

Article

Biofilm removal and bacterial re-colonization inhibition of a novel erythritol/chlorhexidine air-polishing powder on titanium disks.

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Abstract: Air-polishing with low abrasiveness powders is fast arising as a valid and mini-invasive instrument for the management of biofilm colonizing dental implants. The reported advantage is the efficient removal of plaque with respect of the titanium integrity. In the present study, we evaluated the *in-situ* plaque-removal and continual the post-treatment anti-bacterial efficacy of an innovative erythritol/chlorhexidine air-polishing powder and compared it with sodium bicarbonate. Two peri-implantitis-linked biofilm formers strains *Staphylococcus aureus* and *Aggregatibacter actinomycetemcomitans* were selected and used to infect titanium disks before and after the air-polishing treatment. Cells number and viability were assayed by colonies forming units (CFUs) count and metabolic-colorimetric (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) (XTT) assay. Air-polishing performed with either sodium bicarbonate or erythritol/chlorhexidine was effective in reducing bacteria biofilm viability and number onto pre-infected specimens, while erythritol/ chlorhexidine showed a higher post-treatment biofilm re-growth inhibition. Surface analysis via mechanical profilometry failed to show an increase in titanium roughness, regardless of the powder selected.

Keywords: air-polishing; titanium; erythritol; chlorhexidine; biofilm; implants;

1. Introduction

Dental implants placement makes them part of the intra-oral microenvironment, where a complex microbial community tends to adhere to any available surface and build a biofilm [1–3].

Bacterial colonisation at implant surface occurs within 30 minutes after the implant transmucosal portion is connected to the surgical site, while a mature sub-gingival microbiota can be observed within a week [2,4]. Periodontal pathogens (*P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *P. intermedia*) can be early detected, along with peculiar implant-colonising bacteria such as *E. coli* and *S. aureus* [2,4,5]. Shifts in the microbial load or in the relative amount of pathogens can lead to the development of peri-implant mucositis and peri-implantitis [3,6,7]. A regular and accurate disruption of the biofilm at the implant surface is therefore mandatory to prevent and treat peri-implant inflammatory diseases. Nowadays, there is no irrefutable evidence of the superiority of any mechanical or chemical biofilm control mean over the others [8,9]. Although mechanical debridement with manual and/or power-driven instruments can lead to resolution of the inflammatory process [9], it may fail to restore the biocompatibility of the implant due to surface alteration and deposit of debris from the instruments, thus impairing cell viability and attachment [10,11]. Furthermore, the increased surface roughness can lead to higher biofilm adhesion and accumulation [12].

Air-polishing seems to constitute a valid tool for the supra- and sub-gingival management of biofilm at teeth and implants [13–15]. *In-vitro* application of air-polishing on micro-structured titanium surfaces and in simulated peri-implant defects seems to achieve a more successful biofilm removal when compared with various mechanical instrumentation means (i.e. plastic curettes and ultrasonic tips with chlorhexidine irrigation, Vector system) and lasers (Er:YAG and Er,Cr:YSGG) [16–20]. Air-polishing also grants higher osteoblast viability on titanium surface compared with hand and ultrasonic instrumentation [16,20] and Er:YAG laser [18]. However, powders with a different composition or from different manufacturers can exploit peculiar effects on the treated surface [16,21,22]. Sodium bicarbonate is useful in biofilm removal at micro-structured titanium discs but alters the surface morphology [16,23], while glycine powder grants the same efficacy with minimal damage to the titanium, thanks to the low abrasiveness [11,16,22,23]. Also, glycine seems able to reduce the bacterial re-colonisation of the treated surface [23,24].

Erythritol is a biocompatible [25,26], non-cariogenic [27], non-toxic sugar-alcohol [26] recently introduced in the formulation of a low-abrasiveness powder, in combination with chlorhexidine (erythritol/CHX). Amongst the polyols family, erythritol shows the highest inhibitory activity towards cariogenic bacteria [28] and *P. gingivalis* [29] both *in-vitro* and *in-vivo*. It can also decrease the adherence ability of several oral streptococci [30]. Air-polishing with erythritol/CHX in periodontal maintenance therapy showed comparable clinical outcomes to ultrasonic and manual debridement [14,15] and a higher decrease of sites positive for *A. actinomycetemcomitans* [14].

Currently, comparative studies about the efficacy of air-polishing with erythritol/CHX in titanium decontamination are still limited. Recent *in vitro* studies showed that erythritol/CHX causes no changes in the topography of the implant neck [11] and exploits an inhibitory and microbicide activity against bacteria previously cultivated on sandblasted titanium discs [24,31], with a stronger anti-biofilm activity than what obtained by glycine [24].

The present *in vitro* study aimed to test the efficacy of air-polishing with an erythritol/CHX powder (AIR-FLOW® PLUS, EMS Electro Medical Systems, Nyon, Switzerland) in biofilm removal and prevention of bacterial re-growth onto titanium disks; surface roughness changes were evaluated by profilometer. The antibacterial activity was assayed towards *S. aureus* and *A. actinomycetemcomitans* strains, two common peri-implant biofilm formers. Sodium bicarbonate powder served as a comparison.

