Effects on titanium implant surfaces of chemical agents used for the treatment of peri-implantitis

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Abstract: The treatment of peri-implantitis, which causes tissue deterioration surrounding osseointegrated implants, involves surface decontamination and cleaning. However, chemical cleaning agents may alter the structure of implant surfaces. We investigated three such cleaning solutions. Commercially pure (grade 4) machined titanium discs (CAMLOG Biotechnologies AG, Switzerland) were treated with 3% H_2O_2 (5 min), saturated citric acid (pH = 1) (1 min) or chlorhexidine gel (5 min), and their surface properties were examined by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). Human epithelial cell attachment (24-h observation) and proliferation (72-h observation) were investigated via dimethylthiazolyl-diphenyltetrazolium bromide (MTT) and bicinchoninic acid (BCA) protein content assays. AFM revealed no significant difference in roughness of the three treated surfaces. XPS confirmed the constant presence of typical surface elements and an intact TiO_2 layer on each surface. The XPS peaks after chlorhexidine gel treatment demonstrated C—O and/or C=O bond formation, due to chlorhexidine digluconate infiltrating the surface. MTT and BCA assays indicated similar epithelial cell attachments in the three groups; epithelial cell proliferation being significantly higher after H_2O_2 than after chlorhexidine gel treatment (not shown by BCA assays). These agents do not harm the Ti surface. Cleaning with H_2O_2 slightly enhances human epithelial cell growth, in contrast to chlorhexidine gel. © 2010 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 94B:222–229, 2010.

Key Words: peri-implantitis, chemical agent, H_2O_2, citric acid, chlorhexidine gel, atomic force microscopy, X-ray photoelectron spectroscopy, epithelial cell culture

INTRODUCTION

Achievement of the biointegration of alloplastic materials is one of the most important targets of research in the medical, dental, and biological sciences. The most frequently used medical implants are dental implants that serve to replace human teeth. As the average human lifespan is currently increasing, ever more people have missing teeth or need tooth replacement.

Titanium (Ti) and Ti alloy prostheses are widely employed as they possess the best osseointegration properties with a predictably long lifetime.[1,2] The long-term benefits of such implants rely on the responses of the various surrounding tissues (the alveolar bone, or the conjunctive and epithelial parts of the mucosa). The failure of a dental implant is caused mainly by the inflammatory processes affecting the soft and hard tissues.[3]

Peri-implant infections involve peri-implant mucositis, defined as a reversible inflammatory change of the peri-implant soft tissues without bone loss, and peri-implantitis, an inflammatory process resulting in loss of supporting bone and associated with bleeding and suppuration.[3–5] Several studies have evaluated peri-implant infections, but only a few were cross-sectional and provide information on the prevalence of peri-implant diseases among patients with implants functioning for ~10 years. The incidence of peri-implant mucositis has been reported to be in the range of 60% of implant recipients and in 48% of implants.[6,7] The prevalence of peri-implantitis was found to be around 15, 16, and 28% with respect to the recipients,[6–8] and 7 and 12% regarding implant sites.[7,8] The differences in the prevalence of peri-implantitis may be explained by differing criteria used for the diagnosis of peri-implantitis, as well as variations in maintenance procedures.[5,9]

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The etiology of marginal peri-implantitis is based mainly on an infectious factor and a biomechanical factor.[10] Although the causes may differ in both cases, microbial colonization occurs on the surface of the implant.[4,11] If the conditions become pathogenic, bacteria start to proliferate, leading to inflammation around the implant. Peri-implant diseases have been primarily linked to Gram-negative anaerobic microflora.[12] The process is aggravated by microorganism colonization and their toxins, and extensive bone destruction will occur. The inflammation spreads apically thus, in very severe cases, therefore, the patient may lose the implant. Methods which remove the bacteria and the toxins from the surface of challenged implants would prevent or terminate the development of peri-implant bony defects.

The therapy of peri-implantitis in the surgical phase is a complex process, starting with surgical debridement of devitalized peri-implant tissue and continuing with decontamination of the exposed implant surface. The implant surface can be cleaned by mechanical (an air-powder abrasive) or chemical (citric acid, H_2O_2, chlorhexidine digluconate (CHX) or EDTA) procedures or with laser irradiation (CO_2, diode, Er:YAG or Nd:YAG).[13,14] To support antimicrobial treatment, topical, and/or systemic antibiotics may be administered.[13] After removal of damaged tissues from the peri-implant pocket, surgical treatment (guided tissue regeneration with or without the use of bone grafts and barrier membranes) promotes regeneration of any bone defect.[13,15]

