.2Pd. Their experimental results demonstrated that even a low F^- concentration causes corrosion in an acidic environment. If Ti alloy contains at

least 0.2% Pd, this process does not take place. The high corrosion resistance of this alloy is because of the surface enrichment of Pd promoting the repassivation of Ti.

The studies by Huang²³ indicated that, when the NaF concentration was $>0.1\%$, the protectiveness of TiO_2 on Ti was destroyed by F^- , leading to the severe corrosion of Ti. In 2003, Huang²⁴ investigated the effects of F^- and albumin concentrations on the corrosion resistance of Ti-6Al-4V in acidic artificial saliva (pH 5). The X-ray photoelectron spectroscopy (XPS) results showed that when the NaF concentration was $>0.1\%$, a hexafluorotitanate complex (Na_2TiF_6) was formed on the Ti surface, which destroyed the stable TiO_2 layer.

As the pH of the rinses and gels used for caries prevention in dentistry ranges from 3.5 up to neutral, and the F^- concentration in these materials is between 1000 and 10,000 ppm,²¹ it is essential for the dental practitioner to know whether a F^- -containing material can attack the Ti surface or can modify the corrosion resistance of the Ti surface of a dental implant, a prosthesis, or the wires of orthodontic braces. Besides 0.1–0.15% (1000–1500 ppm) F^- , toothpastes contain other constituents, such as rubbing, cleaning, foaming materials, and calcium complexes, which reduce the effectiveness of toothpastes by 25–50%.²⁵

Although all the above-mentioned studies point to the deleterious effect of F^- -containing prophylactic gels, there are a huge number of data documenting that F^- exerts a bone-promoting activity. Ellingsen et al. proved that, when F^- is incorporated in the titanium oxide layer, the retention of implants is significantly increased, even as compared with rough surface implants.^{26,27} The success of a TiO_2 -blasted surface with a F^- -modified TiO_2 layer (OsseoSpeed implants, Astratech) is because of the ability of the F^- coating to stimulate the bone response, leading to binding between Ti and the phosphate from tissue fluids. The free F^- catalyzes this reaction and induces the formation of fluoridated hydroxyapatite and fluorapatite in the surrounding bone.²⁶

The studies by Cooper et al., demonstrated that the F^- modification of TiO_2 grit-blasted CP Ti surfaces enhanced osteoblastic differentiation and interfacial bone formation.²⁸ As far as we are aware, there has so far been no study of the behavior of epithelial cell growth on F^- -treated Ti implant surfaces.

In this work, the effects of different F^- -containing caries-preventive prophylactic rinses and gels on the surface structure and roughness of CP Ti were investigated, through the use of XPS and atomic force microscopy (AFM). A further aim was to survey the attachment and proliferation of human epithelial cells after treatment of the Ti surface with an acidic NaF solution, a widely used F^- -containing mouthwash or a gel. The epithelial cell attachment and proliferation

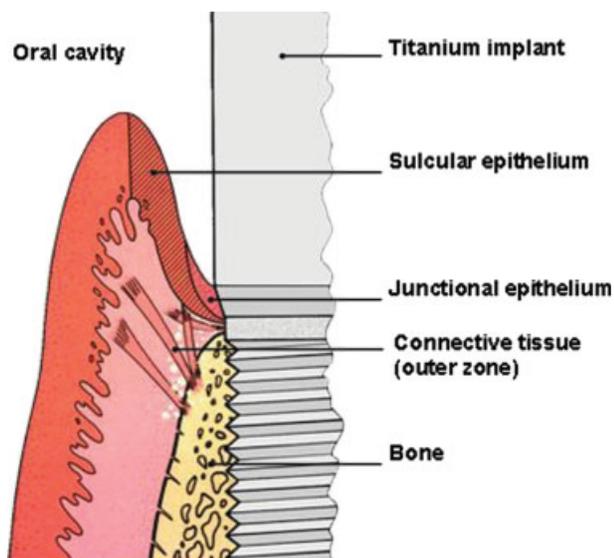


Figure 1. Epithelial attachment on a Ti implant surface, illustrating that F^- -containing agents may come into contact with the neck part of dental Ti implants, which may extend into the oral cavity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

were examined by means of dimethylthiazol-diphenyl tetrazolium bromide (MTT) and protein content assays. For the visualization of cells, scanning electron microscopy (SEM) was applied.