2. Materials and Methods

2.1. Materials

For experiments, 1 cm diameter, 2mm thickness titanium grade II have been used. Air-polishing treatments have been performed using an AIR-FLOW® HANDY 3.0 PERIO system (EMS Electro Medical Systems, Nyon, Switzerland) equipped with two different powders: (i) a sodium bicarbonate-based powder (AIR-FLOW® CLASSIC COMFORT, EMS Electro Medical Systems, Nyon, Switzerland) and (ii) an erythritol/CHX powder (AIR-FLOW® PLUS, EMS Electro Medical Systems, Nyon, Switzerland) with a ~14 µm granulometry and 0.3 % of chlorhexidine content. All other reagents were purchased from Sigma (Sigma-Aldrich, Milan, Italy).

2.2. Profilometry

Powders' abrasiveness was evaluated by profilometry via mechanical profilometer (Surtronic 3+, Taylor-Hobson, Leicester, UK). Each titanium disk surface roughness was first evaluated to set-up single-disk basic parameters. Air-polishing treatment was then performed on the prior analysed disks using one of the selected powders for 5 seconds, keeping 5 mm of distance and a 35° angle

towards the surface. After the air-polishing phase, disks roughness was again evaluated by profilometer to verify any surface change.

2.3. Strains and growth conditions

Two strong biofilm formers, *Staphylococcus aureus* e *Aggregatibacter actinomycetemcomitans* strains, were purchased from the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Bacteria were cultivated in Luria-Bertani (LB) agar plates and stored at 4°C until use. Fresh broth culture was prepared prior to each experiment by dissolving some single colonies into fresh LB medium until a 0.01 optical density (corresponding to 1×10^7 cells/ml) was obtained at 600nm wavelength.

2.4. Preventive anti-adhesion activity

To verify the ability of the test powders to prevent bacteria adhesion, titanium disks were pre-treated with air-polishing technique prior to being infected. Autoclaved sterile disks were treated with either sodium bicarbonate powder or erythritol/CHX powder for 5 seconds keeping 5 mm of distance and a 35° angle towards the surface, and then seeded onto 24 wells plates and immediately submerged with 1ml of fresh bacteria culture containing 1×10^7 cells/ml (prepared as described in 2.3). The plate was incubated for 24 hours at 37°C and then bacteria number and viability were evaluated through the colonies forming units (CFUs) count and the metabolic-colorimetric (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide) (XTT) assay. For the CFUs count, biofilm was detached from disks surface by sonication and vortex (30 seconds each), collected and used to perform 6 ten-folder dilutions. From each dilution, 20 µl were collected and spotted onto LB agar plates and incubated for 24 hours at 37°C; the day after, the number of viable colonies was counted as follow [32,33]:

$$CFU = [(number\ of\ colonies\ x\ dilution\ factor)^{(serial\ dilution)}]$$

where:

number of colonies = countable single round colonies;

dilution factor = dilution made from the initial 1 mL suspension;

serial dilution = 1–6 ten-fold dilution areas where colonies were counted.

For the XTT analysis, after 24 hours of cultivation, the XTT solution (1mg/ml in PBS) was added to the wells containing the infected disks; the plate was incubated 4 hours in the dark and then supernatants optical density was evaluated by spectrophotometer (Victor, PerkinElmer, Waltham, MA, USA) at a 570 wavelength. Not treated disks were used as a control and considered as 100% viability; test specimens' results were normalized in function of control and expressed as % of it [25].

2.5. Plaque removal

To test air-polishing decontamination ability, autoclaved sterile disks were infected as described in 2.4 for 24 hours. Then, the air-polishing procedure was applied for 30 seconds and cells number and viability were assayed by CFUs count and XTT evaluation as prior described.

2.6. Statistical analysis of data

Each experiment was performed using at least 3 specimens; obtained data were analysed by the SPSS software (IBM, Chicago, USA) using the *ONE-way* ANOVA test followed by the Sheffè *post-hoc* analysis. Significance level was set at $p < 0.05$.

3. Results

3.1. Surface analysis by Profilometry

Profilometry analysis results are reported in Figure 1. After the air-polishing treatment, no statistically significant differences were noticed between the not treated specimens (considered as control) and the sodium bicarbonate and erythritol/CHX test specimens. Thus, it can be speculated that the titanium disks surface roughness was not significantly affected by any air-polishing treatment ($p>0.05$).

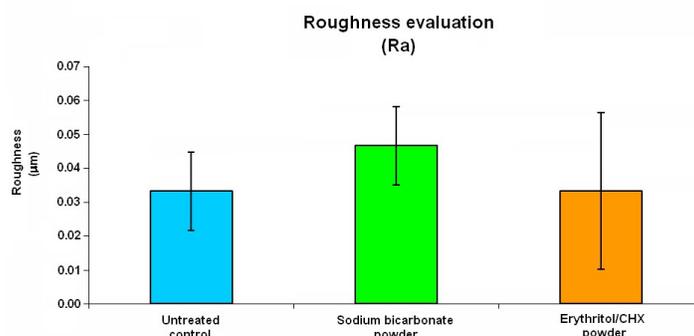


Figure 1. Surface roughness of air-polishing treated titanium disks. No significant differences were noticed between untreated control specimens and the test (sodium bicarbonate and erythritol/CHX) ones ($p>0.05$). Bars represent means and standard deviations.

3.2. Preventive anti-bacteria adhesion activity evaluation

The preventive use of the sodium bicarbonate and erythritol/CHX powders by means of air-polishing procedure was efficient in bacteria adhesion prevention. Results are reported in Figure 2a-d.