For the chemical detoxification of implants, various cleaning solutions are used: CHX, H_2O_2, citric acid, phosphoric acid gel, delmopinol, Listerine^R, iodine, saline irrigation, beta-isodona, chloramine-T, and so forth. Besides these chemical agents, a number of systemic antibiotics can be applied to support the therapy: for example tetracycline, amoxicillin, augmentin, metronidazol, penicillin, and so forth.[5,13]

CHX is a commonly administered antimicrobial agent with a wide range of medical applications. It is used in dentistry as a mouthwash and topical antimicrobial. In the treatment of peri-implantitis it can serve as a rinsing solution,[16,17] or more often as an implant irrigation solution, in combination with systemic antibiotics.[13,15] Renvert et al.[18,19] investigated the difference in effectiveness of minocycline microspheres and CHX gel, and concluded that the adjunctive use of these microspheres led to improved probing depths and bleeding scores, CHX alone resulting in only a limited reduction of the bleeding scores. CHX is also effective in the surgical treatment of late peri-implant defects using guided tissue regeneration.[20,21]

Recognizing the increasing interest in the functionalization of dental implant surfaces with antimicrobial agents prior to implantation, Barbour et al.[22] investigated the adsorption of CHX to TiO_2 crystals of anatase and rutile. Their results proved that CHX in 4-morpholinoethanesulfonic acid (MES) and phosphate-buffered saline (PBS) buffers adsorbed rapidly to anatase and rutile TiO_2, equilibrium being attained in less than 60 s, with gradual desorption over a period of several days. More CHX adsorbed to anatase than to rutile, and the CHX desorbed more rapidly from anatase than from rutile, depending on the buffer used.

The study by Burchard[23] revealed that fibroblasts adhere more readily to surfaces exposed to CHX or saline than to those exposed to stannous fluoride.

Saturated citric acid can also be applied for the decontamination of Ti surfaces in the surgical treatment of peri-implantitis with bone grafts and membranes.[24,25] In a comparison of the effects of citric acid and 10% H_2O_2, Alhag et al.[26] demonstrated that rough surfaces (with an enhanced TiO_2 layer and textured surface; Nobel Biocare AB®, Gothenburg, Sweden) which were plaque-contaminated and cleaned with either solution, can re-osseointegrate. H_2O_2 can be used successfully at a concentration of 3% in the surgical treatment of peri-implantitis, employing bone substitutes with, or without, resorbable membranes.[27,28]

Some authors, including Khoury,[15] have even used a combination of these three different cleaning solutions in the surgical therapy of peri-implantitis. After removal of the granulomatous tissue, the surgical site was repeatedly rinsed with CHX, after which citric acid (pH = 1) was applied for 1 min to decontaminate the implant surface, this then being rinsed with H_2O_2 and 0.9% saline.

Dennison et al.[29] found that machined implants (without a surface coating) are decontaminated by a variety of methods (air-powder abrasive, citric acid solution, or CHX) more readily than hydroxyapatite-coated surfaces.

The above-mentioned chemical agents are commonly applied in the therapy of peri-implantitis, but only investigations relating to the adsorption of CHX on different TiO_2 crystals (anatase and rutile) appear to have been conducted. When used for implant surface decontamination, these materials may alter the morphology and chemical structure of the surface. The aim of our investigation, therefore, was to study the effects of three cleaning solutions in clinical use for peri-implantitis therapy.

In vitro studies are essential in the development of such treatments, as these are the basic steps with which to reveal the action of cleaning solutions on the implant surface. Additionally, fewer animal experiments would be required.

In the present investigation, the effects of three different cleaning solutions (3% H_2O_2 solution, saturated citric acid (pH = 1) and CHX gel) on the chemical structure and surface roughness (R_a) of CP Ti were investigated, through the use of X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). A further aim was to survey the response of the biological environment to these changes, by examining the attachment and proliferation of human epithelial cells after treatment of the Ti surfaces with these solutions. The epithelial cell attachment and proliferation was examined by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and protein-content assays (the latter with bicinchoninic acid).

MATERIALS AND METHODS

Ti discs (9 mm in diameter and 1.5 mm in thickness) were made from CP grade 4 implant material (CAMLOG^R Biotechnologies AG, Switzerland). The surfaces of the discs were machined (through turning), with a roughness (R_a) < 0.2 μm, a typical roughness for the abutment of a dental

implant.[30] The discs were cleaned in acetone and absolute ethanol in an ultrasonic bath for 15 min. After cleaning, the discs were treated with 3% H_2O_2, saturated citric acid (pH = 1) or CHX gel (Corsodyl^R dental gel; SmithKline Beecham Consumer Healthcare, UK). Corsodyl^R contains 1% w/w CHX. As in the usual clinical protocols, the durations of treatment were 5 min for H_2O_2 and CHX gel, and 1 min for citric acid. The control group was rinsed with ultrapure water for 5 min. After treatment, the samples were washed three times with ultrapure water and dried.