MATERIALS AND METHODS

Ti discs (9 mm in diameter and 2 mm in thickness) were made from implant material (CP grade 4, CAMLOG Biotechnologies AG, Switzerland). The discs were mechanically polished to a surface roughness not exceeding $0.2 \mu\text{m}$, the roughness needed for the neck of a dental implant.²⁰ The discs were cleaned in acetone and absolute ethanol in an ultrasonic bath for 15 min. Each sample was immersed for 1 h in one or other of (1) a caries-preventive prophylactic mouthwash

(Elmex, GABA International AG, Switzerland) containing 250 ppm F^- (pH 4.4), which contains fluoride in form of Olaflur (bis-(hydroxyethyl)-aminopropyl-*N*-(hydroxyethyl)-octadecylamin dihydrofluoride) and potassium-fluoride; (2) an aqueous solution of 1% NaF (3800 ppm F^- , pH 4.5), where the pH of the NaF solution was set to 4.5 with lactic acid; or (3) a gel (Elmex, GABA GmbH, Germany) containing a total of 12,500 ppm (1.25%) F^- [pH 4.8 (10% in water); www.gaba.com], 2500 ppm (0.25%) in the form of the amine fluorides Olaflur and Dectaflur (hexadecylamine hydrofluoride), and the rest in the form of sodium fluoride (1%).

After 1 h, the samples were removed from the F^- -containing medium, thoroughly washed with ultrapure water and dried. As the suggested use of the prophylactic rinse and the gel is once a day for 30 s and once a week for 2 min, respectively, our application time corresponds to the accumulated effect of regular usage of 4 months for the rinse and 7.5 months for the gel. The 1-h treatment time seems to be too long, but if we take in consideration that these prophylactic solutions are not rinsed after application, then we may think about shorter cumulative periods.

The chemical composition of the Ti surfaces was studied by XPS. The photoelectrons were generated by Mg $K\alpha$ primary radiation ($h\nu = 1253.6 \text{ eV}$) and were analyzed with a hemispherical electron energy analyzer (Kratos XSAM 800). The X-ray gun was operated at 210 W (14 kV, 15 mA). The binding energies were normalized with respect to the position of the C (1s) peak of adventitious carbon, which was taken as 285.1 eV. The changes in the XPS spectra were measured after 10 min of Ar^+ bombardment, which was repeated several times. Ar^+ was generated with an ion gun energy of 3 kV, and the incident ion beam current density was $4 \mu\text{A}/\text{cm}^2$. The bombardment led to the removal of about 10 nm from the surface material in 10 min. Wide-range scans and higher-resolution narrow scans of the Ti 2p characteristic peaks were recorded.

A PSIA XE-100 atomic force microscope (South Korea) was used to acquire information on the roughness of the sample surface. AFM offers a new tool to study these surfaces on the micron to nanometer scale, using a technique that measures forces on the AFM probe tip as it approaches and retracts from the investigated surface. The tips were contact silicon cantilevers (type: P/N 910M-NSC36) purchased from

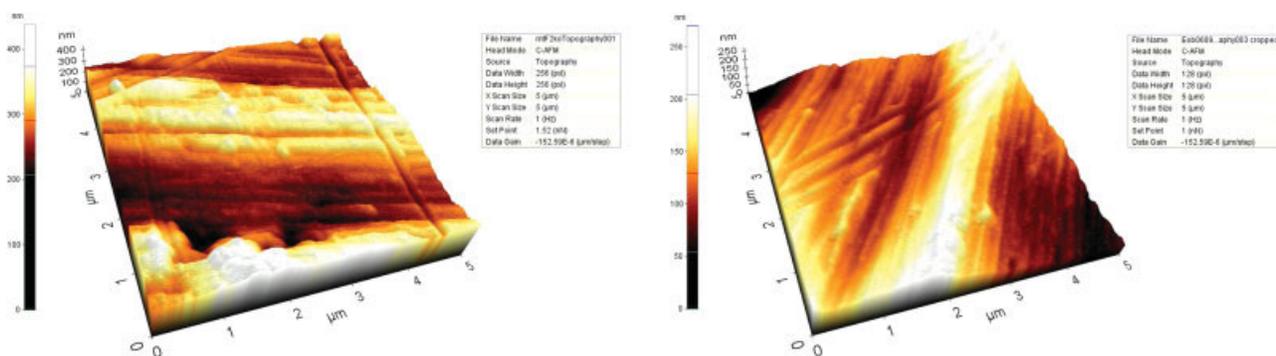


Figure 2. 3D AFM pictures of typical (A) control (untreated) and (B) mouthwash-treated (250 ppm F^- , pH 4.4) Ti samples. The almost parallel grooves originate from the mechanical machining of the samples. The color becomes lighter on proceeding from the depths of the grooves toward the surface. Image size: $5 \times 5 \mu\text{m}$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