Regarding *S. aureus* biofilm viability, significant differences were noticed between untreated controls and the erythritol/CHX treated specimens (Fig. 2a, $p<0.05$, indicated by the *); moreover, significant differences were also noticed by comparing sodium bicarbonate and erythritol/CHX specimens (Fig. 2a, $p<0.05$, indicated by the #).

Considering *A. actinomycetemcomitans* viability, significant differences were noticed only by comparing the untreated and the erythritol/CHX treated specimens (Fig. 2a, $p<0.05$, indicated by the *). No significant differences were noticed between untreated controls and the sodium bicarbonate neither between the two test powders ($p>0.05$).

XTT data seems to be confirmed by the CFUs count (Fig. 2c-d). In fact, the number of *S. aureus* colonies was significantly decreased by erythritol/CHX in comparison with untreated controls (Fig. 2c, $p<0.05$, indicated by the *) and sodium bicarbonate (Fig. 2c, $p<0.05$, indicated by the #).

A similar trend was confirmed for *A. actinomycetemcomitans* colonies number, that was decreased mostly by erythritol/CHX powder with results significant in comparison with both untreated controls and sodium bicarbonate (Fig. 2d, $p<0.05$, indicated by the * and by the # respectively).

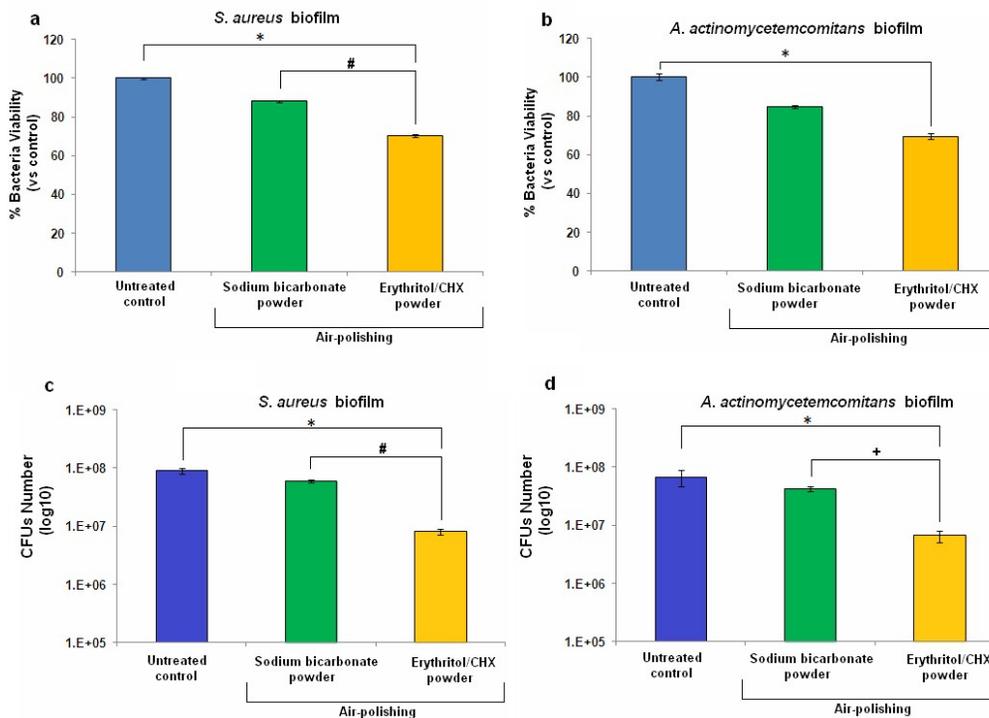


Figure 2. Preventive anti-bacteria adhesion ability evaluation. The application of erythritol/CHX powder by air-polishing procedure was able to reduce *S. aureus* viability (a) and number (c) in a significant way in comparison with untreated controls ($p < 0.05$, indicated by the *) and sodium bicarbonate ($p < 0.05$, indicated by the #). Similarly, significant differences were noticed in *A. actinomycetemcomitans* viability (b) between erythritol/CHX and untreated controls ($p < 0.05$, indicated by the *) and between erythritol/CHX and untreated control ($p < 0.05$, indicated by the *) or sodium bicarbonate ($p < 0.05$, indicated by the #) when the CFUs count was applied (d). Bars represent means and standard deviations.

3.3. Plaque removal evaluation

Air-polishing technique performed with either sodium bicarbonate or erythritol/CHX was effective in reducing bacteria biofilm viability and number onto pre-infected specimens. Results are reported in Figure 3a-d.

Similar results were obtained considering *S. aureus* (Fig. 3a-c) and *A. actinomycetemcomitans* (Fig. 3b-d) biofilm; by XTT assay, it was demonstrated that bacteria viability was significantly ($p < 0.05$) reduced after air-polishing treatment. The viability of treated groups (sodium bicarbonate and erythritol/CHX) resulted as significant in comparison with untreated control in both *S. aureus* (Fig. 3a, indicated by the *) and *A. actinomycetemcomitans* (Fig. 3b, indicated by the #). No significant differences were noticed between the two test powders ($p > 0.05$).

Bacteria viability data were then confirmed by the CFUs count (Fig. 3c-d): in fact, the number of bacteria colonies was reduced in a significant manner ($p < 0.05$) by the application of air-polishing procedure for both *S. aureus* (Fig. 3c, indicated by the *) and *A. actinomycetemcomitans* (Fig. 3d, indicated by the #). As previously shown by the XTT analysis, also with CFUs count did not evidence any significant differences between the sodium bicarbonate and the erythritol/CHX powders ($p > 0.05$).