For AFM a PSIA XE-100 instrument (PSIA Inc., South Korea) was used to acquire information on R_a. AFM is a high resolution imaging technique to study such surfaces on the micron to nanometer scale, via 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 Mikro-Masch 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 × 10 μm and 5 × 5 μm were captured. R_a was determined via the AFM software program (at least six independent measurements) as the arithmetic average of the surface height relative to the mean height.

The chemical composition of the Ti surfaces was studied by XPS. The photoelectrons were generated by Al Kα primary radiation (hν = 1486.6 eV) and analyzed with a hemispherical electron energy analyzer (PHOIBOS 150 MCD 9; manufactured by SPECS). The X-ray gun was operated at 150 W (12 kV, 12.5 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 30-60 min of He^+ bombardment, which was repeated several times. He^+ ions were generated with an ion gun energy of 5 kV, and the incident ion beam current was measured at 200 nA. The bombardment led to the removal of a thickness of ~10 nm from the surface material during the operation. Wide-range scans and high-resolution narrow scans of the Ti 2p, O 1s, and C 1s characteristic peaks were recorded.

Cell-culturing techniques

Adult epidermal epithelial cells were isolated and cultured from inflammation-free oral mucosa of healthy donors (age, 18–46) undergoing dento-alveolar surgery. The protocol of the experiments was approved by the Human Investigation Review Board at the University of Szeged: it complied with the ethical standards of research, in accordance with the Helsinki Declaration. All subjects enrolled in the research gave their signed informed consent.

Mucous membrane specimens were first washed in Salsol A solution (Human Rt, Gödöllö, Hungary) supplemented with 2% antibiotic, antimycotic solution (Sigma-Aldrich GmbH, Germany). Overnight incubation in dispase solution (Grade II, Roche Diagnostics, Mannheim, Germany) was carried out at 4°C to separate the dermis from the epidermis.[31] Next day, the epidermis was peeled off the dermis. The epidermis was placed in 0.25% trypsin-EDTA solution (Sigma-Aldrich GmbH, Germany) for 5 min at 37°C. Following trypsinization, the epidermis was torn apart mechanically and washed vigorously to release epidermal cells. The epidermal cell suspension was centrifuged at 200*g* for 10 min at 4°C. The epidermal cells were then placed in 25 cm^2 tissue culture dishes (Orange Scientific, Belgium).

The oral epithelial cell culture medium consisted of keratinocyte serum-free medium with L-glutamine (Gibco BRL, Eggstein, Germany), supplemented with recombinant epidermal growth factor 2.5 μg/500 mL (Gibco BRL, Eggstein, Germany), bovine pituitary extract 25 mg/500 mL (Gibco), L-glutamine and antibiotic/antimycotic solution containing penicillin G sodium 1%, streptomycin sulfate 1%, and amphotericin B 0.0025% (Sigma-Aldrich GmbH, Germany).

Fresh culture medium was added to the cells three times per week. The primary epithelial cell cultures reached ~90% confluence in 8–16 days. Confluent primary cultures were treated with phosphate-buffered saline (pH = 7.4, Gibco) and cells were harvested by a 2–4 min trypsinization with 0.25% trypsin-EDTA solution (Sigma-Aldrich GmbH, Germany). Harvested cells were divided into two to four equal parts at passages. Cultures were grown at 37°C in a humidified atmosphere containing 5% CO_2.

Optical microscopic images of epithelial cells were recorded with a Nikon TS 100 (Japan) microscope at magnifications of 200×.

Measurement of cell growth and proliferation

The control and treated Ti discs were sterilized on both surfaces under UV-C radiation (20 min) before the epithelial cell culturing experiments.

The growth of cultured epithelial cells was measured with a rapid colorimetric assay, which determines living cell numbers by the reduction of MTT.[32] Cells were seeded into 48-well culture plates at a density of 10^4 cells/well and grown on Ti discs in culture media for 24 or 72 h. The supernatant was removed and replaced with 0.5 mg/mL MTT solution (Sigma-Aldrich GmbH, Germany) in RPMI media without phenol red. After incubation for 4 h at 37°C, the medium was removed gently from each well and the crystallized dye was solubilized with 2% sodium dodecyl sulfate (SDS) and 0.04 m*M* HCl in absolute isopropanol. The optical density of the color reaction at 540 nm (OD_{540}) was determined with a Multiscan Ex spectrophotometer (Thermo Labsystems, Vantaa, Finland) and Ascent Software (Thermo Labsystems, Vantaa, Finland) at the Department of Dermatology and Allergology (University of Szeged).