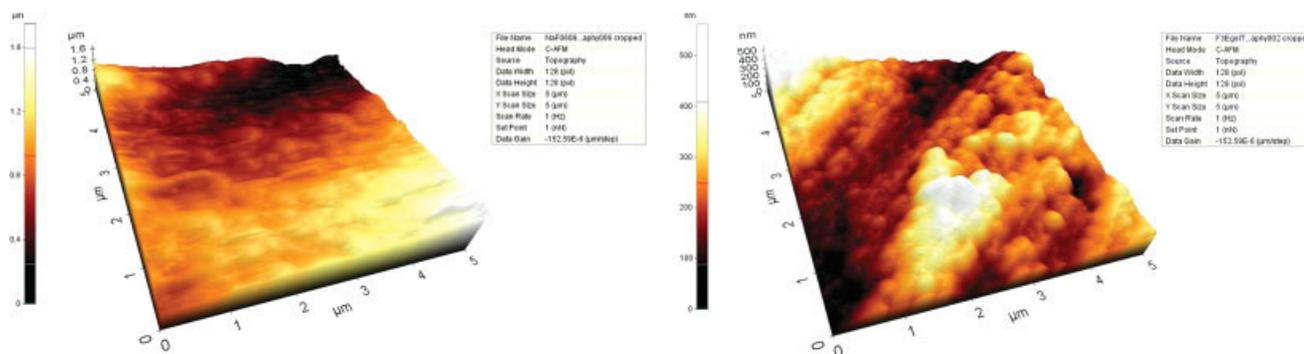


Figure 3. 3D AFM pictures (A) of a Ti disc treated with 1% NaF (3,800 ppm F^- , pH 4.5), and (B) of a characteristic gel-treated (12,500 ppm F^- , pH 4.8) Ti sample. The Ti discs treated with NaF solution displayed an almost 10 times increase in R_a and for gel-treated samples the AFM picture revealed deep corrosive regions and granular forms. Image size: $5 \times 5 \mu\text{m}$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MikroMasch Eesti OU (Estonia). Cantilevers with spring constants of 0.95 and 1.75 N/m were used. The measurements were performed in contact mode, and the height, deflection, and 3D images with areas of $10 \mu\text{m} \times 10 \mu\text{m}$ and $5 \mu\text{m} \times 5 \mu\text{m}$ were captured. The surface roughness (R_a) was determined via the AFM software program (at least 10 independent measurements), and was defined as the arithmetic average of the surface height relative to the mean height. R_{pv} was also determined, as the difference between the highest (peak) and deepest (valley) values of the surface. R_a was depicted graphically following section analysis.

The control and treated Ti discs were sterilized under UV-C radiation (20 s) for the epithelial cell culturing experiments. The cell cultures involved human gingival mucosa from healthy consenting adult (age 18–24) donors. All subjects enrolled in this research have responded to an Informed Consent, and the scheme of the experiments has been approved by the Human Investigation Review Board of University of Szeged, as it complied with the ethical standards of the research, in accordance with the Helsinki Declaration.

A quantity of 1×10^4 cells/mL/disc from the cell culture in the 3rd passage was plated on the Ti discs, in 48-hole cell culture plates. The cell adhesion was determined at 24 h, and the cell proliferation at 72 h. Three independent experiments were performed, and for each treatment five Ti samples were used.

MTT measurements and protein content assays were used to investigate how the cells survived and proliferated on the surfaces treated with the materials containing different amounts of F^- . The MTT is a rapid colorimetric assay widely used for cellular growth and survival study.²⁹ MTT gives a yellowish solution that is converted to the dark-blue water-insoluble MTT-formazan by mitochondrial dehydrogenases in living cells. The blue crystals are solubilized with acidified isopropanol and the intensity is measured colorimetrically at 570 nm. The adhering cells were removed by a lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA , 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , and 1 $\mu\text{g/mL}$ leupeptine) and their protein content was determined with a micro Coomassie (Bradford) protein assay kit (Pierce, Rockford, IL) by following the instructions of the supplier, with bovine serum albumin as standard.

The samples were subsequently dehydrated in graded ethanol and acetone, and dried in critical point dryer (type SPI 1320). Mounted specimens were gold-coated by using an Edwards sputter coater, and viewed in a Hitachi S 2400 scanning electron microscope.

