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# Implant Surfaces Exposed to the Oral Cavity and Treated with Toothpaste Containing Oxygen Releasing Compound: A Morphological Controlled Clinical Trial

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## Abstract

Aim of the present *in vivo* study is to assess if a toothpaste containing an oxygen releasing compound (AX) is able to reduce the biofilm formation on implants with rough surface compared to a control toothpaste, without affecting the microstructure of the tested surface.

**Methods**: In this double blind, cross-over, controlled clinical trial, a total of fourteen healthy volunteers were recruited. For each subject, two mandibular splints (test and control) were created with one implant fixed on the right lingual side of the mandibular arch. The splint was continuously worn for 5 days and the daily hygiene was performed wearing the splint and using the test (AX) or control toothpaste. Implants were analyzed at scanning-electron-microscopy and at laser profilometer for the assessment of biofilm adhesion (% of areas free from biofilm-FA) and surface changes (morphology and roughness).

**Results:** FA resulted significantly higher in test than in control implants. No differences were found between groups in term of biofilm organization, surface microstructure and roughness.

**Discussion**: Daily use of toothpaste containing AX seems to reduce the amount of biofilm adherent to the rough implant surface without corrosion or degradation of the titanium surface.

**Keywords:** Corrosion; Dental Implants; Dental Plaque; Oxygen Compounds

## Introduction

Peri-implantitis is an inflammatory condition due to Gram positive and Gram-negative bacteria that adhere to implant crowns, abutments and even to the dental implant [1-4]. On the surface of these components, pathogenic bacteria organized in a biofilm that provides protection from antibacterial components of saliva, including bacterial agglutinins, lactoferrin, lysozyme and secretory IgA [5]. The accurate daily oral hygiene should disrupt and remove mechanically the bacterial plaque preventing peri-implant disease [6]. However, the additional use of antibacterial agents contained in mouthwashes or toothpastes may be useful to support this hygiene procedure, especially in cases where the areas are difficult to clean or when implant surface is exposed to the oral cavity. In fact, the roughness of the titanium surface increases the bacterial adhesion thus encouraging the pathogenicity of the peri-implant microbiota as well as the progression of peri-implant disease [7,8].

The development of toothpaste containing molecules that reduce the biofilm formation on these hardly cleanable surfaces without degrading the titanium-based surface may help the patient to prevent this disease progression. Ardox-X® (AX) is an antibacterial compound with sodium perborate (peroxo-borate) that generates active oxygen in aqueous solutions. This molecule exerts an inhibitory activity on oral bacteria, reduces the formation of dental biofilm and shift its microbial composition toward a less diverse and less mature plaque [9].

Morphological changes and corrosion of titanium surface after exposition to detoxifying solution may potentially aggravate the peri-implant inflammation and compromise the osteointegration [10-11]. The study of new decontaminating compounds should investigate the antibacterial/anti-biofilm activity as well as its ability to not alter the implant surface. Aim of the present *in vivo* study was to assess if a toothpaste containing AX is able to reduce the biofilm formation on implants with rough surface com-

pared to a control toothpaste, without affecting the microstructure of the tested surface.

## Methods

### Population and design of the study

In this double blind, cross-over, controlled clinical trial, a total of fourteen healthy volunteers were recruited. The sample size was calculated using $\alpha = 0.05$ (5%) and a power of 80%. For variability, the value of 3 (standard deviation of bacterial index of density score) obtained in previous reports was considered [12]. The minimum significant value considered was 4. Based on these data, the number of patients required to be enrolled was 14 for group test and control.

To be included in the study, subjects were with age >18 years, no smokers, no pregnant and no edentulous, having an overall good systemic health (absence of endocrine, hormonal, hematologic, immune or nutritional disorder or any disease or drugs that influence salivary flow). They were recruited among the staff of the University of Milano after signing an informed consent (Ethical committee University of Milan, n° 11/19). The volunteers presented a full mouth plaque score below 20%, no carious lesions and were periodontally healthy. Individuals who had less than 4 mm of probing depth full mouth bleeding score=0 and who did not present clinical attachment loss were considered periodontally healthy. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

### Splint preparation

For each subject, after alginate impressions, two mandibular splints (test and control) were created. On each splint, one implant of 3,3x8mm with rough surface (Dyna, Amsterdam, Holland) was fixed on the right lingual side of the mandibular arch. The mandibular-jaw positioning was chosen to favor the food accumulation with the consequent plaque formation. Before use, the splints were disinfected with ethanol 100% (Sigma Aldrich, Saint Louis, Missouri, USA) and UV light for 24 h.

### Treatment procedure

Two toothpastes were used for this study: test and control. The test toothpaste was formulated with Ardox-X®, lactoferrin, low concentration of fluorine and neutral pH (Implaclean®, Dyna, Amsterdam, Holland). Control toothpaste was prepared with the same formulation as the test except for Ardox-X.