The protein content of the cells (both living and dead) was also measured. A micro BCA™ protein assay kit containing bicinchoninic acid was applied (Pierce, Rockford, IL). An ascendant set of dilutions with an albumin standard (bovine serum albumin (BSA); Pierce) was made as control. The cells were dissolved with lysis buffer (20 m*M* Tris-HCl, pH 7.5, 150 m*M* NaCl, 1 m*M* Na_2EDTA, 1 m*M* EGTA, 1% Triton X-100, 2.5 m*M* sodium pyrophosphate, 1 m*M* β-glycerophosphate, 1 m*M* Na_3VO_4 and 1 μg/mL leupeptin), the reagent (green) was then applied, according to the manufacturer's instructions, and the solution was incubated for 2 h at 37°C.

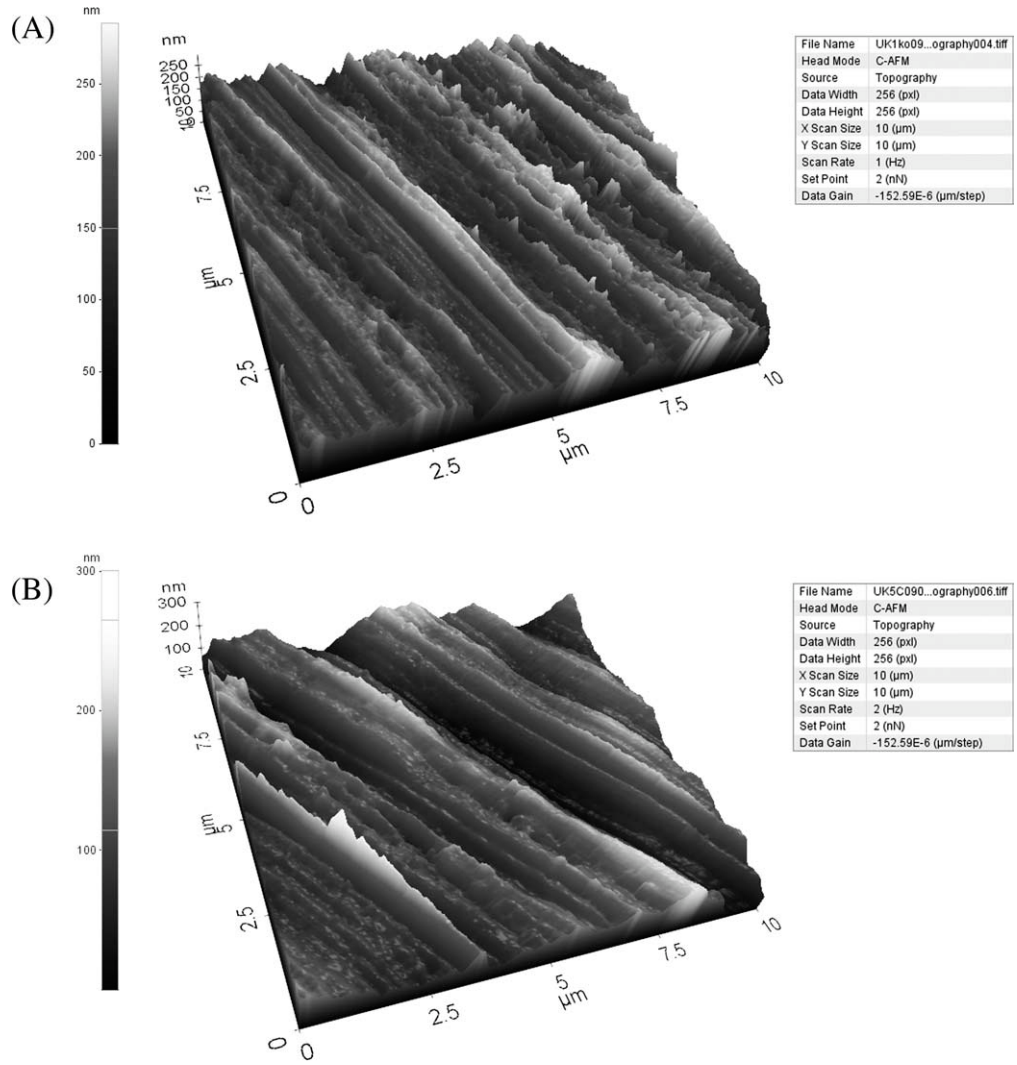

FIGURE 1. 3D AFM pictures of (A) a typical control (untreated) sample and (B) a characteristic citric acid-treated (saturated, pH = 1). The almost parallel grooves originate from the machining of the samples. The color becomes lighter on proceeding from the depths of the grooves toward the surface. Image size: 10 × 10 μm.