Statistical analysis was done using Student's *t*-test for two samples, where $p = 0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

AFM measurements

Before treatment, the polished Ti samples were tested by AFM and XPS. Figure 2(A,B) reveals almost the parallel grooves on the control and mouthwash (250 ppm F^-)-treated samples; these grooves origi-

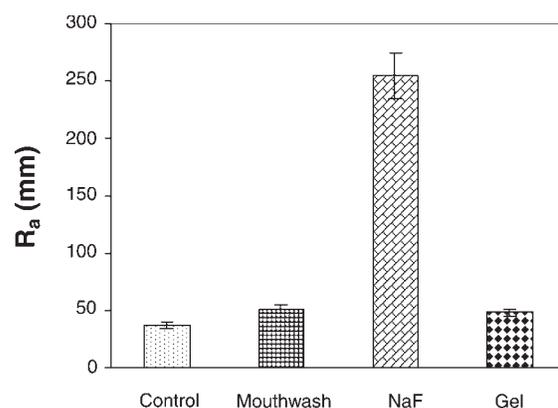


Figure 4. Overall bar-graph of the surface roughness (R_a) of the various samples. The AFM measurements gave $R_a = 37.0 \pm 2$ nm for the control (untreated) samples, 51.3 ± 4 nm for the mouthwash-treated (250 ppm F^- , pH 4.4) samples, 254.8 ± 20 nm for the NaF solution (3800 ppm F^- , pH 4.5) and 48.6 ± 3 nm for the gel-treated (12,500 ppm F^- , pH 4.8) sample.

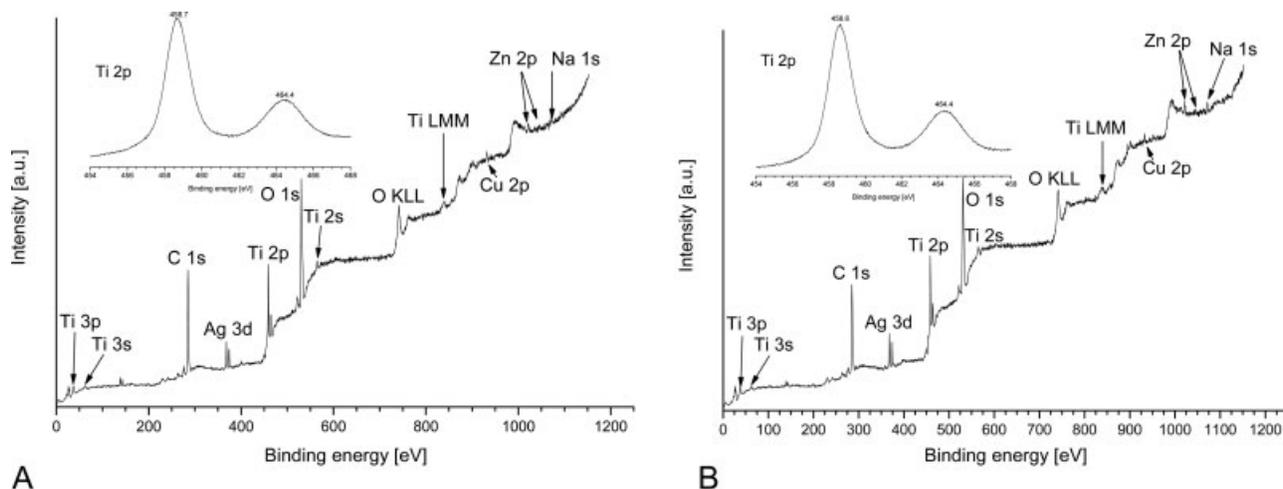


Figure 5. XPS spectra of (A) control and (B) mouthwash-treated (250 ppm F^- , pH 4.4) Ti discs. The XPS survey spectra confirmed the presence of O, C, and Ti. These elements are typically observed on Ti implant surfaces.

nate from the mechanical machining of the samples (the color becomes lighter on proceeding from the depths of the grooves toward the surface). The AFM measurements gave $R_a = 37.0 \pm 2$ nm for the control samples, and 51.3 ± 4 nm for the mouthwash-treated samples (Fig. 4). Although major differences can not be observed between the two samples, R_a was significantly ($p = 0.007$) higher than the control value.

After treatment with 1% NaF solution (3800 ppm F^- , pH = 4.5), the Ti discs displayed the biggest increase in R_a [Fig. 3(A)]: the depth of the grooves was almost 10 times the control depth: $R_a = 254.8$ nm \pm 20 nm ($p < 0.001$, Fig. 4).