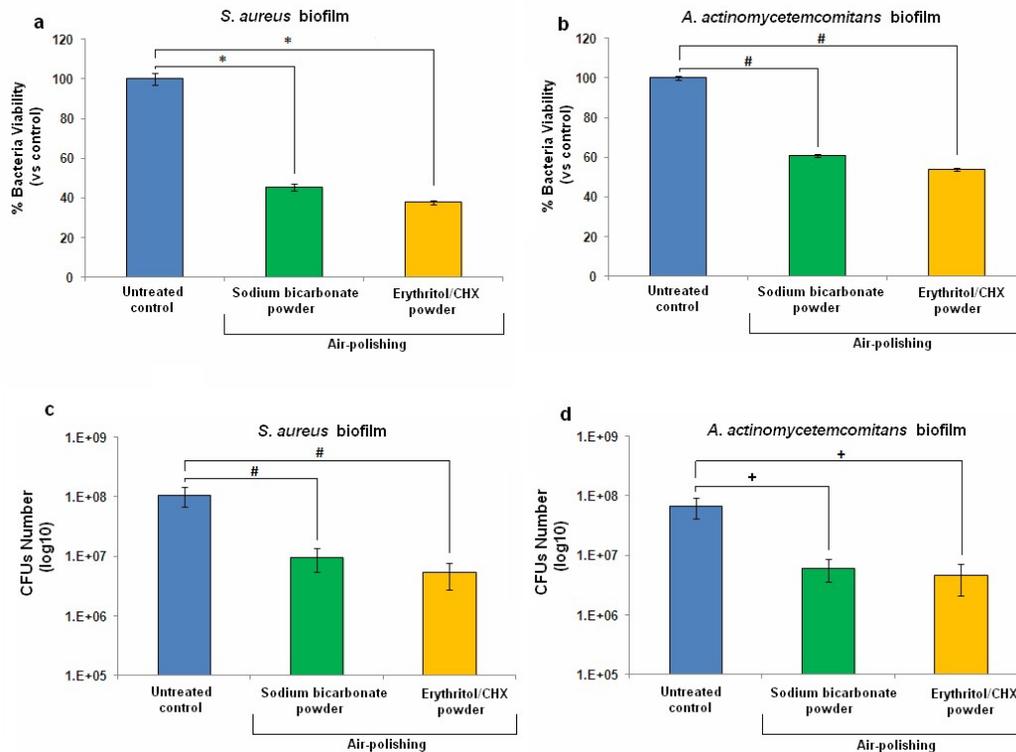


Figure 3. Plaque removal evaluation. The air-polishing usage significantly reduced *S. aureus* (a) and *A. actinomycetemcomitans* (b) viability in comparison with the untreated controls ($p < 0.05$, indicated by the * and #, respectively). Likewise, also bacteria number was significantly reduced ($p < 0.05$) by sodium bicarbonate and erythritol/CHX powders in comparison with controls when both *S. aureus* (c, indicated by the #) and *A. actinomycetemcomitans* (d, indicated by the +) were considered. No significant differences were marked between the two test powders ($p > 0.05$). Bars represent means and standard deviations.

4. Discussion

The development of peri-implant inflammatory disease depends on the presence and growth of biofilm at the implant surface [34]. The peri-implant microbiota can include typical periodontal pathogens (*P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *P. intermedia*) but also peculiar implant-colonising bacteria such as *E. coli* and *S. aureus* [2,4,5].

Air-polishing with low abrasiveness powder is stepping in as a valid and mini-invasive instrument for the management of dental implants biofilm. The advantages reported in literature are the efficient removal of bacterial deposits respecting the treated titanium surface [16–20,23,24]. A recent review of the literature from Schwarz et al. [13] concluded that air-polishing can be successfully applied in mucositis cases as a mono-therapy and can improve the outcome of the treatment of peri-implantitis when combined with ultrasonic debridement. However, different parameters can influence the procedure efficiency and invasiveness, such as the device used, pressure settings, air/water ratio, working distance and angulation, and the powder selected [16,21,22].

In the present study, an erythritol/CHX powder was chosen (AIR-FLOW® PLUS, EMS Electro Medical Systems, Nyon, Switzerland) and compared to a sodium bicarbonate one in terms of biofilm removal efficiency, surface alteration and anti-biofilm activity.

To test the efficacy in biofilm removal, Grade II titanium disks were submerged and incubated for 24 hours in bacteria cultures containing *S. aureus* and *A. actinomycetemcomitans*, then treated with an air-polishing system for 30 seconds. After-treatment bacteria number and viability were evaluated through the colonies forming units (CFUs) count and the metabolic-colorimetric XTT assay. Both erythritol/CHX and sodium bicarbonate powders were found equally and significantly effective in reducing the quantity and viability of bacterial cells. These findings are in line with the results of Drago et al. [24] who tested the bacterial viability of *S. aureus*, *C. albicans* and *B. fragilis* biofilm on titanium sandblasted disks after 5 seconds of air-polishing with glycine and erythritol/CHX, evaluated through confocal laser scanning microscopy and spectrophotometric assay. The treatment with erythritol/CHX powder obtained a significantly higher reduction of all bacterial strains, compared with both glycine powder and a mechanical control treatment.

