

# Original Research Article

## Antimicrobial activity of oxygen active gel against *Porphyromonas gingivalis* contamination at the implant-abutment interface

### ABSTRACT

**Aims:** The purpose of this study *in vitro*, was to evaluate the antimicrobial activity of oxygen active gel (BlueM®) against *Porphyromonas gingivalis* (*Pg*) at the implant-abutment interface (IAI), in three different types of implant-prosthetic connections.

**Methodology:** A total of 45 dental implants with three different types of connections were divided into three groups according to filling product at the interface: Control (C): unfilled (n=15), BlueM (BM): oxygen active gel (n=15), Chlorexidine (CX): 2% chlorhexidine gel (n=15). They were incubated with a solution containing *Pg* for 5 days under an aerobic condition. Bacterial contamination at IAI was carried out for qPCR.

**Results:** All 45 implants showed contamination at the IAI by *Pg* after 5 days of incubation independent of connection type. EH type connections showed greater contamination by *Pg* compared MT connections ( $p=0.0098$ ). No differences were observed between different types of connections in BM and CX groups.

**Conclusion:** The application of active oxygen gel promoted reduction in contamination *P. gingivalis* in EH type connections at IAI *in vitro*, but not eliminate it.

**Keywords:** *Microgap; Bacterial contamination; Interface implant–abutment; Dental implant.*

### 1. INTRODUCTION

A peri-implantitis is shown as a pathological alteration of the tissues around the osteointegrated implants, with the increase in biofilm being considered as one of its main etiological factors that lead to failure and consequently the loss of implants [1]. The peri-implantitis sites present a microbiome very similar periodontal diseases, It is composed by gram-anaerobic microflora and the *Porphyromonas gingivalis* was the most frequently red complex organism found in peri-implantitis followed by *Tannerella forsythia* and *Prevotella intermedia* [2–4].

The bacterial microleakage from microgap at the implant abutment interface (IAI) on two piece of dental implants system could acts as a bacterial reservoir affecting the soft tissues, intensifying the loss of periodontal support and may have a role to the peri-implantitis onset [4–8]. A microleakage at the IAI between the different prosthetic connections and implants have been shown by previous studies *in vitro* [9,10] and *in vivo* [11–14] even in healthy implant sites [4]. Among commercially available prosthetic connections, morse tapered implants seem to be more effective in reduce microgap at the IAI and consequently bacterial load reduction [15], also marginal bone loss [2].

In the search for new methods and products to prevent a bacterial microleakage at the IAI, the dental industries and researchers seek to improve connectors and implants designs, as well as to develop products and / or add substances with potential for chemical action or

sealing agents, to assist in the control of microbial infiltration [16]. As ideal product to reduce bacterial infiltration at the IAI has to present properties as fast and broad spectrum of antimicrobial action, being non-toxic, odorless, easy to use, not causing surface damage to the implant, stability, lower degradation on body fluid and slow release at site of application [2].

Clinically, the 2% chlorhexidine digluconate is most common studied as antiseptic agents at the IAI. However, the effectiveness of chlorhexidine to prevent bacterial accumulation at the IAI is controversial in the literature [4,13,14].

Recently, chemical agent with the active ingredient based on oxygen, Blue<sup>®</sup>M (Bluem Europe BV, Zwolle, Overijssel, Netherlands), presented in the form of toothpaste, mouthwash, mouth foam and oral gel with bactericidal and anti-inflammatory properties wound healing action in infectious and surgical processes and has been indicated as agent at the IAI.

Therefore, the aim of this study was evaluated the antimicrobial activity of oxygen active gel against *Porphyromonas gingivalis* at the IAI, in three different types of implant-prosthetic connections, in vitro.

## **2. MATERIAL AND METHODS**

### **2.1 Bacterial strain and Growth condition**

Strains of bacteria of *Porphyromonas gingivalis* W83 were grown in solid tryptic soy agar medium (TSA-Difco) supplemented with 0.2% yeast extract (Difco), 7% sheep's defibrinated blood, 5 µg / mL of hemin (Sigma - Merck KGaA, Darmstadt, Germany) and 1 mg / mL of menadione (Sigma - Merck KGaA, Darmstadt, Germany) under anaerobic conditions (10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>), at 37 ° C for 18hours, generate in an anaerobic chambers (MiniMacs, Don Whitley Scientific, Shipley, UK).

