Evaluation of the antimicrobial activity of iodoform paste on the contamination of the implant-abutment interface by *Porphyromonas gingivalis*- an *in vitro* study

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Abstract

Aims: To evaluate the antimicrobial activity of an iodoform-based paste applied to the surface of three types of implant-abutment connections.

Materials and Methods: 45 dental implants were used, of which 15 were with Morse Cone tapered (MT), 15 internal hexagon (IH) and 15 external hexagon (EH) connections. Five implants from each of the connection were distributed into three groups according to the antimicrobial material used control group (GC), chlorhexidine (CX) and iodoform paste (PH). Microbial contamination of all dental implants was evaluated through DNA extraction, identification and quantification using qPCR.

Results: Connections MT and IH required less PH and CX compared to the control ($p < 0.0001$ and $p < 0.0005$, respectively), whereas connection EH required the least amount of PH ($p = 0.0002$). When comparing the antimicrobial materials, there were no differences between the control and CX groups ($p = 0.0535$, $p = 0.0742$, respectively) as well as between the control and PH groups for MT and EH connections ($p < 0.0001$).

Conclusion: The MT connection was less susceptible to bacterial contamination. In addition, PH proved to be as effective as CX in controlling the growth of *P. gingivalis* in the connections tested.

Keywords. Iodoform paste, antimicrobial activity, dental implants

Introduction

Inadequate control of the dental biofilm is considered a biological risk factor for development of peri-implant diseases (Schwarz *et al.*, 2017). Inflammation in peri-implant tissues can be caused by microleakage of the bacterial reservoir at the implant-abutment interface. Although undesirable, it is inevitable that the connections of two-piece implants become colonized by bacteria because of the space at the interface between the implant fixture and abutment with the periodontal tissue, which can result in peri-implant disease (do Nascimento *et al.*, 2012; Canullo *et al.*, 2015). Microbial accumulation around dental implants can result in stimulating damage to peri-implant tissues, leading to peri-implant mucositis when involving soft tissues and to peri-implantitis when involving bone loss (Renvert *et al.*, 2017).

Some factors can interfere with the increase or decrease in bacterial contamination around dental imnplants, such as: internal design of the implant, type of connection, surface treatment, surgical technique and use of antimicrobial agents in combination with filling materials (Amoroso *et al.*, 2006).

Implant systems differ according to the geometry of the interface between abutment and implant. The connection between implants and their abutments can be categorized as external connections (which have a standard external hexagon on the implant platform), internal connections (which comprise a variety of morphologies, such as internal hexagon, internal octagon and internal trilobe) and conical internal connection or Morse Cone tapered (MT) (Schmitt *et al.*, 2014).

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However, no connection system currently in use can be considered clearly superior to others in all aspects. It is not yet clear what the real link is between the internal morphology of the interface and peri-implant bone loss (Caricasulo *et al*., 2018).

Although filler materials associated with antimicrobial agents have been used clinically at this interface in an attempt to decrease or prevent microbial accumulation, there are a few studies that have assessed the antimicrobial activity of these materials (Pereira *et al*., 2017). Thus, this study aimed to evaluate the antimicrobial activity of an iodoform paste to support the use of antimicrobial agents on the surface of three types of implant-abutment connections.

Materials and methods

Inoculum of Microorganism

Strains of *Porphyromonas gingivalis* W83 were grown on plates containing solid medium Tryptic soy agar (TSA-Difco) supplemented with 0.2% yeast extract (Difco, Becton Dickinson, USA), 7% defibrinated sheep blood, 5 µL of hemin (Sigma - Merck KGaA, Darmstadt, Germany) and 1 mg/mL of menadione (Sigma - Merck KGaA, Darmstadt, Germany) under anaerobic conditions at 37°C, that is, 10% CO_2, 10% CO_2H_2 and 80% N_2 (MiniMacs Anaerobic Workstation, Don Whitley, Shipley, UK). The bacterial colonies were resuspended in TSB-BHI-HM medium and cultured for 18 hours in anaerobiosis. The inoculum turbidity was adjusted to an OD of 0.5 (660 nm).

Microbial Contamination Test

A total of 45 dental implants (Supreme Line, Dentoflex) measuring 11.5 mm in length and 3.75 mm in diameter were used, of which 15 were with Morse Cone tapered connection (MT), 15 with internal hexagon (IH) connection 15 with external hexagon (EH) connection. All dental implants were submitted to a microbial contamination test within an external medium. Five implants from each of the connection were distributed into three groups according to the antimicrobial material used.