After being included in the study, each volunteer was randomly assigned by an operator (GP), according to pre-defined randomization tables, to one of the treatment modalities: test for the first phase and control for the second phase or conversely control for the first phase and test for the second phase of the study.

**First phase**: In the first phase of the study, each volunteer received one splint, and the test or the control toothpaste for daily hygiene according to randomization tables and was asked to comply with the following instructions: to wear the splint for five days without interruptions, with the exception of the few minutes required for meals, to perform daily hygiene wearing the splint and using treatment or control toothpaste at least one hour after meal and do not brush the dental implants during hygiene procedures. At the end of the five days, each volunteer came directly at Lab of Thin Section at Department of Biomedical, Surgical and Dental Science wearing the splint and returned the first splint to the blind operator (EC).

**Second phase**: One month after the first phase, the volunteers repeated the experiments with the same modality but switching the treatment. The blind operator assigned the second splint and the remaining test or control toothpaste to the volunteer. The volunteers complied with the same instructions of the first part of the study, and at the end of the fifth day they returned the second splint to the blind operator (EC).

### Splint processing

Before the removal of the splint, a plaque-disclosing pill was administered to the volunteers in order to highlight the residual plaque on implant surface. After removal, standardized macrophotographs of the disposal were taken.

### Analysis of plaque

For the morphological and semi-quantitative analysis of the plaque covering the implant surface, the splints were prepared for observation at Scanning Electron Microscope (SEM) by cutting a block containing the implant. Briefly, after a rapid fixation period in 10% formalin and a passage in 70% alcohol, blocks containing implants were photographed at backscattered scanning electron microscope (Jeol Neoscope JCM-6000 Nikon, Japan). Ten microphotographs of each implant in the area exposed to the oral cavity were acquired at 100x magnification. Each photo was analyzed using an image analysis program (Image J) that allowed the computation of the total area of the implant surface, and then of the areas free from organic residues. The ratio between the areas free from biofilm and total implant surface was found and finally expressed in percentage (% of implant surface free from the residual biofilm) (FA-free area).

### Morphological analysis of surface and profilometry

To assess micro-morphological alterations of implant surface that may occur after treatment with the toothpastes, microphotographs of the test and control implants at magnification up to x1000 were taken before placement of the splints in mouth on the overall implant surface, and after oral exposition in the areas free from the residual biofilm. In case of morphological changes in any photomicrograph, the surface of the observed implant was considered altered.

To assess the roughness differences of the implants after treatments, the UBM Microfocus Laser profilometer (UBM, Sunnyvale, CA, USA) was used. The following parameters were considered: Ra that is the arithmetic average of the roughness profile, Rmax that is the largest single roughness depth within the evaluation length.

### Data and statistical Analysis

For descriptive analysis, mean and standard deviation of FA, Ra and Rmax was computed for each group (test and control). The non-parametric Mann-Whitney test for paired data (Kyplot 2.0 software) was used for the between groups analysis. The significance of data was set at $p<0.05$.

## Results

### Study population

Forteen volunteers with a mean age of 28 years, systemically and periodontally healthy and no smokers were enrolled. All patients complied with the study.

### Analysis

**Morphological analysis of plaque**. At five days after treatment, bacterial colonization was present as a thick layer coating the surface of both test and control implants (Figure 1a and b). In both groups biofilm appeared highly organized with a dominance of coccal shaped cells (Figure 2) and filamentous forms penetrating the intercellular matrix. In all the analyzed samples, the bacterial aggregates were covered by a matrix (Figure 3), probably salivary proteins and bacterial extracellular compounds, involved in the adhesion of micro-organisms onto the titanium.

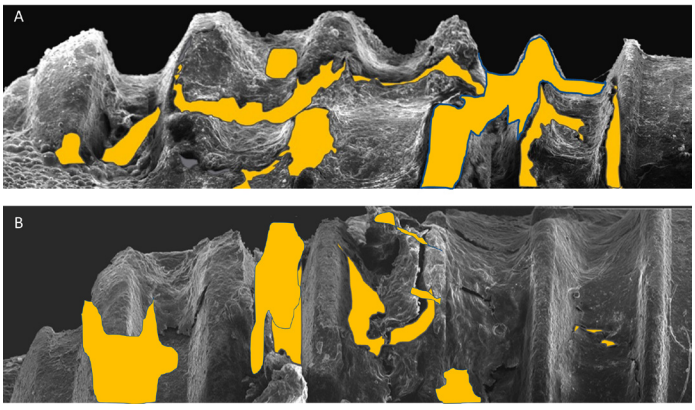


**Figure 1:** shows implant covered by plaque after test (a) and control (b) treatments. The surface of the implant free from bacteria is indicated in yellow.