Both erythritol and chlorhexidine are known to exert an anti-biofilm/antimicrobial activity, therefore prevention/delay of re-growth of biofilm could be an additional advantage of their application on implants. Chlorhexidine has a well-known immediate and posterior antimicrobial effect thanks to its substantivity [35,36], while erythritol shows different mechanisms of action. Söderling et al. [30] demonstrated that erythritol decreases the polysaccharide-mediated bacterial adherence of different Streptococcus strains, raising the interest in the cariology field. Of periodontal relevance, Hashino et al. [29] showed that erythritol is able to suppress the growth of a *P. gingivalis*-*S. gordonii* biofilm interfering with different metabolic pathways and inhibiting the nucleotide and matrix biosynthesis. Regular administration of erythritol has been advocated as a caries-prevention regimen: Mäkinen et al. [28] showed how a 6-months-long daily use of erythritol-containing chewable tablets and dentifrice is able to reduce the plaque and saliva levels of *S. mutans* streptococci, while Falony et al. [37] proved the caries-preventive effect of a daily dose of erythritol during a 3-year intervention, and observed persistence of the benefit up to 3 years after the end of the administration. However, there is currently no evidence of erythritol substantivity after a single administration or through its application as a single-component via air-polishing, being available just in combination with CHX. In the second part of their study, Drago et al. [24] analysed the recovery level of the residual biofilm after 16-18 hours of incubation. The disks treated with erythritol/CHX showed around 50% less viable cells than the control specimens. Positive results were observed in the present study as well, where bacterial growth was analysed on sterile titanium disk treated with 5 seconds of air-polishing and then incubated for 24 hours in fresh bacteria culture. *S. aureus* viability was significantly lower on specimens previously treated with erythritol/CHX powder compared with sodium bicarbonate and control disks, with confirmation by the CFUs count showing significantly fewer colonies. In regard to *A. actinomycetemcomitans*, the XTT assay test confirmed a significant reduction of viability on erythritol/CHX treated disks compared with untreated controls, but failed to reach the statistical significance when compared with sodium bicarbonate. This differs from the CFUs count, where erythritol/CHX treated specimens showed significantly fewer colonies than untreated and sodium bicarbonate ones too. The authors speculate this outcome might be due to the substantivity of the CHX on the titanium disks surface. At this stage, translation of in-vitro results to in-vivo recommendations should still be cautious. The application of erythritol/CHX through air-polishing for periodontal maintenance therapy showed small positive shifts of the bacterial population on the short term but failed to reach the statistical significance compared with classic ultrasonic instrumentation [14,15]. *In vivo* clinical trials involving the use of the erythritol/CHX powder with a focus on the microbiology of the peri-implant environment are still not available.

Of major concern during plaque removal at the implant surface is the possible surface alteration, since increased roughness is related to augmented plaque accumulation [38-40]. In the present study, a mechanical profilometer was used to evaluate the titanium disks roughness before and after the application of air-polishing with sodium bicarbonate or erythritol/CHX powder for 5 seconds. The profilometry failed to prove any statistically significant difference in surface roughness between treated and untreated specimens, regardless of the powder used, thus excluding the possibility to

favour the bacterial adhesion. This finding does not mean that sodium bicarbonate and erythritol/CHX powders have an equal effect on titanium surface. Cochis et al. [23] analysed the morphological change induced by air-polishing on titanium disks through both laser profilometry and scanning electron microscopy (SEM). Even if the mean and maximum roughness measured by the profilometer did not reach a statistically significant difference after application sodium bicarbonate powder, the SEM observation revealed newly formed roughness and craters. Morphological changes after sodium bicarbonate treatment were reported at the SEM not only on machined but also on rough titanium surface [16]. Low abrasiveness powders such as glycine and erythritol/CHX seem to cause almost no observable morphological changes at SEM analysis [11,23]. Schmidt et al. [11] in an in-vitro study investigated the alterations caused to implant abutments by different instrumentations. Two different glycine powders and an erythritol/CHX powder were tested. SEM images revealed that the erythritol/CHX powder results in the least surface modification.

The clinical significance of the surface alternation is uncertain and only biofilm viability tests performed after the application of air-polishing can give an insight. Cochis et al. [23] in the in-vivo part of their study exposed the treated titanium disks to the oral environment. The microbiological evaluation could not reveal any statistically significant difference in plaque accumulation between untreated and sodium bicarbonate-treated specimens, confirming no increase in bacterial retention. The results are confirmed by the present study in which the TTX analysis and CFUs count could not reveal any statistically significant difference in *S. aureus* and *A. actinomycetemcomitans* growth between untreated and sodium bicarbonate-treated specimens. Even if the present study failed to prove any roughness and plaque accumulation increase after air-polishing with sodium bicarbonate, it's important to remember that the application lasted for 5 seconds, simulating a single session of prophylaxis. In the clinical reality, patients with implant-supported restorations should undergo a session of maintenance therapy every 5-6 months [41]. As a consequence, with a long-term application of sodium bicarbonate, a cumulative increase of surface alteration can be supposed and could become of clinical relevance. It's important to remember that any damage to the titanium may decrease fibroblast adhesion and, hence, implant biocompatibility [42]. Moreover, sodium bicarbonate powder is proven to cause damage to the gingival tissue [43] making necessary to aim the air-polishing jet away from soft tissues and keep a distance from the gingival margin during the in-vivo application.