Initially, the interior of the pre-sterilized implants was filled with the materials according to the experimental groups as follows:

Proheal Group (PH) (n = 15): The Proheal® paste (BioMacmed) was introduced into the implants by using a sterile spatula until reaching their edges (Proheal containing 15.5% iodoform and 5% Marigold oil).

Chlorhexidine Group (CX) (n = 15): Chlorhexidine gel 2% (Chlorhexoral, Biodynamic) was introduced into the implants by using sterile needle and syringe until reaching their edges.

Control Group (n = 15): Group without filler material at the implant-abutment interface.

Next, all the connections were screwed to the implant with a torque of 32 N (as recommended by the manufacturer). Excess material was removed from the outside of the interface using sterile gauze and then individually placed into glass tubes containing 4.5 mL of TSH-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 bacterial suspension.

The tubes were agitated on an orbital shaker 211DS (Labnet) for 30 minutes at 150 rpm and 37°C before being incubated in anaerobiosis. The tubes were removed from the incubator and agitated every 24 hours on an orbital shaker, as described above, and incubated again in anaerobiosis. The implants and their connections were gently removed from the tubes with the aid of sterile forceps and placed into new tubes containing TSH-BHI-HM medium every 48 hours. These procedures were performed up to the fifth day of incubation.

After five days of incubation, implant and abutment were removed and rinsed twice by immersing them in sterile 0.9% NaCl solution. Next, the implant was unscrewed, and a sample was collected from the inside of the implant connection by using a sterile microapplicator (KG Sorensen) and transferred to polystyrene tubes containing 485 µL of 1 x PBS solution and stored at -20°C.

DNA Extraction

As for the DNA extraction protocol (genomic purification), PureLink Genomic DNA mini kits (Invitrogen, Carlsbad) were used according to the manufacturer's instructions.

Bacterial Detection and Quantification Using the qPCR

The primers and probe (Thermo Fischer Scientific) used for detection and quantification of periodontal pathogens 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.

Table 1. Primers and real-time PCR probe used in this study.

Primers	**Sequence**
P. gingivalis F	ACCTTACCCGGGATTGAAATG
P. gingivalis R	CAACCATGCAGCACCTAGAA
Probe	**Sequence**
P. gingivalis Pr	VIC-ATGACTGATGGTGAAAAC-CGTCTTCCCTTC-TAMRA

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: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute each. In this process, the Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) was used for amplification and monitoring to allow fluorescence emissions to be quantitatively analyzed.

Statistical Analysis

Statistical analyses were performed by using the GraphPad Biostat Prism software, version 5 (GraphPad Software). The data obtained were submitted to Kolmogorov-Smirnov test for normality in the groups. The comparison of the amount of bacterial strains found in the different experimental groups and different types of implant-abutment interface was performed by using ANOVA followed by Tukey's post-test at a significance level of 5%.

Results

All dental implants showed contamination regardless of the type of connection and filling material used. Both iodoform paste and chlorhexidine showed antimicrobial activity against *P. gingivalis* when applied to the different types of implant-abutment connections. In this study, a smaller amount of *P. gingivalis* was observed in external hexagon (EH) connections treated with iodoform paste.

Differences in bacterial quantification for *P. gingivalis* bacteria found in the connections of the experimental groups are shown in Figure 1. It can be seen that the conical Morse cone and internal hexagon (IH) connections showed a higher bacterial quantity in relation to the control group with a significant difference (p <0.0001 and p = 0.0001 respectively) whereas EH connection with iodoform paste had a lower bacterial amount (p = 0.0002).

The results regarding the intra-group connections (i.e. control, CX and PH) are shown in Figure 2. No statistically significant differences in antimicrobial activity were observed in the different types of implant-abutment connections regarding chlorhexidine and control groups (Figure 2a, 2b).

In the iodoform group, there were statistical differences between IH, MT and EH connections (p < 0.0001), with the lowest bacterial growth being observed in the two latter ones and the highest in the former (Figure 2c).

