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

Background: Bacterial contamination at the dental implant abutment interface through microgap may lead to peri-implant tissue infections resulting to marginal bone loss and affecting the long term success of implants.
Aims: The purpose of this in vitro 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 (n=15/each) according to filling product at the interface: Control (C) - unfilled, BlueM (BM) - oxygen active gel, Chlorexidine (CX) - 2% chlorhexidine gel. They were incubated with a solution containing *Pg* for 5 days under an aerobic condition. Bacterial contamination at the interface were detected and quantificated by qPCR.
Results: All 45 implants showed contamination at the IAI by *Pg* after 5 days of incubation, independent of prosthetic connection type. EH type connections showed greater contamination by *Pg* compared to MT type connections (p=0.0098). No differences were observed among different types of connections in BM and CX groups.
Conclusion: The application of active oxygen gel promoted a reduction in *P. gingivalis* contamination in EH type connections at the IAI *in vitro*, but did not eliminate it completely .

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 dental 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 novel methods and products to prevent a bacterial microleakeage at the IAI, the dental industries and researchers are striving to improve connectors and implants

designs. Additionally, they are developing products and/or incorporating substances with potential for chemical action or as sealing agents to aid 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®M (Bluem Europe BV, Zwolle, Overissel, 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 in vitro study was to evaluated the antimicrobial activity of oxygen active gel (BlueM®) 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% CO2, 10% H2 and 80% N_2), 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 37ºC. 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 -20ºC.

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 µL reaction mixture containing 2.5 µL of DNA, 2.5 µL of TaqMan Universal Master Mix II with UNG, 1.5 µL of MgCl2, 1 dNTP µL, 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: 95ºC for 10 minutes, followed by 40 cycles at 95ºC for 15 seconds and 60ºC 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 using Graphpad statistical software 8 (Graphpad Software inc., San Diego, CA, USA). The variables exhibited a normal distribution as determined by Kolmogorov-Smirnov test. The Kruskal-Wallis test was utilized, 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 among different types of connections in the BM (Figure1B) and CX groups (Figure1C).
Considering antimicrobial products treatment, the application of chlorhexidine gel significantly reduced infiltration at IAI in all three 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).

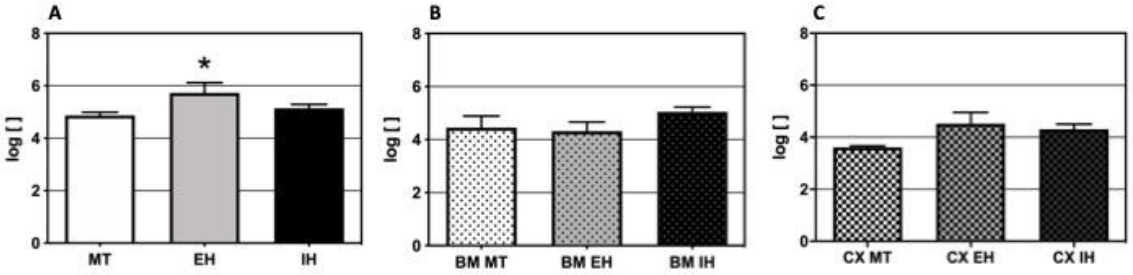

Figure 1. Quantification of *Porphyromonas gingivalis* at implant abutment interface in different type of prosthetic connections. (1A) Control group. (1B) BM group. (1C) CX group. (*) p<0.05

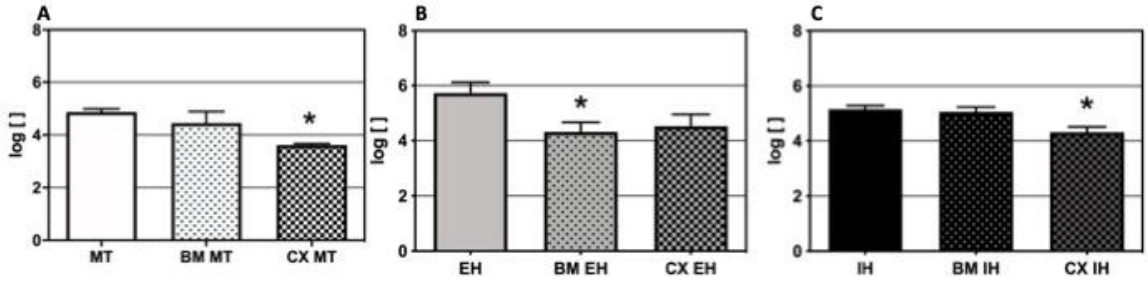

Figure 2. Quantification of *Porphyromonas gingivalis* at IAI in different type of connections according to the antimicrobial products treatment. (2A) Morse tapered type connection. (2B) External hexagon type connection. (2C) Internal hexagon type connection. (*) p <0.05

