Dental Implants Surface *in vitro* Decontamination Protocols

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Abstract

Objective The number of patients rehabilitated with dental implants has contributed to increased incidence of peri-implant diseases. Due to complex and difficult treatment, peri-implantitis is a challenge and an efficient clinical protocol is not yet established. Aim of this study was to evaluate the efficacy of two protocols for *in vitro* decontamination of dental implants surface.

Materials and Methods Twenty titanium implants (BioHE-Bioconect) were used. Implants were divided into five groups (n = 4). NC group (negative control): sterile implants; PC group (positive control): biofilm contaminated implants; S group: biofilm contaminated implants, brushed with sterile saline; SB group: biofilm contaminated implants, brushed with sterile saline and treated with air-powder abrasive system with sodium bicarbonate (1 minute); and antimicrobial photodynamic therapy (aPDT) group: biofilm contaminated implants, brushed with sterile saline and treated with antimicrobial photodynamic therapy (red laser + toluidine blue O). The implants were contaminated *in vitro* with subgingival biofilm and distributed in groups PC, S, SB, and aPDT. Each group received the respective decontamination treatment, except groups NC and PC. Then, all implants were placed in tubes containing culture medium for later sowing and counting of colony-forming units (CFUs).

Statistical Analysis One-way analysis of variance and Tukey tests were performed, at 5% significance level.

Results Significantly fewer CFUs were observed in the aPDT group (19.38×10^5) when compared with groups SB (26.88×10^5), S (47.75×10^5), and PC (59.88×10^5) ($p < 0.01$). Both the aPDT and SB groups were statistically different from the NC group ($p < 0.01$).

Conclusion Proposed protocols, using air-powder abrasive system with sodium bicarbonate and aPDT, showed to be efficacious in the decontamination of dental implants surface *in vitro*.

Keywords
- peri-implantitis
- sodium bicarbonate
- photodynamic therapy
- dental implants
- decontamination

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Introduction

Osseointegrated dental implants are well established as a predictable treatment option, with high success[1] and survival rates.[2] However, failures can occur and, with increased number of implants installed, an increase in related complications is also expected.[3]

Peri-implant diseases are among the main complications, with incidence and prevalence rates following the increase in dental implant installation.[4] Recently, peri-implant diseases were classified into peri-implant mucositis and peri-implantitis[5] and present the biofilm as the main etiologic agent.[5,6] Peri-implantitis is considered a clinically complex and irreversible condition,[4,7] and is characterized by inflammation of the soft tissue around the implant with subsequent and progressive bone loss.[8]

The management of peri-implantitis can vary according to disease severity and extent, and nonsurgical or surgical approaches are indicated depending on the clinical and radiographic findings.[9] Nonsurgical therapy includes the use of mechanical methods that promotes biofilm removal by using curettes, ultrasonic scalers, air abrasive systems, and titanium brushes.[9] Chemical methods like citric acid and lasers, besides antiseptics and antibiotics[9] are suggested to be used in association with mechanical methods. Although nonsurgical therapy is an option, studies have shown that surgical treatment is recommended for peri-implantitis to achieve more favorable outcomes.[9]

One of the main difficulties in the peri-implantitis treatment is the implant surface decontamination, which seems to be a major concern[7,10-14] in the process that aims at reosseointegration.[15,16] Thus, several methods of implant surface decontamination have been proposed, both mechanical and chemical, in surgical access.[10,15,17] However, to date, no protocol has been established as the gold standard for peri-implantitis treatment.[15,18,19]

The literature shows that best decontamination results can be achieved when both mechanical and chemical methods are used.[9,12,20,21] As a mechanical method, sandblasting with abrasive particles has been used to treat peri-implantitis with positive effects.[16,22,23] The sandblasting systems use abrasive powder introduced into a stream of compressed air, and these powder particles gain kinetic energy from a flow of water and compressed air.[24] So, powder is an important parameter in the sandblasting effectiveness.[16] Among the available types, sodium bicarbonate proved to be very effective in removing bacteria from implant surfaces.[16] Regarding peri-implantitis treatment, studies show positive results with air-powder abrasive system with sodium bicarbonate using different time protocols from 60 seconds to 2 minutes.[25-27] Disadvantages as implant surface changes are also presented.[16,25] Thus, concerns about the time and efficacy are still raised in the literature, as well as changes in implant surface topography.

In the last decades, lasers associated with photosensitizing agents, in antimicrobial photodynamic therapy (aPDT), have been used as a chemical method for dental implant surface decontamination with promising results.[19,28] The aPDT is a minimally invasive, nontoxic, and safe method[14] based on the use of a photosensitizer, usually a dye activated by light with a specific wavelength, which can kill bacteria by generating highly reactive oxygen species.[14,29] The anti-infective treatment with aPDT uses a low-level laser after application of photosensitizing agents like toluidine blue and methylene blue or indocyanine green,[13,30] the only three photosensitizers clinically approved for use in humans in combination with light.[30,31] Recent studies have shown that aPDT seems to be effective in reducing bacterial load in peri-implantitis and is a potential alternative therapy.[14] However, some studies showed no difference in the effects of aPDT when compared with conventional therapies in peri-implantitis clinical treatment.[32] Thus, despite the promising results of aPDT, there is no consensus and a great diversity of protocols in related parameters, and an ideal protocol is not yet established.