A possible limitation of the present study is the use of mono-bacteria biofilm. Exposure of the titanium disks to the actual oral biofilm in its complexity would give a more realistic picture of the anti-biofilm activity of the erythritol/CHX powder.

5. Conclusions

Both sodium bicarbonate and erythritol/CHX powders are good tools for air-polishing at the implant surface. None of the powders determined a significant increase of titanium surface roughness, thus reducing the possibility to favour bacteria adhesion. Sodium bicarbonate and erythritol/CHX resulted effective in plaque removal and adhesion prevention, with a superior anti-biofilm effect towards the considered strains showed by the erythritol/CHX powder.

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References

1. Listgarten, M. The Structure of Dental Plaque. *Periodontol.* 2000 **1994**, *5*, 52–65.
2. Fürst, M. M.; Salvi, G. E.; Lang, N. P.; Persson, G. R. Bacterial Colonization Immediately after Installation on Oral Titanium Implants. *Clin. Oral Implants Res.* **2007**, *18* (4), 501–508. doi:10.1111/j.1600-0501.2007.01381.x.
3. Belibasakis, G. N. Microbiological and Immuno-Pathological Aspects of Peri-Implant Diseases. *Arch. Oral Biol.* **2014**, *59* (1), 66–72. doi:10.1016/j.archoralbio.2013.09.013.
4. Quirynen, M.; Vogels, R.; Peeters, W.; Van Steenberghe, D.; Naert, I.; Haffajee, A. Dynamics of Initial Subgingival Colonization of “Pristine” Peri-Implant Pockets. *Clin. Oral Implants Res.* **2006**, *17* (1), 25–37. doi:10.1111/j.1600-0501.2005.01194.x.
5. Heuer, W.; Stiesch, M.; Abraham, W. R. Microbial Diversity of Supra- and Subgingival Biofilms on Freshly Colonized Titanium Implant Abutments in the Human Mouth. *Eur. J. Clin. Microbiol. Infect. Dis.* **2011**, *30* (2), 193–200. doi:10.1007/s10096-010-1068-y.
6. Mombelli, A.; van Oosten, M. A.; Schurch, E. Jr; Land, N. P. The Microbiota Associated with Successful or Failing Osseointegrated Titanium Implants. *Oral Microbiol Immunol* **1987**, *2* (4), 145–151.
7. Hultin, M.; Gustafsson, A.; Hallström, H.; Johansson, L. Å.; Ekfeldt, A.; Klinge, B. Microbiological Findings and Host Response in Patients with Peri-Implantitis. *Clin. Oral Implants Res.* **2002**, *13* (4), 349–358. doi:10.1034/j.1600-0501.2002.130402.x.
8. Grusovin, M.; Coulthard, P.; Worthington, H. V.; Esposito, M. Interventions for Replacing Missing Teeth: Maintaining Health around Dental Implants 26. *Cochrane.Database.Syst.Rev.* **2004**, No. 1469–493X, CD003069. doi:10.1002/14651858.CD003069.pub2.
9. Esposito, M.; Grusovin, M. G.; Worthington, H. V. Interventions for replacing missing teeth: treatment of peri-implantitis. *Cochrane Database of Systematic Reviews* **2012**, Issue 1. Art. No.: CD004970. DOI: 10.1002/14651858.CD004970.pub5
10. Louropoulou, A.; Slot, D. E.; van der Weijden, F. Influence of Mechanical Instruments on the Biocompatibility of Titanium Dental Implants Surfaces: A Systematic Review. *Clin. Oral Implants Res.* **2015**, *25* (10), 1149–1160. doi:10.1111/clr.12224.
11. Schmidt, K. E.; Ausschill, T. M.; Heumann, C.; Frankenberger, R.; Eick, S.; Sculean, A.; Arweiler, N. B. Influence of Different Instrumentation Modalities on the Surface Characteristics and Biofilm Formation on Dental Implant Neck, in Vitro. *Clin. Oral Implants Res.* **2016**, 1–8. doi:10.1111/clr.12823.
12. Louropoulou, A.; Slot, D. E.; Van der Weijden, F. a. Titanium Surface Alterations Following the Use of Different Mechanical Instruments: A Systematic Review. *Clin. Oral Implants Res.* **2012**, *23* (6), 643–658. doi:10.1111/j.1600-0501.2011.02208.x.
13. Schwarz, F.; Becker, K.; Renvert, S. Efficacy of Air Polishing for the Non-Surgical Treatment of Peri-Implant Diseases: A Systematic Review. *J. Clin. Periodontol.* **2015**, *42* (10), 951–959. doi:10.1111/jcpe.12454.
14. Müller, N.; Moëne, R.; Cancela, J. A.; Mombelli, A. Subgingival Air-Polishing with Erythritol during Periodontal Maintenance: Randomized Clinical Trial of Twelve Months. *J. Clin. Periodontol.* **2014**, *41* (9), 883–889. doi:10.1111/jcpe.12289.
15. Hägi, T. T.; Hofmänner, P.; Eick, S.; Donnet, M.; Salvi, G. E.; Sculean, A.; Ramseier, C. A. The Effects of Erythritol Air-Polishing Powder on Microbiologic and Clinical Outcomes during Supportive Periodontal Therapy: Six-Month Results of a Randomized Controlled Clinical Trial. *Quintessence Int.* **2015**, *46* (1), 31–41. doi:10.3290/j.qi.a32817.
16. Schwarz, F.; Ferrari, D.; Popovski, K.; Hartig, B.; Becker, J. Influence of Different Air-Abrasive Powders on Cell Viability at Biologically Contaminated Titanium Dental Implants Surfaces. *J. Biomed. Mater. Res. - Part B Appl. Biomater.* **2009**, *88* (1), 83–91. doi:10.1002/jbm.b.31154.
17. Schwarz, F.; Nuesry, E.; Bieling, K.; Herten, M.; Becker, J. Influence of an Erbium, Chromium-Doped Yttrium, Scandium, Gallium, and Garnet (Er,Cr:YSGG) Laser on the Reestablishment of the Biocompatibility of Contaminated Titanium Implant Surfaces. *J. Periodontol.* **2006**, *77* (11), 1820–1827. doi:10.1902/jop.2006.050456.
18. Schwarz, F.; Sculean, A.; Romanos, G.; Herten, M.; Horn, N.; Scherbaum, W.; Becker, J. Influence of Different Treatment Approaches on the Removal of Early Plaque Biofilms and the Viability of SAOS2