On the discs immersed in the gel (12,500 ppm F^-) the AFM [Fig. 3(B)] picture revealed deep corrosive regions and granular forms, and the average roughness of the gel-treated surface was significantly

increased, $R_a = 48.6 \pm 3$ nm ($p = 0.005$), as compared with the control samples (Fig. 4).

XPS measurements

The XPS survey spectra of control and rinse-treated samples in Figure 5(A,B) confirmed the presence of O, C, and Ti. The C 1s signal indicates the presence of carbonaceous contamination, because of C-containing molecules remaining after chemical cleaning or adsorbed later on the air-exposed surfaces. These elements are typically observed on Ti implant surfaces.³⁰ Trace amounts of Ag, Cu, Zn, and Na could also be detected³¹ originating from external contamination.

The double peaks of Ti (Ti 2p at 458 and 464 eV binding energy) and the O 1s signal (530 eV) demon-

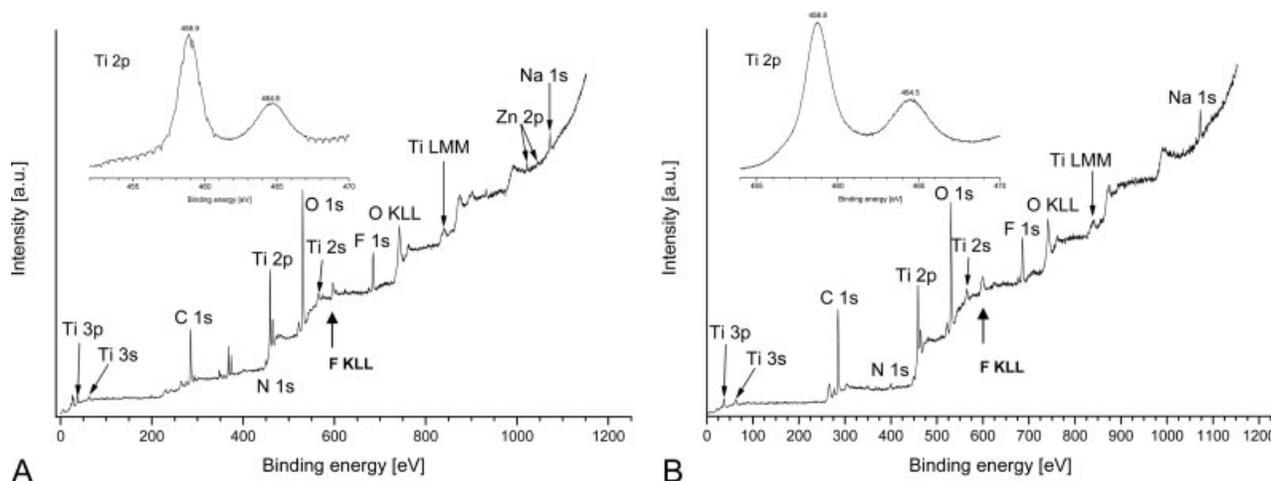


Figure 6. XPS spectra of (A) NaF (3800 ppm F^- , pH 4.5) and (B) gel-treated (12,500 ppm F^- , pH 4.8) Ti discs. Three new peaks can be observed on the spectra originating from Na_2TiF_6 , which modifies the TiO_2 layer of the surface.

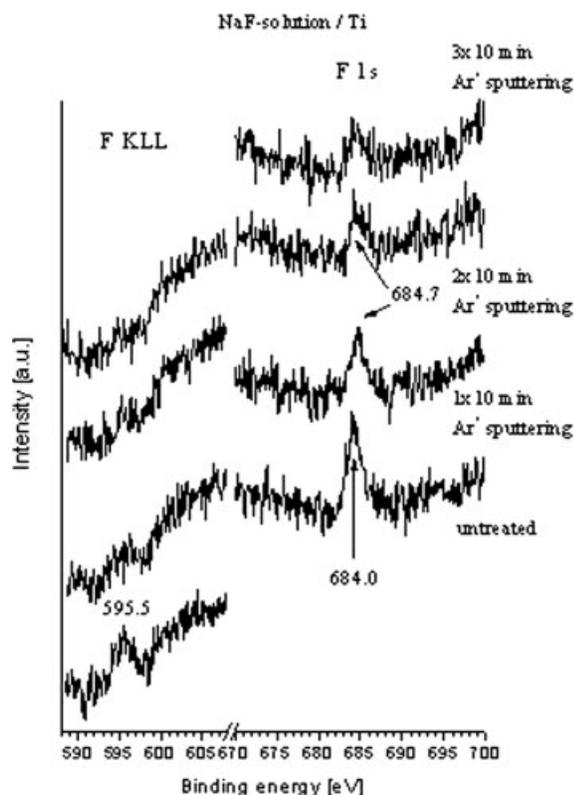


Figure 7. High-resolution XPS spectra of the NaF-treated (3800 ppm F^- , pH 4.5) Ti disc after 10, 20, and 30 min of Ar^+ bombardment. After 10 min of Ar^+ bombardment, repeated XPS investigation revealed that about 10 nm was removed from the surface of the material, but the F 1s peak at 684.7 eV persisted, proving that the binding between the Ti and F^- was very strong.