Because of this reaction, the color changed to purple. OD_{540} was determined with a Multiscan Ex spectrophotometer (Thermo Labsystems, Vantaa, Finland) and Ascent Software (Thermo Labsystems, Vantaa, Finland) at the Department of Dermatology and Allergology (University of Szeged).

A quantity of 10^4 cells/0.5 mL medium/disc from the cell culture in the third 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. In all, four independent experiments were performed, and five Ti samples were used for each treatment.

Data presentation and statistical analysis

The means ± the standard errors of the mean (SEM) were calculated for the R_a (nm) values measured by AFM. The MTT and protein content assay data are presented as means ± SEM of OD_{540}. After normality testing, data were compared via one-way analysis of variance (ANOVA), followed by Tukey's and Scheffe *post hoc* tests to determine statistical differences after multiple comparisons (SPSS 15.0, SPSS, Chicago, IL). A probability value < 0.05 was considered significant.

RESULTS

AFM measurements

Before the cell culture experiments, the Ti samples were tested by AFM and XPS. Figure 1(A,B) reveal the almost parallel grooves on each sample, originating from the machining (the color becomes lighter on proceeding from the depths of the grooves toward the surface). The AFM measurements gave $R_a = 22 \pm 3$ nm for the control samples [Fig. 1(A) and 2], 25 ± 7 nm for the citric acid-treated samples (Fig. 1(B) and 2), 30 ± 5 nm after treatment with 3% H_2O_2 solution (Fig. 2), and 14 ± 4 nm for the CHX gel-treated discs (Fig. 2), probably a result of gel adsorption to the Ti surface.[22] The differences were not significant statistically.

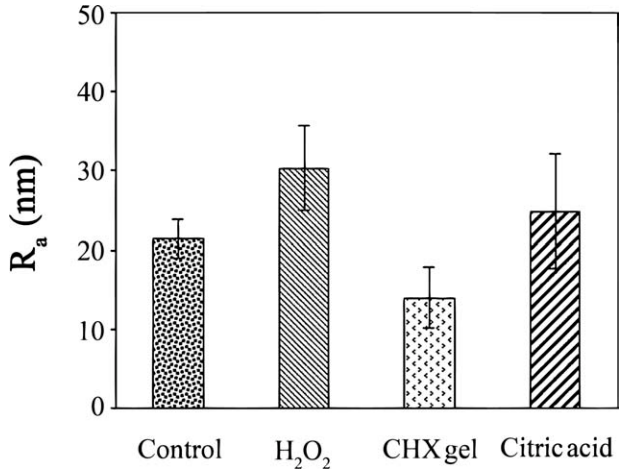

FIGURE 2. Overall bar-graph of the surface roughness (R_a) of the various samples. The AFM measurements gave $R_a = 22 \pm 3$ nm (mean ± SEM) for the control (untreated) samples, 30 ± 5 nm for the H_2O_2 (3%)-treated samples, 14 ± 4 nm for the chlorhexidine gel-treated samples, and 25 ± 7 nm for the citric acid (pH = 1)-treated samples. Statistical analysis did not reveal any significant differences between the groups.

XPS measurements

The XPS measurements revealed Ti, O, C, and N in the topmost atomic layers of all samples (untreated and treated).

The binding energy of Ti 2p 3/2 electrons, which corresponds to Ti^{4+}, was measured at 458.6 ± 0.1 eV for each sample (Fig. 3). The double Ti peaks (Ti 2p at 458.6 and 464 eV) and the O 1s signal (530 eV) demonstrate the presence of the TiO_2 layer.[33,34] The immersion in the different cleaning solutions did not change the Ti 2p signal of the surface (Fig. 3).

Major changes were observed in the O 1s peak, which could be deconvoluted into three peaks (Fig. 4). The most intense one, at ~530.1 eV, is that of lattice O in TiO_2, while the peak at ~531.7 eV is due to surface OH groups. The third peak, at 532.9–533.0 eV, corresponds to the O in C—O and/or C=O bonds. The latter is most intense for the CHX gel-treated sample [Fig. 4(B)], which can be explained in terms of the possible adsorption of CHX to the surface.[22] This is supported by the deconvolution of the C 1s signal (data not shown) which gave four peaks for all samples, the peak at 287 eV for the gel-treated sample proving more intense than those for the other samples.

The decrease in the C 1s signal (Fig. 5) after a 30–60 min He^+ bombardment of the untreated sample indicates the presence of carbonaceous contamination, due to C-containing molecules remaining after cleaning or adsorbed later on the air-exposed surfaces. These elements are observed typically on Ti implant surfaces.[35]

Microscopic images

Optical microscopic images of epithelial cells are to be seen in Figure 6 at magnifications of 200×. Figure 6(A) reveals there are only few attached cells as it is a primary epithelial cell culture, while Figure 6(B) shows a confluent epithelial cell culture.