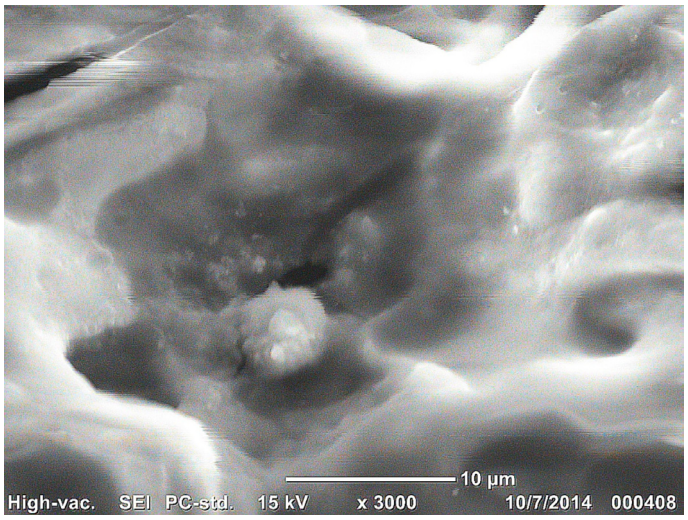


**Figure 2:** Microphotograph of a cell coccal-shape like immerse in the plaque.

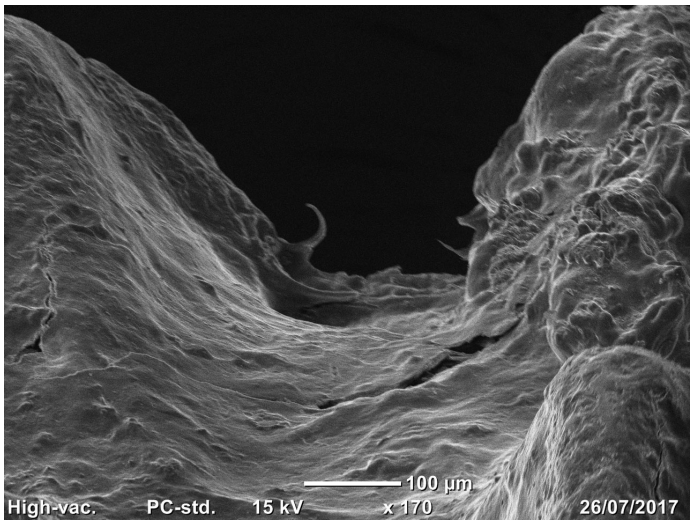


**Figure 3:** Microphotograph of the space between two implant threads covered by plaque.

**Morphological analysis of implant surface**. At SEM microphotograps, no morphological sign of surface micro-degradation such as crater-like structures, pores or detachment of the surface layer appeared on any implant after treatments with test and control toothpastes (Figure 4a and b).

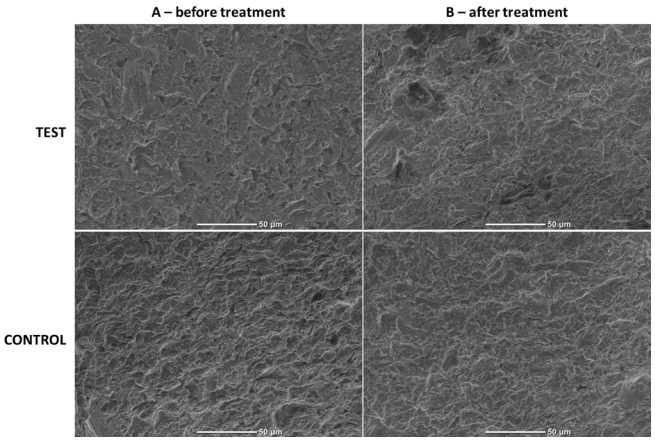


**Figure 4:** Microphotograps of implant surface before oral exposition (a) and after treatment with the test toothpaste (b). No sign of disgregation of the titanium surface has been observed.

**Semi-quantitative analysis of areas free from biofilm**. Data on % of areas free from biofilm in both groups is reported in figure 5. This data resulted significantly higher in test than in control group ($p<0.001$, Mann-Whitney test for paired data).