### **2.2 Contamination of *P. gingivalis* in the abutment- implant interface**

A total of 45 dental implants with 3.75mm diameter and 11,5mm length (Dentoflex, São Paulo, Brazil) with three different types of connections, 15 of morse tapered (MT), 15 of internal hexagon (IH) and 15 of external hexagon (EH) were studied. To examine the effects of oxygen active gel as sealing agent and the connection geometry on bacterial leakage, five implants from each connection and their abutment were divided into three groups according to filling product in the interface Control Group (C): unfilled (n=15), BlueM Group (BM): oxygen active gel (n=15), Chlorexidine Group (CX): 2% chlorhexidine gel (n=15).

Immediately after removing implants from sterile pack, the inner part of each implant was filled up with products by using a sterile syringe until the edge of the implants. Then each abutment was screwed to the implant with an insertion torque as recommendations of the manufacturers.

The specimens were immersed individually in glass tubes containing 4.5 mL of TSB-BHI-HM (1.55% Tryptic Soy Broth TSB-, Difco Co., Detroit, MI, USA), 1.48% Brain-Heart Infusion (BHI, Difco Co., Detroit, MI, USA), 0.2% yeast extract, 5 µg/mL of hemin and 1 µg / mL of menadione (HM, Sigma Aldrich - St. Louis, Missouri, USA) medium for prepared bacterial suspension. The tubes were incubated in anaerobic conditions for 5 days and every 24hours were removed and agitated in the orbital shaker for 30minutes at 150rpm hours at a temperature of 37°C. During the incubation period, the culture medium was changed with new bacterial suspension every 48hours. After incubation period, the specimens were removed from the tubes, and washed by immersing in sterile 0.9% saline solution. The abutment-implant connections were unscrewed, and samples were collected from the inner part of implant using sterile microbrush (KG Soresen) and transferred to a polystyrene tube containing 48 µL of PBS and stored at -20°C.

### 2.3 DNA extraction, detection and quantification of *P. gingivalis* contamination of the abutment-implant interface by qPCR

A total DNA from sample was extracted using a PureLink Genomic DNA mini kit (Invitrogen, Carlsbad) according to the manufacturer's instructions. DNA was eluted in TE buffer, the quantity and quality were estimated by spectrometry (Nanodrop ND1000, Thermo Fisher Scientific Inc., Wilmington, Delaware).

The presence and absolute quantification of *Porphyromonas gingivalis* in sample was performed by real time polymerase chain reaction (qPCR) using *Pg (W83)* as control, using the thermal cycler Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, California). The determination of DNA genome copies in controls was based on the genome size of bacteria. The samples were amplified in a 25  $\mu$ L reaction mixture containing 2.5  $\mu$ L of DNA, 2.5  $\mu$ L of TaqMan Universal Master Mix II with UNG, 1.5  $\mu$ L of MgCl<sub>2</sub>, 1 dNTP  $\mu$ L, 12.5 pmol of the primers and 3.75 pmol from the Custom TaqMan TAMRA probe. For PCR cycling, the conditions used were as follows: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute each. The primers and probe used for detection and quantification of *Porphyromonas gingivalis* are shown in Table 1 and were selected by using the Primer Express V 1.0 software (Applied Biosystems International) based on highly conserved regions specific to 16S rRNA gene species.

### 2.4 Statistical Analysis

All statistically analysis was performed with Graphpad statistical software 8 (Graphpad Software inc., San Diego, CA, USA). The variables presented normal distribution by Kolmogorov-Smirnov test, Kruskal-Wallis test was used followed by the Dunn test for comparisons between different groups and connections. Differences were considered significant for values of  $P < 0.05$ .

## 3. RESULTS AND DISCUSSION

All implants showed contaminations at the implant-abutment interface by *P. gingivalis* after 5 days of incubation independent of treatment groups (Figure 1). EH type connections showed greater contamination by *P. gingivalis* compared MT connections in the control group ( $P=0.0312$ ) (Figure 1A). No differences were observed between different types of connections in BM (Figure1B) and CX groups (Figure1C).