Discussion

Despite the evolution of dental implants in recent years regarding their design, chemical and mechanical characteristics, failures still occur associated with infections such as peri-implant mucositis and peri-implantitis, which can lead to loss of the implant (Asensio *et al.*, 2019). Infiltration of bacteria into the gaps occurring

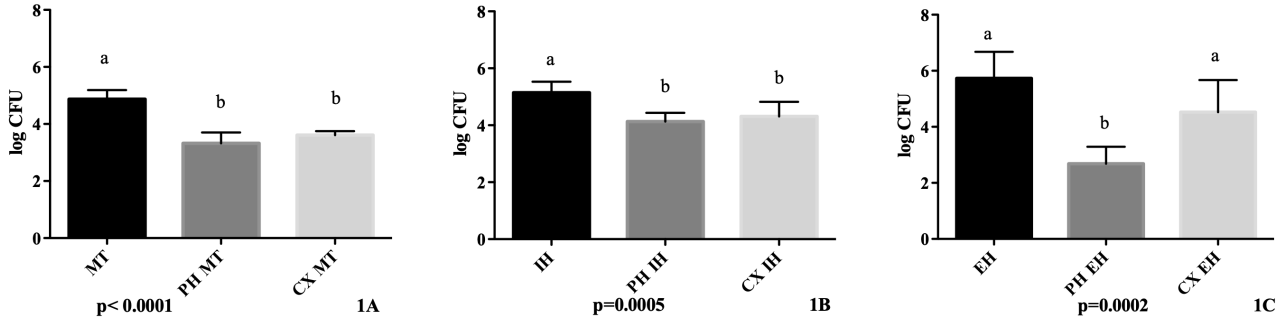

Figure 1: Bacterial quantification of P. gingivalis in the MT (1A), IH (1B) and EH (1C) connections in relation to the use of iodoform paste and chlorhexidine.

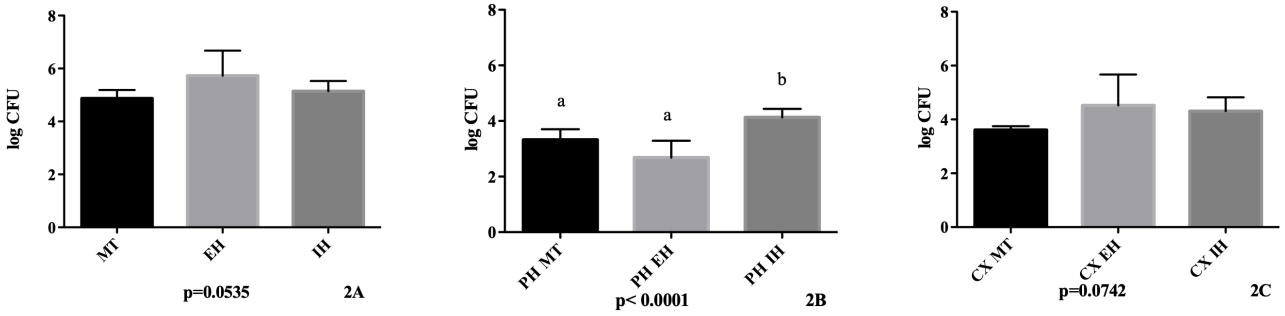

Figure 2: Bacterial quantification of P. gingivalis in the different connections in relation to control (2A), chlorhexidine (2B) and iodoform (2C) groups.

at the implant-abutment interface is one of the causes of these infections (do Nascimento *et al*., 2012, Canullo *et al*., 2015, Mohammadi *et al*., 2019, Smith and Turkyilmaz, 2014). The present study demonstrated that there are differences between the types of implant-abutment connections in terms of bacterial infiltration. It was also found that iodoform and chlorhexidine have similar antimicrobial activities, although they did not completely reduce the contamination by *P. gingivalis*.

Torque tightening does not statistically influence bacterial microleakage at the implant-abutment interface with EH connections (Silva-Neto *et al*., 2012). In the present study, all connections were screwed to implants with a torque of 32 N, as recommended by the manufacturer.

In the present study, three types of connections were compared: Morse Cone tapered (MT), internal hexagon (IH) and external hexagon (EH). It was found that the MT connection showed the lowest numerical levels of infiltration by *P. gingivalis* in the control and chlorhexidine groups. Previous studies have demonstrated similar results, reporting that MT connection allowed less leakage than other types (Koutouzis *et al*., 2011; Koutouzis *et al*., 2015; Tripodi *et al*., 2015; D'Ercole *et al*., 2014; Larrucea Verdugo *et al*., 2014).