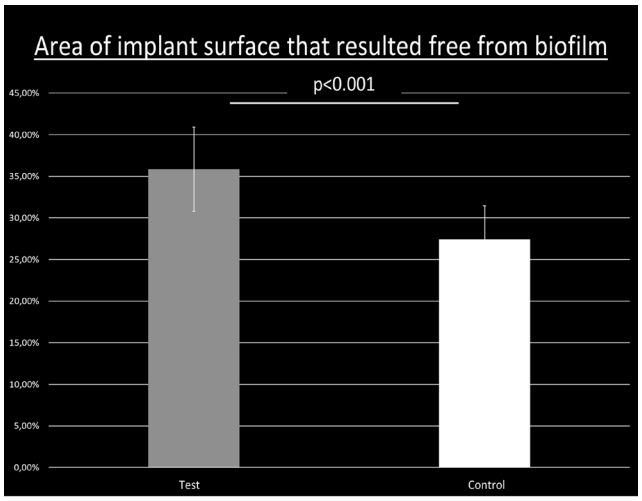


**Figure 5:** Graphic shows the percentage (%) areas of implant surface that resulted free from biofilm. A statistical difference ($p<0.001$) was found between groups (non-parametric Mann-Whitney test).

**Analysis of the surface roughness**. At the analysis by means of profilometry, Ra was 0.781±0.1 µm for test and 0.783±0.09 µm for control group, Rmax was 5.04±0.8 µm for test and 5.03±0.9 µm for control group (mean ± standard deviation). No differences resulted between groups (Mann-Whitney test for paired data).

## Discussion

In the present study, morphological aspects of biofilm formed *in vivo* on titanium implants with rough surface and treated with a toothpaste containing an oxygen releasing compound were analyzed by means of scanning electron microscopy. At the analysis, implants treated with the test agent showed a significant higher percentage of areas free from biofilm than control. These data indicate that the daily use of toothpaste containing AX may exert an antibacterial activity against bacteria of the oral plaque thus to inhibit the amount of biofilm adherent to the rough implant surface even after 5 days of exposition to the oral cavity. These data accord to literature that demonstrated the effects of oxygenating compounds in countering the infection of the oral tissues acting directly against anaerobes and indirectly by promoting tissue healing stimulating endogenous antibacterial activity of saliva and enhancing leukocyte functions at the tooth/gingiva interface [13]. Clinical and *in vitro* studies demonstrated that oxygenating agents are able to affect bacterial composition and metabolic activity of the microcosm biofilm and to improve clinical and microbiological parameters of pockets instrumented and then irrigated [14,15].

*In vivo* study on biofilm formation observed that hydrogen peroxide exerts an anti-adherence activity by reducing the total number of bacteria attached to the titanium surface and a bactericidal effect increasing the percentage of dead bacterial cells [16]. Clinical trials reported an improvement in peri-implant inflammatory clinical parameters after decontamination with hydrogen peroxide [17]. *In vitro* study on oxygen releasing compound Ardox-X® technology showed that this molecule selectively inhibits the growth of oral bacteria, with anaerobe Gram-negative species being the most sensitive. These promising findings were confirmed in an *in vivo* microbiological trial [9].

The further finding of the present study, regards the absence of morphological alterations of titanium surfaces both in test and control samples, showing that the toothpaste containing an oxygen releasing compound does not seem to corrode or degrade the titanium. The aggressive potential to modify the implant surface is an important factor to consider for chemical as well as for mechanical antibacterial treatments. In fact, the released Ti particles are able to induce inflammatory response in tissues surrounding implants that results in peri-implant bone loss [18]. Titanium disks were found to release Ti particles after mechanical treatments [18]. Similarly, chemical agents can be corrosive for metals, leading to degradation of dental implant surface and to release of metallic ions that results toxic for peri-implant tissues [19]. These morphological alterations can be detected after observation under microscope. Damaging signs were found at optical microscopy observation on Ti surface after immersion in acid decontaminating solutions containing peroxyacetic acid, citric acid, hydrogen peroxide 15%, tetracycline, and doxycycline [11]. Morphological aspects of degradation by excessive oxidation and material loss of titanium disks were observed at SEM after exposition to fluoridate medium [19]. A study reported no localized corrosion of implant systems after immersion in hydrogen peroxide although the profilometry showed increase in roughness [20].

The damaging potential of reacting oxygen species on metals has been largely demonstrated at SEM analysis, in contrast with findings of the present study [20,21]. This data is probably due to the nature of the tested oxygen compound. The tested toothpaste releases active oxygen in aqueous solutions generated by sodium perborate (peroxo-borate), but do not release the reactive oxygen species. Furthermore, in the present study the exposition of the implant surface to the oxygen releasing compound was tested *in vivo* thus the effect of salivary antioxidant molecules including peroxidase, superoxide dismutase, uric acid has to be considered [22].

To conclude, the daily use of toothpaste containing AX seems to inhibit the amount of biofilm adherent to the rough implant surface and formed after 5 days of exposition to the oral cavity. Furthermore, this molecule does not seem to corrode or degrade the titanium. Further morphological investigations could be done to assess if AX is able to disrupt a biofilm already formed on rough surfaces.

**Conflict of interest**

The authors declare there is no conflict of interest regarding the publication of this article

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