Considering antimicrobial products treatment, the application of chlorhexidine gel significantly reduced infiltration at IAI in all 3 connections (Figure 2). In MT type connection, a statistical difference was observed between the Control and CX ( $P < 0.0001$ ) (Figure 2A). (Figure 2B). In the IH connection, CX statistically reduced contamination the Control ( $P=0.0059$ ) and BM ( $P=0.0153$ ) (Figure 2C). In the EH type, BM reduced the contamination by *P. gingivalis* similar to CX ( $P=0.0098$ ).

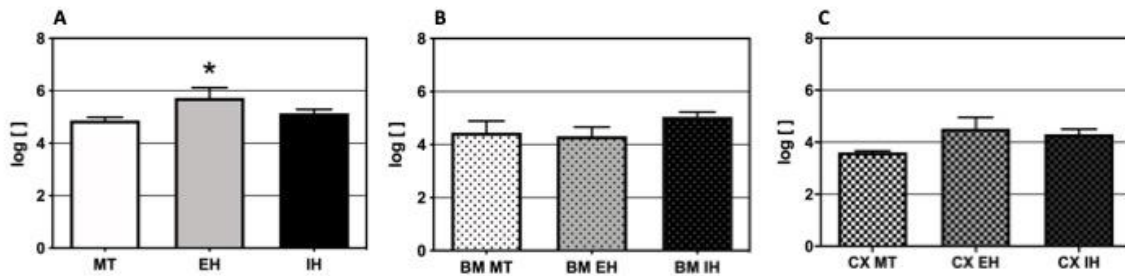


Figure 1. Quantification of *Porphyromonas gingivalis* at implant abutment interface in different type of prosthetic connections. (1A) Control group. (1B) BM group. (1C) CX group. (\*)  $p < 0.05$

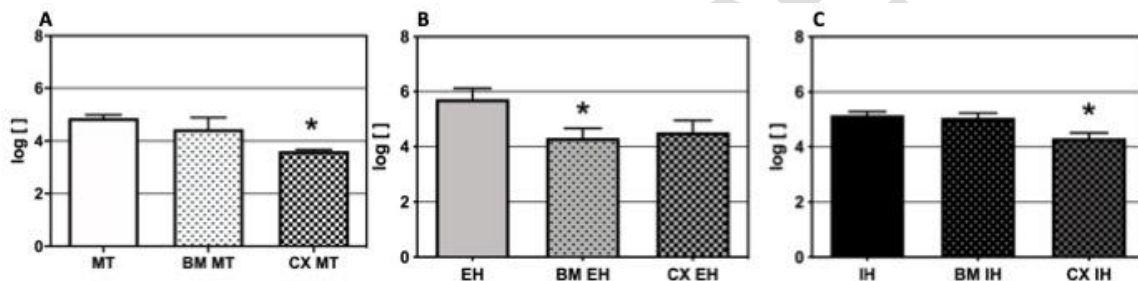


Figure 2. Quantification of *Porphyromonas gingivalis* at IAI in different type of connections according to the antimicrobial products treatment. (2A) Morse tapered type connection. (2B) External hexagon type connection. (2C) Internal hexagon type connection. (\*)  $p < 0.05$

Table 1. qPCR primers and probe used in this study.

<u>Primers</u>	<u>Sequence</u>
<i>Porphyromonas gingivalis</i> F	ACCTTACCCGGGATTGAAATG
<i>Porphyromonas gingivalis</i> R	CAACCATGCAGCACCTAGAA
<u>Probe</u>	<u>Sequence</u>
<i>Porphyromonas gingivalis</i> Pr	VIC-ATGACTGATGGTGAAAACCGTCTTCCCTTC-TAMRA

The reduction bacterial contamination at the IAI through microgap is one of the challenges to be overcome for the success of the patient's oral rehabilitation with dental implants. Few studies in the literature analyzed bacterial infiltration through the implant-prosthetic connector interface, and concomitant investigating alternatives to prevent or minimize contamination, using substances with potential antimicrobial action [17,18].