- Osteoblasts Grown on Titanium Implants. *Clin. Oral Investig.* **2005**, *9* (2), 111–117. doi:10.1007/s00784-005-0305-8.
19. Ronay, V.; Merlini, A.; Attin, T.; Schmidlin, P. R.; Sahrman, P. *In Vitro* Cleaning Potential of Three Implant Debridement Methods. Simulation of the Non-Surgical Approach. *Clin. Oral Implants Res.* **2016**, n/a-n/a. doi:10.1111/clr.12773.
 20. Schwarz, F.; Papanicolau, P.; Rothamel, D.; Beck, B.; Hertzen, M.; Becker, J. Influence of Plaque Biofilm Removal on Reestablishment of the Biocompatibility of Contaminated Titanium Surfaces. *J Biomed Mater Res* **2006**, *77A*, 437–444. doi:10.1002/jbm.a.30628 Abstract:
 21. Barnes, C. M.; Covey, D.; Watanabe, H.; Smetich, B.; Schulte, J. R.; Chen, H. An in Vitro Comparison of the Effects of Various Air Polishing Powders on Enamel and Selected Esthetic Restorative Materials. *J. Clin. Dent.* **2014**, *25* (4), 76–87.
 22. Salerno, M.; Giacomelli, L.; Derchi, G.; Patra, N.; Diaspro, A. Atomic Force Microscopy in Vitro Study of Surface Roughness and Fractal Character of a Dental Restoration Composite after Air-Polishing. *Biomed. Eng. Online* **2010**, *9* (1), 59. doi:10.1186/1475-925X-9-59.
 23. Cochis, A.; Fini, M.; Carrassi, A.; Migliario, M.; Visai, L.; Rimondini, L. Effect of Air Polishing with Glycine Powder on Titanium Abutment Surfaces. *Clin. Oral Implants Res.* **2013**, *24* (8), 904–909. doi:10.1111/j.1600-0501.2012.02490.x.
 24. Drago, L.; Del Fabbro, M.; Bortolin, M.; Vassena, C.; De Vecchi, E.; Taschieri, S. Biofilm Removal and Antimicrobial Activity of Two Different Air-Polishing Powders: An in Vitro Study. *J. Periodontol.* **2014**, *85* (11), e363-9. doi:10.1902/jop.2014.140134.
 25. Munro, I. C.; Bernt, W. O.; Borzelleca, J. F.; Flamm, G.; Lynch, B. S.; Kennepohl, E.; Bär, E. a.; Modderman, J. Erythritol: An Interpretive Summary of Biochemical, Metabolic, Toxicological and Clinical Data. *Food Chem. Toxicol.* **1998**, *36* (12), 1139–1174. doi:10.1016/S0278-6915(98)00091-X.
 26. Awuchi, C. G. A. Sugar Alcohols: Chemistry, Production, Health Concerns and Nutritional Importance of Mannitol, Sorbitol, Xylitol, and Erythritol. *Int. J. Adv. Acad. Res. | Sci. Technol. Eng.* **2017**, *3* (2), 2488–9849.
 27. Bradshaw, D. J.; Marsh, P. D. Effect of Sugar Alcohols on the Composition and Metabolism of a Mixed Culture of Oral Bacteria Grown in a Chemostat. *Caries Res.* **1994**, *28* (4), 251–256. doi:10.1159/000261977.
 28. Mäkinen, K. K.; Saag, M.; Isotupa, K. P.; Olak, J.; Nömmela, R.; Söderling, E.; Mäkinen, P. L. Similarity of the Effects of Erythritol and Xylitol on Some Risk Factors of Dental Caries. *Caries Res.* **2005**, *39* (3), 207–215. doi:10.1159/000084800.
 29. Hashino, E.; Kuboniwa, M.; Alghamdi, S. A.; Yamaguchi, M.; Yamamoto, R.; Cho, H.; Amano, a. Erythritol Alters Microstructure and Metabolomic Profiles of Biofilm Composed of Streptococcus Gordonii and Porphyromonas Gingivalis. *Mol. Oral Microbiol.* **2013**, *28* (6), 435–451. doi:10.1111/omi.12037.
 30. Söderling, E. M.; Hietala-Lenkkeri, A. M. Xylitol and Erythritol Decrease Adherence of Polysaccharide-Producing Oral Streptococci. *Curr. Microbiol.* **2010**, *60* (1), 25–29. doi:10.1007/s00284-009-9496-6.
 31. Drago, L.; Bortolin, M.; Taschieri, S.; De Vecchi, E.; Agrappi, S.; Del Fabbro, M.; Francetti, L.; Mattina, R. Erythritol/chlorhexidine Combination Reduces Microbial Biofilm and Prevents Its Formation on Titanium Surfaces in Vitro. *J. Oral Pathol. Med.* **2017**, *46* (8), 625–631. doi:10.1111/jop.12536.
 32. Azzimonti, B.; Cochis, A.; El Beyrouthy, M.; Iriti, M.; Uberti, F.; Sorrentino, R.; Landini, M.M.; Rimondini, L.; Varoni, E.M. Essential Oil from Berries of Lebanese Juniperus excelsa M. Bieb Displays Similar Antibacterial Activity to Chlorhexidine but Higher Cytocompatibility with Human Oral Primary Cells. *Molecules* **2015**, *20*, 9344–9357; doi:10.3390/molecules20059344.
 33. Ferraris, S.; Truffa Giachet, F.; Miola, M.; Bertone, E.; Varesano, A.; Vineis, C.; Cochis, A.; Sorrentino, R.; Rimondini, L.; Spriano, S. Nanogrooves and keratin nanofibers on titanium surfaces aimed at driving gingival fibroblasts alignment and proliferation without increasing bacterial adhesion. *Mater. Sci. Eng. C* **2017**, *76*, 1–12. <http://dx.doi.org/10.1016/j.msec.2017.02.152>.
 34. Pontoriero, R.; Tonelli, M.; Carnevale, G.; Mombelli, A.; Nyman, S.; Lang, N. . Experimentally Induced Periimplant mucositis. A Clinical Study in Humans. *Clin Oral Implant. Res* **1994**, *5* (4), 254–259.
 35. Brambilla, E.; Ionescu, A.; Gagliani, M.; Cochis, A.; Arciola, C.R.; Rimondini, L. Biofilm formation on composite resins for dental restorations: an in situ study on the effect of chlorhexidine mouthrinses. *Int. J. Artif. Organs* **2012**, *35*, 792–799. doi:10.5301/ijao.5000165.
 36. Quintas, V.; Prada-López, I.; Donos, N.; Suárez-Quintanilla, D.; Tomás, I. In Situ Neutralisation of the Antibacterial Effect of 0.2% Chlorhexidine on Salivary Microbiota: Quantification of Substantivity. *Arch. Oral Biol.* **2015**, *60*, 1109–1116. doi:<https://doi.org/10.1016/j.archoralbio.2015.04.002>.