strate the presence of the TiO_2 layer.^{32,33} The immersion in the mouthwash containing 250 ppm F^- did not change the XPS spectrum of the surface [Fig. 5(B)].

After the NaF and gel treatments, three new peaks were to be seen on the spectra [Fig. 6(A,B)]. One of them, at a binding energy of 1080 eV, is the characteristic line of Na 1s, which resulted from NaF. Two other peaks appeared in the 600–700 eV binding energy region. The line at 600 eV originates from F KLL,²³ and the F 1s peak at 685.3 eV from Na_2TiF_6 , which modifies the TiO_2 layer of the surface.^{23,24}

After 10 min of Ar^+ bombardment, repeated XPS investigation revealed that about 10 nm was removed from the surface of the material, but the F 1s peak at 684.7 eV persisted (Fig. 7), proving that the binding between the Ti and F^- was very strong.

MTT and protein content measurements

The results of MTT and protein concentration experiments relating to cell attachment (24-h observation) are illustrated in the bar-graphs of Figure 8(A,B). The MTT results indicated that the epithelial cell

attachment on the Ti surface was not disturbed significantly by immersion in the mouthwash or NaF ($E_{540,control} = 0.216 \pm 0.007$, $E_{540,mouthwash} = 0.231 \pm 0.011$, $E_{540,NaF} = 0.192 \pm 0.016$). Following immersion in the gel, however, the attachment was significantly stronger ($E_{540,gel} = 0.255 \pm 0.013$; $p = 0.015$). The protein concentration after 24 h was the same for all Ti samples, independently of the F^- material applied ($c_{control} = 4.60 \pm 0.47$).

The MTT and protein content assay results concerning cell proliferation (72-h observation) are presented in Figure 9(A,B). The level of cell proliferation revealed by the MTT measurement was decreased significantly ($p < 0.001$) only in the case of the NaF-treated sample ($E_{540,control} = 0.268 \pm 0.022$, $E_{540,NaF} = 0.137 \pm 0.004$, $E_{540,mouthwash} = 0.271 \pm 0.01$, $E_{540,gel} = 0.221 \pm 0.019$). The protein content assays demonstrated the same tendency as the MTT measurements for the gel-treated sample: a significant (but slight) decrease ($c_{gel} = 4.59 \pm 0.41 \mu\text{g/mL}$; $p = 0.0312$) relative to the rinse-treated sample ($c_{mouthwash} = 5.82 \pm 0.38 \mu\text{g/mL}$). A significant change was not detected for the NaF-treated sample ($c_{NaF} = 5.25 \pm 0.39 \mu\text{g/mL}$; $c_{control} = 5.31 \pm 0.18 \mu\text{g/mL}$).

The influence of the surface roughness on epithelial cell growth has been studied by many authors and it has been proved that epithelial cells do not approach so closely to acid-etched and sand-blasted surfaces as to smooth (polished, $S_a < 0.5 \mu\text{m}$) surfaces.³⁴ Baharloo et al.³⁵ observed that surfaces with smooth topography promote epithelial-cell growth, spreading, and the production of focal contacts on Ti surfaces.

Although our treatments were rather strong (for the gel and NaF, even the presence of Na_2TiF_6 was detected), the roughness never exceeded $0.5 \mu\text{m}$, and the protein concentration was not decreased as compared with the control. This is in accordance with the findings of the above-mentioned authors.

The MTT method revealed a significant increase in cell attachment for the gel-treated sample, and a decrease in proliferation for the NaF-treated sample. The difference between the results obtained with these two methods is not yet understood, but may well be associated with the inherent differences between the methods. MTT assay measures the amount of living cells, while in the protein related measurements all the cells (viable and nonviable) are included.

SEM observations

The SEM micrographs showed the same pictures of the surface structure of the differently treated Ti samples as those seen on AFM: a comparatively smooth surface for the mouthwash-treated discs [Fig. 10(A)] and a rougher surface with granules because of the corrosive effect of the F^- -containing gel [Fig. 10(B)].