Among the three types of connections analyzed in this study, all of them presented bacterial contaminations. The lack of complete adaptation between implant and prosthetic components may be responsible for infiltration at IAI [19]. Although no prosthetic connection geometry that can be considered superior performance than other, EH connection showed large amount of infiltrated bacteria than the others connections in control group, it indicates the largest microgap width at IAI of EH type connections, corroborating with previous studies [10,20].

The EH-type implants, due to the geometry of the abutment-implant connection, have the largest microgap among three connections, and therefore the large amount of infiltrated periodontal pathogenic bacteria. [21–25]. This deficiency can be attempted with the application of sealing products in its connections, which significantly prevents bacterial penetration [14,21,26].

Ozdiler et al., 2018 [21] compared the different taper angles in internal conical implants and use of sealing products in influences on microleakage along IAI. Their conclusion was the silicon gel Silicone gel as sealant at IAI could improve immediate close of microgap and consequently can reduce bacterial leakage, although a complete hermetic seal is not achieved. Further, the influence of mechanical performance such as screw loosening and long-term results are unclear.

This finding of the present study demonstrated that the oxygen active gel led to a significant reduction in the target bacterial contamination at the abutment-implant interface in the EH type connections. Therefore, it presented similar reduction of *P. gingivalis* to 2% chlorhexidine gel, although they did not completely eliminate the bacterial contamination. Chlorhexidine is chemical agent commonly used in dentistry with a wide spectrum of activity and low toxicity. In higher concentrations, It has an antifungal and bactericidal effects, capable of eliminating periodontal pathogens as *P. gingival* in different formulations (refx) However, in the oral cavity, it is related some adverse effect such staining of the tongue and/or teeth, dysgnesia and desquamative gingivitis [27,28]. Sinjari et al., 2018[14] evaluated the clinical application of 0,2% chlorhexidine gel at the IAI. The authors observed that the substance reduced marginal bone loss in first year, suggesting that the reduction of microorganism infiltration at the IAI implicated in a reduction of the inflammatory process in situ, resulting in diminished marginal bone loss. Nonetheless, the authors emphasized the continuous use and the side effects of chlorhexidine over time are not known yet.

According to our result, the effect of active oxygen gel was similar to 2% chlorhexidine gel in all connections geometries, reducing the amount of *P. gingivalis*. Active oxygen gel releases gradually of active oxygen that inhibits bacteria metabolism. Due to its smaller molecule dimension compared to a chlorhexidine molecule, it has a much greater power to penetrate to biofilm reaching the deepest regions, where it acts on the bacteria. It is important to note that in its formulation does not contain any anti-bacterial agent, therefore does not cause any adverse reactions, such as hypersensitivity, toxicity or bacterial resistance. In addition, it has a wound healing and anti-inflammatory effect, probably attributed to the penetration of a high concentration of oxygen in the tissues.

The formulation of the product chosen for this research was oral gel, in this way, it allowed greater gradual release of oxygen and less solubility compared to other formulations such as toothpaste or mouth foam. However, due to its consistency, like the chlorhexidine gel used in this work, it is unable to perform as mechanical barrier that prevents bacterial infiltration by the microgap as sealing agent.

Bacterial leakage through IAI can compromise the long-term success of osseointegrated implants. Whereas this is related to the crestal bone remodeling at implant sites [14].

Therefore, more studies are necessary to verify not only the quantitative capacity to prevent bacterial infiltration, but also on its properties, such as viscosity, stability and permeability in clinical application.

On the other hand, mechanical factors, such as screw loosening, torque loss affecting implant-abutment stability, increasing microgap width dynamic loading have to be considered in the application of the products at IAI.

Within the limitations of the present study, the application of active oxygen gel promoted reduction in contamination *P. gingivalis* in all types of connections at IAI, but not eliminate it, in vitro. It may reduce periodontal bacterial microleakage compared with the interface without sealing material.

#### 4. CONCLUSION

Within the limitations of the present study, the application of active oxygen gel promoted reduction in contamination *P. gingivalis* in all types of connections at IAI, but not eliminate it, in vitro. It may reduce periodontal bacterial microleakage compared with the interface without sealing material.

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