The present study aims to contribute to the advancement of knowledge on peri-implantitis treatment, evaluating the efficacy of two protocols, mechanical and chemical, for *in vitro* decontamination of metallic implants surface.

Materials and Methods

This study was approved by the research ethics committee of the Inga University Center (number 3.072.210) regarding the biofilm collection. A sample size calculation was performed, and the result showed that 20 implants were necessary for this study. Therefore, 20 grade IV commercially available pure titanium implants were used, with a surface treated with double acid etching, cylindrical, external hexagon, measuring 5 mm in diameter and 18 mm in length (BioHE-Bioconect; Itapira, Brazil), sterilized from the factory. The dental implants were randomly assigned to the following groups ($n = 4$):

- NC group (negative control): sterile implants.
- PC group (positive control): biofilm contaminated implants.
- S group: biofilm contaminated implants, brushed with sterile saline.
- SB group: biofilm contaminated implants, brushed with sterile saline and treated with air-powder abrasive system with sodium bicarbonate (1 minute).
- aPDT group: biofilm contaminated implants, brushed with sterile saline and treated with aPDT.

The implants were contaminated with biofilm, except for the NC group. After signing the informed consent form, a subgingival biofilm sample was collected from a volunteer, diagnosed with severe periodontitis who underwent periodontal treatment at the Ingá University Center. The collection was performed using proper Gracey curettes (HuFriedy Mfg. Co.; Chicago, United States). The selection of the volunteer followed the inclusion criteria: adult subject (> 18 years), absence of systemic problems, and nonsmoker. Exclusion criteria were pregnancy, breastfeeding, and use of antibiotics in the last 6 months.

After collection, the biofilm was cultured in sterile brain heart infusion (BHI) broth (Kasvi, São José dos Pinhais, Brazil)

and gram morphotinturial analysis was performed. The implants were then distributed in groups PC, S, SB, and aPDT, and contaminated in test tubes containing 10 mL of BHI broth medium and subgingival biofilm, and maintained for 7 days, in a 37°C oven, for the formation of biofilm on the implant surfaces. After the *in vitro* contamination, the implants received the respective treatments, except for the PC implants that did not receive any decontamination treatment. Throughout the process, sterile forceps and gloves were used to avoid contamination of the implants with bacteria other than the biofilm.

Implants from the S, SB, and aPDT groups were brushed with a soft bristle toothbrush (Dentalclean; Londrina, Brazil) with 20 mL of sterile saline (Eurofarma; São Paulo, Brazil). Twenty brush strokes were performed, covering all implant surfaces (►**Fig. 1**). After brushing, the SB group implants were treated with high-pressure air-powder abrasive system (Practical Jet–Kondentech; São Carlos, Brazil) with extra-fine granulation sodium bicarbonate (Profhylaxis–Formaden; São José dos Pinhais, Brazil), for 1 minute (►**Fig. 2**) and rinsed with 10 mL of sterile saline. The aPDT group implants, after brushing, were placed in 12-well acrylic plates for 1 minute, immersed in 3 mL of toluidine blue O (Sigma-Aldrich; São Paulo, Brazil) at a concentration of 100 µg/mL diluted in distilled water[19] (►**Fig. 3**). Subsequently, implants were irradiated in scan mode with Whitening Lase II (DMC; São Carlos, Brazil) with 600 µm diameter fiber optics at a distance of 5 mm of the surface and divided into four faces (buccal, lingual, mesial, and distal), according to the adapted protocol[19] (660 nm; 30 mW; 50 J/cm^2; 47 seconds) (►**Fig. 4**), and rinsed with 10 mL of sterile saline.

After decontamination treatments were performed, all implants were inserted in 10 mL of sterile BHI broth medium. After 24 hours incubated at 37°C, dilutions and sowing acrylic plates were done in duplicate. These plates were stored in a CO_2 anaerobic jar, guaranteeing a condition of microaerophilia, and kept for 48 hours incubated at 37°C, allowing colonies to grow. Then, the colony-forming units (CFUs) were counted, with the naked eye, by an experienced examiner. The intergroup comparison of CFUs was performed by one-way analysis of variance and Tukey tests, with a significance level of 5%.

Results

The aPDT group presented the lowest number of CFUs ($19.38 \times 10^5 \pm 1.493$) when compared with the SB group ($26.88 \times 10^5 \pm 2.496$), S group ($47.75 \times 10^5 \pm 4.735$), and the PC group ($59.88 \times 10^5 \pm 1.436$), with statistically significant differences ($p < 0.01$). The SB group had a significantly lower number of CFUs when compared with groups S and PC ($p < 0.01$) and all the

Fig. 1 Dental implant brushed with a soft bristle toothbrush and sterile saline.