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

Primers	**Sequence**
Porphyromonas gingivalis F	ACCTTACCCGGGATTGAAATG
Porphyromonas gingivalis R	CAACCATGCAGCACCTAGAA
Probe	**Sequence**
Porphyromonas gingivalis 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 while simultaneously 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 can be considered superior in performance to others, EH connections showed a large amount of infiltrated bacteria than the other connections in the control group. It indicates a wider microgap width at IAI of EH-type connections, which is consistent with findings from 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 that using silicon gel Silicone gel as sealant at IAI could improve the immediate closure of microgap, thereby potentially reducing bacterial leakage, although it does not acheieve a complete hermetic seal. Furthermore, the influence of mechanical performance factors such as screw loosening and long-term outcomes remains 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. However, in the oral cavity, it is related a some adverse effect such staining of the tongue and/or teeth, dysguesia 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 may have contributed to a decrease in 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, it possess a significantly greater ability to penetrate biofilm, reaching even the deepest regions where it acts on bacteria. It is important to note that its formulation does not contain any antibacterial agent, thus avoiding adverse reactions such as hypersensitivity, toxicity or bacterial resistance. Additionally, it exhibits wound healing and anti-inflammatory effects, likely attributed to the penetration of a high concentration of oxygen into 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]. Hence, further studies are necessary to evaluate not only the quantitative efficacy in preventing bacterial infiltration, but also its properties, including viscosity, stability and permeability, in clinical application.Additionally, mechanical factors such as screw loosening, torque loss which affect implant-abutment stability and may increase microgap width under dynamic loading, need to be considered when applying products at the IAI.

4. CONCLUSION

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

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors WHH, SHFJr, DP and YJK conceptualized the study, did formal analysis and helped in project administration. Authors WHH, SHFJr, RDPC, AM, RN, KCM, MHT, DP and YJK did the Investigation and performed the methodology. Authors WHH, SHFJr, MHT, DP and YJK wrote the original draft. Authors MHT, DP and YJK reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Details of the AI usage are given below:

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REFERENCES

[1] Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Clin Periodontol. 2018 Jun;45 Suppl 20:S278-S285. https://doi: 10.1111/jcpe.12956.

[2] Canullo L, Penarrocha-Oltra D, Soldini C, Mazzocco F, Penarrocha M, Covani U. Microbiological assessment of the implant-abutment interface in different connections: cross-sectional study after 5 years of functional loading. Clin Oral Impl Res. 2015;26:426–34. https://doi.org/10.1111/clr.12383.

[3] Lafaurie GI, Sabogal MA, Castillo DM, Rincón MV, Gómez LA, Lesmes YA, et al. Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: A Systematic Review. J Periodontol. 2017;88:1066–89. https://doi.org/10.1902/jop.2017.170123.

[4] Tallarico M, Canullo L, Caneva M, Özcan M. Microbial colonization at the implant-abutment interface and its possible influence on periimplantitis: A systematic review and meta-analysis. J Prosthodont Res. 2017;61:233–41. https://doi.org/10.1016/j.jpor.2017.03.001.

[5] Orsini G, Fanali S, Scarano A, Petrone G, di Silvestro S, Piattelli A. Tissue reactions, fluids, and bacterial infiltration in implants retrieved at autopsy: a case report. Int J Oral Maxillofac Implants. 2000;15:283–6.

[6] Sasada Y, Cochran D. Implant-Abutment Connections: A Review of Biologic Consequences and Peri-implantitis Implications. Int J Oral Maxillofac Implants. 2017;32:1296–307. https://doi.org/10.11607/jomi.5732.

[7] Koutouzis T. Implant-abutment connection as contributing factor to peri-implant diseases. Periodontol 2000. 2019;81:152–66. https://doi.org/10.1111/prd.12289.

[8] Liu Y, Wang J. Influences of microgap and micromotion of implant–abutment interface on marginal bone loss around implant neck. Arch Oral Biol. 2017;83:153–60. https://doi.org/10.1016/j.archoralbio.2017.07.022.

[9] Assenza B, Tripodi D, Scarano A, Perrotti V, Piattelli A, Iezzi G, et al. Bacterial Leakage in Implants With Different Implant–Abutment Connections: An In Vitro Study. J Periodontol.2012;83:491–7. https://doi.org/10.1902/jop.2011.110320.

[10] Ferrari Jr S, Han W, Cogo-Müller K, Sendky W, CARVALHO R, KIM Y, et al. Evaluation of the antimicrobial activity of iodoform paste on the contamination of the implant-abutment interface by Porphyromonas gingivalis- an in vitro study. J Int Acad Periodontol. 2021;23:308–13.

[11] da Silva-Neto JP, Prudente MS, Dantas TS, Senna PM, Ribeiro RF, das Neves FD. Microleakage at Different Implant-Abutment Connections Under Unloaded and Loaded Conditions. Implant Dent. 2017; 26(3):388-392.https:// doi: 10.1097/ID.0000000000000568.

[12] Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Bonaventura GD, et al. Fluids and Microbial Penetration in the Internal Part of Cement-Retained Versus Screw-Retained Implant-Abutment Connections. J Periodontol. 2001;72:1146–50. https://doi.org/10.1902/jop.2000.72.9.1146.