37. Falony, G.; Honkala, S.; Runnel, R.; Olak, J.; Nömmela, R.; Russak, S.; Saag, M.; Mäkinen, P. L.; Mäkinen, K.; Vahlberg, T.; Honkala, E. Long-Term Effect of Erythritol on Dental Caries Development during Childhood: A Posttreatment Survival Analysis. *Caries Res.* **2016**, *50*, 579–588. doi:10.1159/000450762.
38. Quirynen, M.; Van Der Mei, H. C.; Bollen, C. M.; Schotte, A.; Marechal, M.; Doornbusch, G. I.; Naert, I.; Busscher, H. J.; Van Steenberghe, D. An in Vivo Study of the Influence of the Surface Roughness of Implants on the Microbiology of Supra- and Subgingival Plaque. *J. Dent. Res.* **1993**, *72* (9), 1304–1309. doi:10.1177/00220345930720090801.
39. Borsari, V.; Fini, M.; Giavaresi, G.; Tschon, M.; Chiesa, R.; Chiusoli, L.; Salito, A.; Rimondini, L.; Giardino, R. Comparative in vivo evaluation of porous and dense duplex titanium and hydroxyapatite coating with high roughnesses in different implantation environments. *J. Biomed. Mater. Res. A* **2009**, *89*, 550–560. doi:10.1002/jbm.a.31995.
40. Ferraris, S.; Venturello, A.; Miola, M.; Cochis, A.; Rimondini, L.; Spriano, S. Antibacterial and bioactive nanostructured titanium surfaces for bone integration. *Appl. Surf. Sci.* **2014**, *311*, 279–291. doi:10.1016/j.apsusc.2014.05.056.
41. Monje, A.; Aranda, L.; Diaz, K. T.; Alarcón, M. A.; Bagramian, R. A.; Wang, H. L.; Catena, A. Impact of Maintenance Therapy for the Prevention of Peri-Implant Diseases. *J. Dent. Res.* **2016**, *95* (4), 372–379. doi:10.1177/0022034515622432.
42. Dmytryk, J. J.; Fox, S. C.; Moriarty, J. D. The Effects of Scaling Titanium Implant Surfaces with Metal and Plastic Instruments on Cell Attachment. *J. Periodontol.* **1990**, *61* (8), 491–496. doi:10.1902/jop.1990.61.8.491.
43. Petersilka, G.; Faggion, C. M.; Stratmann, U.; Gerss, J.; Ehmke, B.; Haeberlein, I.; Flemmig, T. F. Effect of Glycine Powder Air-Polishing on the Gingiva. *J. Clin. Periodontol.* **2008**, *35* (4), 324–332. doi:10.1111/j.1600-051X.2007.01195.x.