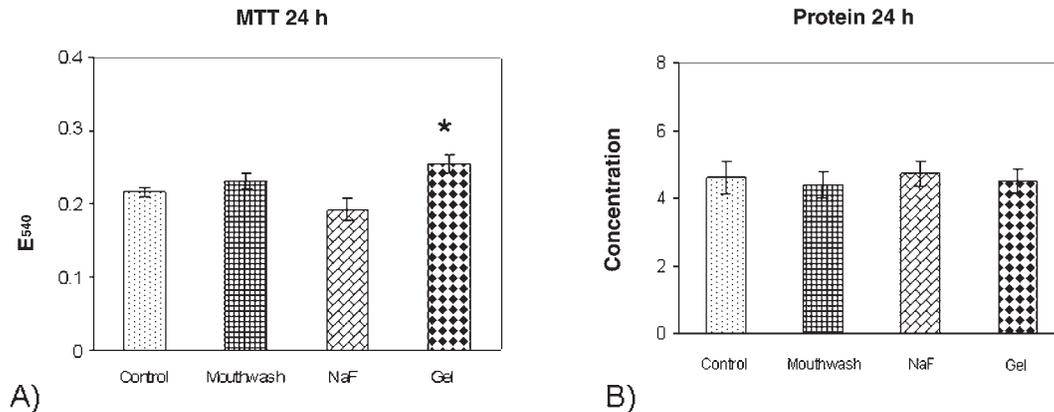


Figure 8. 24-h MTT (A) and protein concentration (B) results. The MTT results indicated that the epithelial cell attachment on the Ti surface was not disturbed significantly by immersion in the mouthwash or NaF, but after immersion in the gel, the attachment was significantly stronger. The protein concentration after 24 h was the same for all Ti samples, independently of the F⁻ material applied.

SEM of the Ti discs after 24-h human epithelial cell binding revealed human epithelial cells bound independently to selected sites on the prepared titanium surface and they exhibited a spherical morphology (not shown). Figure 10 illustrates the SEM pictures of the adhesion of the human epithelial cells to titanium surface after 72-h culturing. Number of the attached and proliferated cells is visible with spreading behaviors. However, we could not see any morphological differences on the adhesion and the growing of cells either on control or treated titanium surfaces [Fig. 10(A,B)].

CONCLUSIONS

R_a was demonstrated by AFM to be increased significantly on all test samples. For the mouthwash-treated

sample, $R_a = 51.3 \pm 4$ nm ($p = 0.007$), and for the gel-treated sample, $R_a = 48.6 \pm 3$ nm ($p = 0.005$), as compared with $R_a = 37.0 \pm 2$ nm for the control surface. The 1% NaF solution-treated Ti discs displayed an almost 10-fold increase in roughness: $R_a = 254.8 \pm 19$ nm ($p < 0.001$), which is due to the fact that in aqueous solution in acidic pH hydrofluoric acid (HF) will form. We suppose that in case of gel, even if the F⁻ concentration is higher, the different agents like Olaflur and Decaflur are fixing and chemically bonding (neutralizing) the F⁻, impeding the formation of this acid.

XPS revealed that the high F⁻ concentration and acidic pH of the gel and the 1% NaF solution resulted in strong corrosion and modification of the composition of the Ti surface. The complex Na₂TiF₆ was formed, bound strongly to the surface.

The MTT results (24-h observation) showed that the epithelial cell attachment on the Ti surface was not