MTT and protein content measurements

The results of MTT and protein concentration experiments relating to cell attachment (24-h observation) and cell proliferation are illustrated in the bar-graphs of Figure 7. The MTT results [Fig. 7(A)] demonstrate that the epithelial cell attachment on the Ti surface was not disturbed significantly by the different cleaning solutions. More (but not

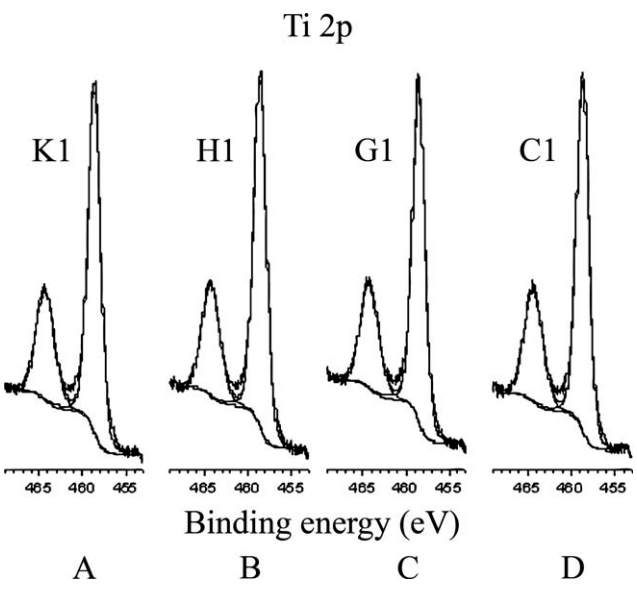

FIGURE 3. Ti 2p signals in XPS spectra of (A) control (K1), (B) H_2O_2-treated (H1), (C) chlorhexidine gel-treated (G1) and (D) citric acid-treated (C1) Ti discs, confirming the presence of TiO_2 on all surfaces.

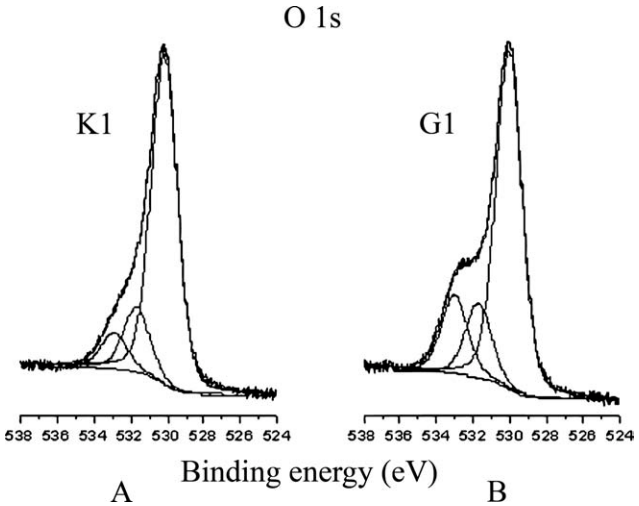

FIGURE 4. O 1s signals in XPS spectra of (A) control (K1) and (B) chlorhexidine gel-treated (G1) Ti discs. The signal was deconvoluted into three peaks: the most intense one (at 530.1 eV) is that of lattice O in TiO_2, while that at ~531.7 eV is due to surface OH groups. The third peak, at 532.9–533.0 eV, corresponds to the O in C—O and/or C=O bonds. The latter is most intense for the chlorhexidine gel-treated sample [Fig. 4(B)], which can be explained in terms of the possible binding of chlorhexidine digluconate to the surface.