[13] Romanos GE, Biltucci MT, Kokaras A, Paster BJ. Bacterial Composition at the Implant-Abutment Connection under Loading in vivo: Bacteria at Implant-Abutment Interface under in vivo Loading Conditions. Clin impl Dent Rel Re.s 2016;18:138–45. https://doi.org/10.1111/cid.12270.

[14] Sinjari B, D'Addazio G, De Tullio I, Traini T, Caputi S. Peri-Implant Bone Resorption during Healing Abutment Placement: The Effect of a 0.20 % Chlorhexidine Gel vs. Placebo—A Randomized Double Blind Controlled Human Study. BioMed Res Int 2018;2018:1–13. https://doi.org/10.1155/2018/5326340.

[15] Tsuge T, Hagiwara Y, Matsumura H. Marginal Fit and Microgaps of Implant-abutment Interface with Internal Anti-rotation Configuration. Dent Mater J. 2008;27:29–34. https://doi.org/10.4012/dmj.27.29.

[16] Mombelli A, Décaillet F. The characteristics of biofilms in peri-implant disease: Biofilms in peri-implant disease. J Clin Periodontol. 2011;38:203–13. https://doi.org/10.1111/j.1600-051X.2010.01666.x.

[17] de Sousa CA, Taborda MBB, Momesso GAC, Rocha EP, dos Santos PH, Santiago-Júnior JF, et al. Materials Sealing Preventing Biofilm Formation in Implant/Abutment Joints: Which Is the Most Effective? A Systematic Review and Meta-Analysis. J Oral Implantol. 2020;46:163–71. https://doi.org/10.1563/aaid-joi-D-19-00121.

[18] Huacho PMM, Nogueira MNM, Basso FG, Jafelicci Junior M, Francisconi RS, Spolidorio DMP. Analyses of Biofilm on Implant Abutment Surfaces Coating with Diamond-Like Carbon and Biocompatibility. Braz Dent J. 2017;28:317–23. https://doi.org/10.1590/0103-6440201601136.

[19] Mao Z, Beuer F, Wu D, Zhu Q, Yassine J, Schwitalla A, Schmidt F. Microleakage along the implant-abutment interface: a systematic review and meta-analysis of in vitro studies. Int J Implant Dent. 2023; 21:9(1):34. https://doi: 10.1186/s40729-023-00494-y.

[20] Ricomini Filho AP, Fernandes FS de F, Straioto FG, Silva WJ da, Del Bel Cury AA. Preload loss and bacterial penetration on different implant-abutment connection systems. Braz Dent J. 2010;21:123–9. https://doi.org/10.1590/S0103-64402010000200006.

[21] Ozdiler A, Bakir-Topcuoglu N, Kulekci G, Isik-Ozkol G. Effects of Taper Angle and Sealant Agents on Bacterial Leakage Along the Implant-Abutment Interface: An In Vitro Study Under Loaded Conditions. Int J Oral Maxillofac Implants. 2018;33:1071–7. https://doi.org/10.11607/jomi.6257.

[22] D'Ercole S, Scarano A, Perrotti V, Mulatinho J, Piattelli A, Iezzi G, et al. Implants With Internal Hexagon and Conical Implant-Abutment Connections: An In Vitro Study of the Bacterial Contamination. J Oral Implantol. 2014;40:30–4. https://doi.org/10.1563/AAID-JOI-D-11-00121.

[23] do Nascimento C, Barbosa RES, Issa JPM, Watanabe E, Ito IY, Albuquerque RF. Bacterial leakage along the implant-abutment interface of premachined or cast components. Int J Oral Maxillofac Surg. 2008;37:177–80. https://doi.org/10.1016/j.ijom.2007.07.026.

[24] do Nascimento C, Miani PK, Pedrazzi V, Gonçalves RB, Ribeiro RF, Faria ACL, et al. Leakage of saliva through the implant-abutment interface: in vitro evaluation of three different implant connections under unloaded and loaded conditions. Int J Oral Maxillofac Implants. 2012;27:551–60.

[25] Laleman I, Lambert F. Implant connection and abutment selection as a predisposing and/or precipitating factor for peri-implant diseases: A review. Clin Implant Dent Rel Res. 2023;25:723–33. https://doi.org/10.1111/cid.13185.

[26] Yu P, Zhi Li, Tan X, Yu H. Effect of sealing gel on the microleakage resistance and mechanical behavior during dynamic loading of 3 implant systems. J Prosthetic Dent. 2022;127:308–17. https://doi.org/10.1016/j.prosdent.2020.05.030.

[27] Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance Toward Chlorhexidine in Oral Bacteria – Is There Cause for Concern? Front Microbiol. 2019;10:587. https://doi.org/10.3389/fmicb.2019.00587.

[28] Pedrazzi V, Escobar EC, Cortelli JR, Haas AN, Andrade AKPD, Pannuti CM, et al. Antimicrobial mouthrinse use as an adjunct method in peri-implant biofilm control. Braz Oral Res. 2014;28. https://doi.org/10.1590/1807-3107BOR-2014.vol28.0022.