The MT system is characterized by a fitting mechanism in which two elements exert an action resulting in close frictional contact when a male conical element is inserted into a female one (Smith and Turkyilmaz, 2014). MT connections have been proposed to improve the implant stability by reducing the micro-movements of the components, which enhances the antibacterial seal. Larrucea Verdugo *et al*., (2014) showed that this is because implants with external connections tend to have larger implant-abutment gaps than implants with Morse-type connections. Implants with a Morse taper connection have hermetic sealing and mechanical advantages. Although there are many Morse taper systems of dental implants, they have considerable variation in wall angles, connection depth and presence of internal locking mechanism (Canullo *et al*., 2015). In addition, implants with external connections have a smaller sealing area and straight walls, which requires a certain degree of tolerance between the parts to join the abutment to them.

When assessing the antimicrobial activity of the iodoform and chlorhexidine paste, both were found to promote a similar reduction in the microbial load at the implant-abutment interface. However, they were not enough to prevent contamination by *P. gingivalis* completely.

Despite the proven efficacy of chlorhexidine, its use has been questioned due to reports of bacterial resistance (Kampf, 2016; Cieplik *et al*., 2019) and adverse allergic reactions (Cai *et al*., 2019). In order to develop alternatives to chlorhexidine, researchers started looking for antiseptics with better antibiotic properties and more stability and durability than chlorhexidine, especially when in contact with organic fluids (McDonnell and Russell, 1999).

In dental implantology, iodoform paste is an active substance that may be an antiseptic alternative for avoiding contamination and consequently future bone resorption around implants, with minimal effect on the peri-implant tissues. Furthermore, it is believed that iodoform paste could act physically to reduce bacterial contamination at the implant-abutment interface and within the implant, in addition to providing chemical control. Such an effect arises because the paste has the function of filling empty gaps, preventing the penetration of microorganisms and chemically controlling microbial multiplication in the intra-implant space and around the inter-components by its antiseptic action (do Nascimento *et al*., 2012, Cuppini *et al*., 2019). In dentistry, the use of iodoform is well reported in the literature, especially for treatment of periapical lesions as it acts as a biological stimulant and as an antiseptic (Cassol *et al.,* 2019). Iodoform is radio-opaque and this allows radiographic visualization and monitoring of the filling of the root canal and degree of penetration of the medication. In addition, it is also possible to observe the leakage of iodoform into the periapical tissues (Cerqueira *et al*., 2008) due to the slow and persistent release of iodine into organic liquids. Iodoform also acts lethally on bacteria, some spores, fungi and viruses through protein inhibition. It is practically insoluble in water, which means that its antiseptic action is reduced. However, the release of iodine is constant when in contact with secretions or infected areas, thus exerting its anti-contamination effects (Cerqueira *et al*., 2008; Pilownic *et al*., 2017)

Pereira *et al*., (2017) conducted an *in vitro* study to evaluate the antibacterial efficiency between gels at different concentrations (i.e. 1%, 2% and 2.5%) of chlorhexidine, tetracycline, Neosporin and ProHeal pastes. The bacterial species used in their study included *Escherichia coli*, *Streptococcus sanguinis*, *Fusobacterium nucleatum* and *Prevotella nigrescens*. All of the antibacterial agents used inside the implants induced zones of bacterial inhibition against aerobic bacteria, but ProHeal® paste had the greatest effect against anaerobic red complex bacteria, which reside inside implants and peri-implant regions.

Studies show that *P. gingivalis* is an important periodontal pathogen (Bassetti *et al*., 2014; Galofré *et al*., 2018). In the present study, chlorhexidine and iodoform were effective in reducing *P. gingivalis*, but they were unable to prevent contamination of the implants completely. Given these results, we propose that these substances would also be able to reduce microbial infiltration by other bacteria involved in the development of peri-implantitis. In addition, despite being an *in vitro*

study, the use of these formulations could be of clinical use in reducing or even preventing contamination of implant-abutment connections. The findings of this study indicate that iodoform paste could be a good alternative to chlorhexidine. Nevertheless, it should be noted is that in this *in vitro* model does not take into account multi-species biofilm interactions, and thus does not fully reproduce what occurs in the oral cavity *in vivo*. Therefore, further clinical studies evaluating these formulations are needed to further explore the clinical effectiveness of iodoform paste for controlling peri-implant infection.

In conclusion, Cone-Morse tapered connections are less susceptible to microbial contamination. Furthermore, iodoform was shown to be effective against the infiltration by *P. gingivalis* in a way comparable to chlorhexidine. Studies evaluating the clinical effectiveness of these formulations are needed to verify these findings.

Disclosure

All authors declare no conflict of interest.

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