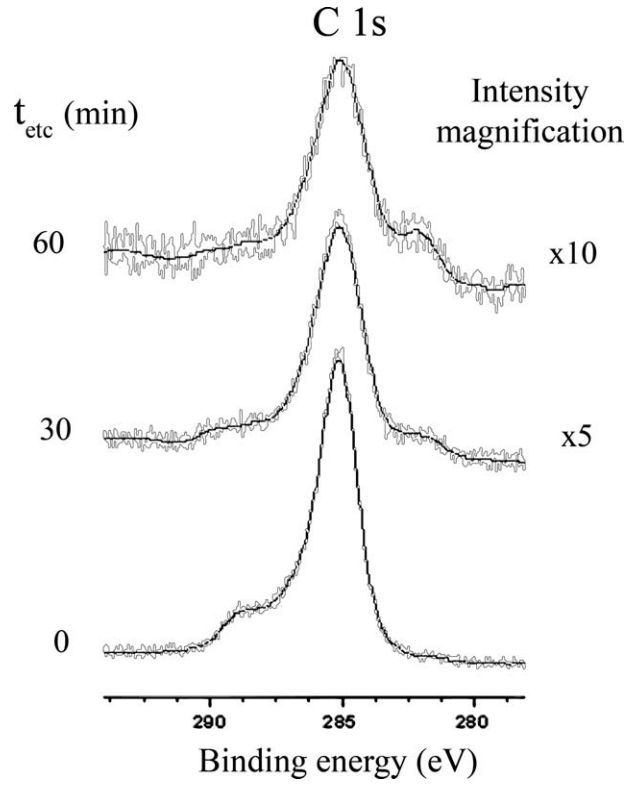

FIGURE 5. C 1s signals in the XPS spectra of the control Ti disc after 0 min (lowestmost curve), 30 min (middle) and 60 min (uppermost curve) of He^+ bombardment. The bombardment led to the removal of ~10 nm from the surface of the material during the operation. The decrease in the C 1s signal indicates the presence of carbonaceous contamination.

significantly more) living cells were observed on the discs treated with H_2O_2 and citric acid than on the control and CHX gel-treated discs. The protein concentration after 24 h [Fig. 7(B)] was similar for all samples. The level of cell proliferation revealed by the MTT measurements [Fig. 7(A)] was increased slightly by the H_2O_2 and citric acid treatments. The H_2O_2-treated sample exhibited a significant ($p = 0.011$) increase as compared to the CHX gel-treated discs. No significant differences were observed among the other groups. The protein concentration after 72 h [Fig. 7(B)] was similar for all Ti samples.

DISCUSSION

R_a was demonstrated by AFM to be similar for all groups. Only for the CHX gel-treated group was R_a lower (14 ± 4 nm), than that of controls. These surfaces are therefore smooth, and equally suitable for epithelial cell attachment and proliferation. The influence of R_a on epithelial cell growth has been studied by many authors and it is known that epithelial cells do not attach so strongly to acid-etched or sand-blasted surfaces as to smooth (polished, $S_a < 0.5$ μm) surfaces.[36] Surfaces with a smooth topography promote epithelial cell growth, spreading, and the production of focal contacts on Ti surfaces.[37]

The XPS measurements proved the presence of an intact TiO_2 layer on both the untreated and the treated samples. A major change was observed for the CHX gel-treated sample, as the O 1s signal included an intense peak corresponding to the O in C—O and/or C=O bonds. This is assumed to be a result of the adsorption of CHX to the surface, as observed by other authors.[22]

The MTT assays (24-h observation) showed that the epithelial cell attachment was not changed significantly on the Ti surfaces treated with the different cleaning solutions, and the protein-content assay supported this. The MTT method revealed differences in cell proliferation (72-h observation) between groups, with a significant increase in proliferation for the H_2O_2-treated sample relative to the CHX gel-treated one. The proliferation for the citric acid-treated samples was higher, but not significantly so, as compared to controls. The protein content assays indicated similar degrees of cell proliferation for the differently treated samples. The variations observed in the proliferation of epithelial cells cannot result from differences in R_a of the Ti samples, as the samples applied in this study had machined surfaces, with R_a between 0.014 and 0.030 μm, depending on the chemical