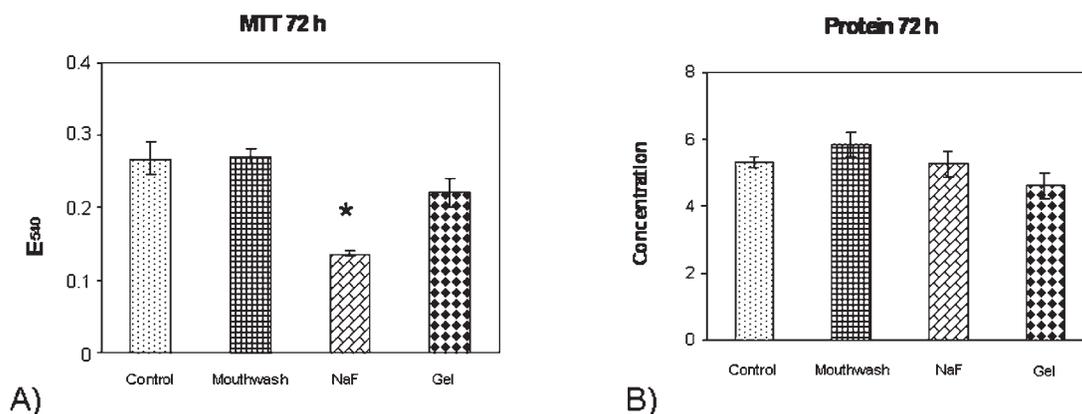


Figure 9. 72-h MTT (A) and protein content assay (B) results. The level of cell proliferation revealed by the MTT measurement was decreased significantly only in the case of the NaF-treated sample. The protein content assays demonstrated the same tendency as the MTT measurements for the gel-treated sample: a significant (but slight) decrease relative to the rinse-treated sample.

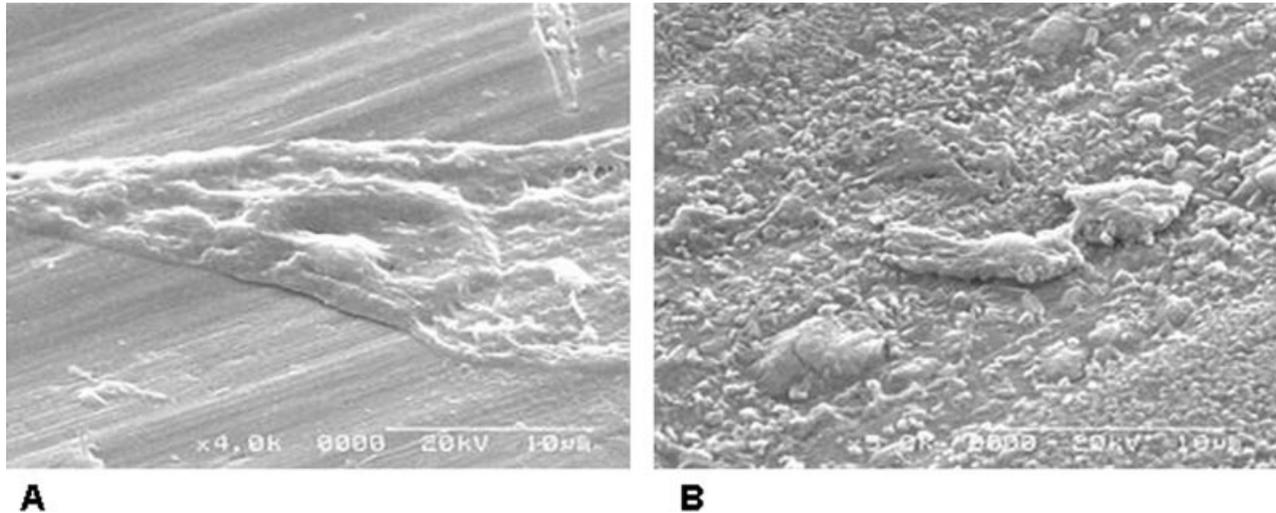


Figure 10. SEM images of epithelial cells on (A) mouthwash- (250 ppm F^- , pH 4.4) and (B) gel-treated (12,500 ppm F^- , pH 4.8) Ti samples (72-h observation). Magnification: $\times 4000$ and $\times 5000$, respectively. Number of the attached and proliferated cells are visible with spreading behaviors, however, we could not see any morphological differences on the adhesion and the growing of cells either on control or treated titanium surfaces.

disturbed significantly by use of the mouthwash or NaF, but following gel treatment, the attachment was significantly stronger. The protein concentration was the same for all the Ti samples, independently of the F^- material applied.

The cell proliferation (72-h observation) determined by MTT measurement was decreased significantly only for the NaF-treated samples. The protein content assays indicated almost the same tendency as the MTT measurements: for the gel-treated samples a significant decrease relative to the mouthwash-treated samples, but no significant change for the NaF-treated samples.

These results suggest that epithelial cell culturing results can depend on the investigation method used, and it is advisable to take the adverse effects of a high F^- concentration and low pH into consideration when prophylactic gels are utilized by patients with implants or other dental appliances made of titanium.

The authors are grateful to the Department of Oral Surgery (Faculty of Dentistry, University of Szeged) for providing the human gingiva. The authors thank Dr. Frédéric Cuisinier (UFR Odontologie, Université Montpellier I, Montpellier, France) for valuable discussions and Attila Dallos (Department of Dermatology and Allergology, University of Szeged) for his help in the protein assays.

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