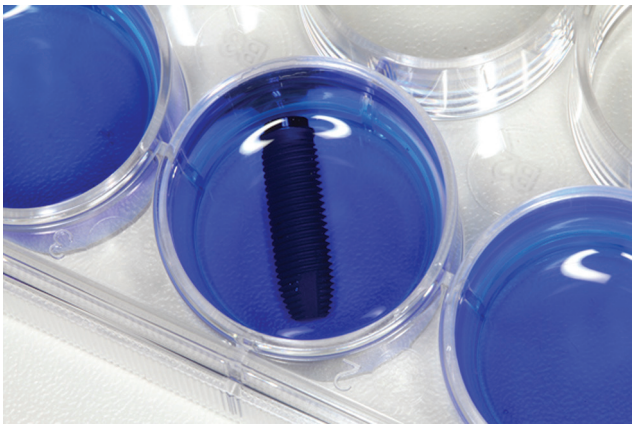

Fig. 3 Dental implant immersed in toluidine blue O (TBO) (100 µg/mL) for 1 minute before irradiation.

Fig. 2 Dental implant receiving air-powder abrasive system with sodium bicarbonate for 1 minute.

Fig. 4 Implant being irradiated with red laser (Whitening Lase II–DMC, São Carlos, Brazil) for 47 seconds.

Table 1 Mean values and standard deviation of colony-forming units (CFUs) according to control and test groups

Experimental groups	Mean ± SD
Negative control	0 ± 0^{a}
Positive control	$59.88 \times 10^{6} \pm 1.44^{b}$
Serum	$47.75 \times 10^{6} \pm 4.74^{c}$
Air-powder abrasive system with sodium bicarbonate	$26.88 \times 10^{6} \pm 1.49^{d}$
Antimicrobial photodynamic therapy	$19.38 \times 10^{6} \pm 2.50^{e}$

Abbreviation: SD, standard deviation.
Note: Different letters indicate significant difference between groups.

groups were statistically different from the NC group, which was not contaminated ($p < 0.01$). The aPDT group, even showing greater efficacy, was not able to completely eliminate the microbial biofilm from the implant surface (►**Table 1**).

Discussion

The air-powder abrasive system with sodium bicarbonate and aPDT, within the conditions presented in this *in vitro* study, were efficacious in decontaminating surface of dental implants, but did not completely eliminate the microorganisms.

aPDT showed a greater reduction in the number of CFUs when compared with the air-powder abrasive system with sodium bicarbonate. The protocol used was adapted from Salmeron et al[19] who also obtained positive results in decontaminating the titanium surface with aPDT. Although these results were significant, the study by Salmeron et al[19] was made with titanium discs. Thus, it was decided to use a similar protocol (adapted for use in newer equipment) only in dental implants, to verify the decontaminating potential in this surface conformation, with the design of a real implant and the difficulty of threads decontamination in clinical practice.

One of the biggest challenges and fundamental phases in peri-implantitis treatment is implant surface decontamination.[7,10-14] The search for more effective and clinically applicable decontaminating methods continues. In this sense, biofilm and its characteristics become extremely relevant in studies on the topic.[33] The similarities found between the microbiota of periodontal and peri-implant diseases[33-35] validated the use of the biofilm in this experimental model. The morphotinturial analysis, performed right after the collection, revealed the presence of Gram-positive and Gram-negative microorganisms, however, there was no biofilm characterization with identification of microorganisms because of the need for more complex analyses that demand higher cost and specific equipment.

Among the mechanical methods available for implant surface decontamination, the air-powder abrasive system with sodium bicarbonate was selected for this study because it is widely used in general clinical routine. The literature brings a wide variety of decontamination protocols[25-27] and some studies report possible changes in titanium surface topography[16,25] and even the risk of emphysema.[36] The present study did not evaluate these possible adverse effects of the air-powder abrasive system with sodium bicarbonate, but its decontamination potential is an easily reproducible *in vitro* model with similar characteristics to peri-implantitis cases.

The results presented here demonstrate that the air-powder abrasive system with sodium bicarbonate was efficacious, corroborating the results presented in the literature.[16,25-27] The advantage of this protocol is the short application time, ease of equipment use, and abrasive particles easily found in dental offices.

Combining chemical and mechanical methods for implant surface decontamination can be an interesting alternative as per the results of the present study and the current literature.[12,20,21] Furthermore, the fact that the S group also presented a decontaminating effect in this study corroborates this hypothesis, suggesting the association of mechanical and chemical methods as an alternative for surface decontamination in cases of peri-implantitis.

Conclusion

According to the results obtained in this study and considering the limitations of the proposed model, it was possible to conclude that the proposed protocols using air-powder abrasive system with sodium bicarbonate and aPDT demonstrated good efficacy in the decontamination of dental implant surfaces *in vitro*, but the ideal clinical application protocol is still under study.

Conflict of Interest
None declared.

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