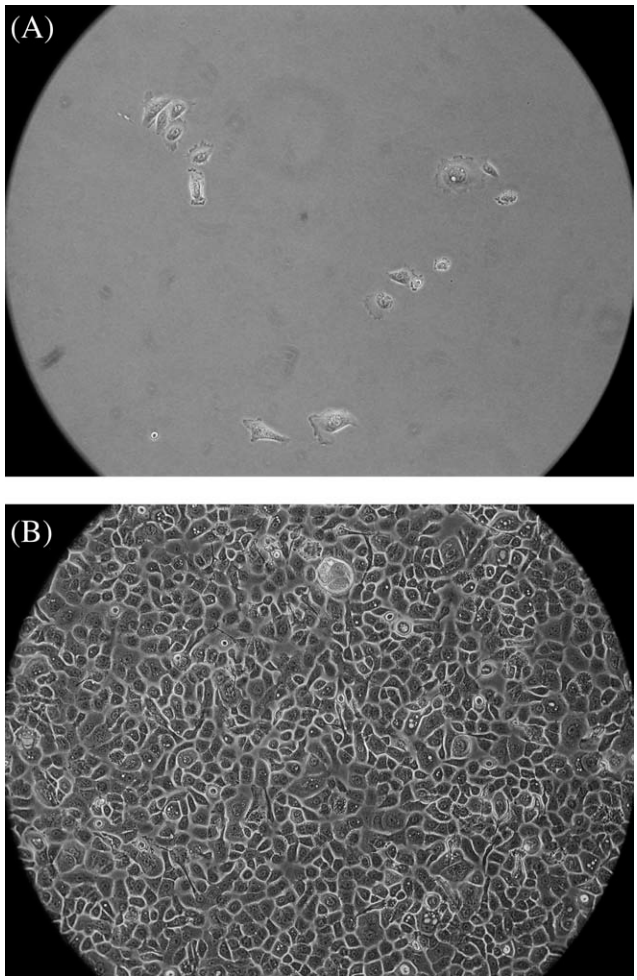

FIGURE 6. Optical microscopic images of A) epithelial cells of primary culture recorded with a Nikon TS 100 (Japan) microscope. There are few attached cells, magnification ×200. (B) Confluent epithelial cell culture (magnification ×200).

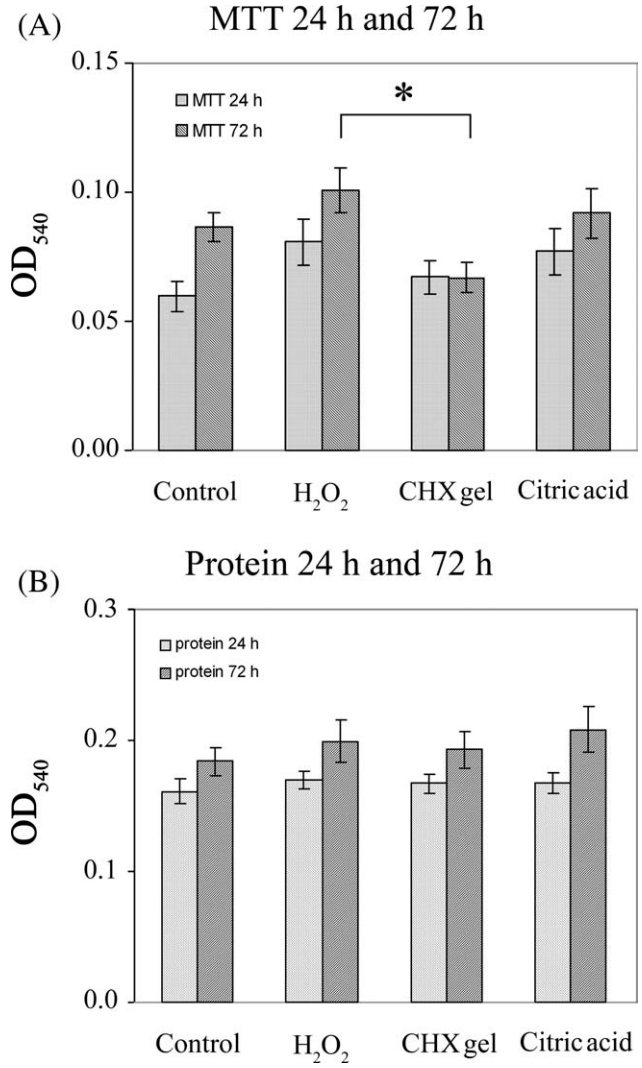

FIGURE 7. (A) 24-h and 72-h MTT and (B) 24-h and 72-h protein concentration results. The MTT data indicated that the epithelial cell attachment to the Ti surface was not disturbed significantly by the different cleaning solutions. H_2O_2 and citric acid treatment of the Ti discs induced slight increases in epithelial cell attachment and viability relative to the control and chlorhexidine gel-treated discs. The level of cell proliferation revealed by the MTT measurements was decreased significantly only in the case of the chlorhexidine gel-treated samples as compared with the H_2O_2-treated discs and the amount of cells was almost the same as at 24 h. The protein concentration after 24 and 72 h was the same for all Ti samples.

agent used. As defined by Klinge et al.[36] these surfaces are all smooth, and it appears improbable that epithelial cells are sensitive to such small changes in R_a. As the R_a values of the two surfaces do not differ significantly, we presume the chemical composition of the CHX gel-treated surface is less favorable for the cells than the H_2O_2-treated surface.

The discrepancy between the results obtained with the two different methods (MTT and protein content assays) in cell proliferation is not yet understood, but may well be associated with the inherent differences between the methods. The MTT assay measures the number of living cells, whereas both viable and nonviable cells are included in the protein-related measurements. Thus, the latter method is effective, but not selective for viable cells.

These findings suggest that the results of epithelial cell-culturing can depend on the investigation method applied, and that it is advisable to take into consideration the adsorption of CHX gel to the Ti surface when this material is used for dental implant decontamination. TiO_2 surface treatment with H_2O_2 or citric acid can result in the same or even better survival and proliferation of epithelial cells than in the untreated case. This is an important finding as these toxic decontamination solutions were not expected to improve cell attachment and proliferation at all.

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