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Iodoform and silver-coated abutments preventing bacterial leakage through the implant-abutment interfaces: *In vitro* analysis using molecular-based method

Cássio do Nascimento, Flávio Henrique Carriço Nogueira Fernandes, Wendel Teixeira, Vinícius Pedrazzi*

Department of Dental Materials and Prosthesis, School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil

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ABSTRACT

Objectives: The aim of this *in vitro* study was to evaluate the effectiveness of an iodoform paste and silver-coated abutments in preventing the microbial colonization and leakage through the implant-abutment interface of morse taper and internal hexagon implants.

Material and methods: Seventy-two implants with morse taper (n = 36) or internal hexagon connections (n = 36) were investigated. Implants were treated with iodoform paste (n = 12), silver-coated abutments (n = 12), or control (n = 12). After saliva incubation, Checkerboard DNA-DNA hybridization was used to identify and quantify up to 43 microbial species colonizing the inner parts of the implants. ANOVA-Type and Wald-Type analyses of variance were used to investigate the relative effects and their interaction. Friedman-Conover test adjusted by Benjamini-Hockberg FDR were performed for pairwise multiple comparisons. Significance was set as $p < 0.05$.

Results: Analyses of variance indicate a significant interaction between connections, antimicrobial treatments, and species. The frequency of contamination was reduced in the implants submitted to the antimicrobial treatments. Iodoform and silver-coated abutments significantly reduced the total microbial counts in the internal hexagon implants. The lower microbial counts were recorded for morse taper implants with silver-coated abutments.

Conclusions: Iodoform paste and silver-coated abutments have influenced the microbial leakage through the implant-abutment interface, by reducing both frequency of contamination and microbial levels. Treatments were not effective in reducing the counts of the target species.

1. Introduction

During the last decades, dental implants have been frequently reported in the literature as a predictable treatment for the replacement of missing teeth (McKinney, Steflick, Koth, & Singh, 1988; Srinivasan et al., 2012; Stanford, 2005). Despite of the large number of existing variables, such as surgical protocols, surface treatment, and implant-based materials, osseointegrated implants are commonly found presenting high long-term survival rates in both partially and totally edentulous patients. Current series of systematic reviews reported a 10-year survival rates of 95.2% for implant-supported single crowns, 93.1% for implants supporting fixed dental prostheses, and 82.1% for implants supporting combined tooth–implant-supported prostheses (Pjetursson & Heimisdottir, 2018). The rates are quite high even in previously failed sites, which reached a survival rate of up to 88.7% (Gomes et al., 2018), and in patients with previous history of periodontal disease (Correia, Gouveia, Felino, Costa, & Almeida, 2017; Gomes, Sartori, Able, Silva, & do Nascimento, 2017). While it is a predictable treatment, implant failures have been reported based on systematic reviews of several studies that performed follow-up from 5 to 15 years (Muddugangadhar, Amarnath, Sonika, Chheda, & Garg, 2015; Snauwaert, Duyck, van Steenbergue, Quirynen, & Naert, 2000).

Although a number of implant-related factors have been discussed in the literature, bacteria have been strictly associated with the late complications of the dental implants (Sridhar et al., 2018). The early colonizers such as *Streptococcus* spp., which are commonly found harboring the oral microbiota, facilitate attachment of the late colonizers during the biofilm formation. As the biofilm matures, putative

* Corresponding author at: Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, CEP: 14040-904, Ribeirão Preto, SP, Brazil.
E-mail address: pedrazzi@forp.usp.br (V. Pedrazzi).

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pathogenic species closely related to peri-implantitis (*e.g. Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia* and *Treponema denticola*) become more prevalent and increase the risks of bone resorption and implant loss (Koo, Xiao, Klein, & Jeon, 2010).

The implants and their components are susceptible to the accumulation of biofilm after exposure in the oral cavity. The hollow spaces resulting from the two-part implant systems, for example the gaps resulted from the implant-abutment interface, act as a trap for microorganism colonization and growth causing inflammation of the peri-implant tissues (do Nascimento, Miani, Pedrazzi, Muller, & de Albuquerque, 2012). The bacterial adhesion on the implant-related surfaces and consequent microleakage through the implant-abutment interface have been extensively reported in several *in vitro* (Mishra, Chowdhary, & Kumari, 2017; Pita, do Nascimento, dos Santos, Pires, & Pedrazzi, 2017; Silva et al., 2018) and *in vivo* studies (do Nascimento et al., 2016; de Freitas et al., 2018; Raffaini et al., 2018). The biological complications associated to the long-term prosthetic loading may result in the implant failure and loss (Vidyasagar & Apse, 2003).

Studies on the microbial colonization of dental implants are complex and their results should be interpreted with caution due to the presence of uncontrolled confounding factors. Numerous implant systems, different study designs, and methodologies used for species identification and quantification (*e.g.* microscopy, culture-based techniques, molecular biology-based methods), as well as the absence of international standardization, makes comparison between studies difficult (da Silva-Neto, Nóbilo, Penatti, Simamoto, & das Neves, 2012). However, there is a consensus in the literature that implants are promptly colonized by microorganisms after surgical insertion, and that controlling biofilm formation is essential for the long-term success of the implant-supported restorations (Renvert & Polyzois, 2000; Wilson, Valderrama, & Rodrigues, 2014).

In fact, preventing bacterial adhesion and microbial leakage through the implant-abutment interface still represents a challenging scenario. Iodoform is a biologically active source for inhibiting microbial growth. In this context, iodoform paste has been largely shown to have antimicrobial effects against endodontic infections, mainly against aerobic and facultative microorganisms (Cassol, Duarte, Pintor, Barcelos, & Primo, 2019; Navit et al., 2016). Silver coating is frequently assessed in the implant dentistry since it presents an antibacterial effect by disrupting the bacterial metabolism (Feng et al., 2000; Park et al., 2009). The resulting silver coating surfaces exhibit antibacterial properties and can prevent the surface adhesion of bacterial cells (Cheng et al., 2019). Although a number of investigations have proposed alternatives to prevent or minimize this pathway of contamination, the sealing capability of different implant connections remains unclear.

Thus, the aim of this *in vitro* study was to evaluate the antimicrobial effect of the iodoform paste and the silver-coated titanium abutments on the microbial leakage of morse taper or internal hexagonal dental implants. The null hypothesis tested was that both iodoform paste and silver-coated abutments significantly reduce the microbial colonization and leakage through the implant-abutment interface.

2. Material and methods

2.1. Experimental design

The variation in this investigation involved the platform connections of the implants (in two levels: morse taper and internal hexagon) and the antimicrobial agents (in three levels: iodoform paste, silver-coated abutments, and control – no antimicrobial agent). Six groups were tested (n = 12), including the following: Iodoform Paste with Internal Hexagon, Iodoform Paste with Morse Taper, Silver-coated Abutments with Internal Hexagon, Silver-coated Abutments with Morse Taper, Control – Internal Hexagon, and Control – Morse Taper. Two response variables were investigated: (1) the total count of microorganisms (a pool of the total target species) and (2) the individual microbial counts, discriminating between the 43 different target species colonizing the implant assemblies.

A total of 72 two-piece conical implants (11.5 mm in length; Pross, Dabi Atlante, Ribeirão Preto, SP, Brazil) were used in this investigation, 36 with an Internal Hexagon (IH) platform of 4.3 mm in diameter and 36 with a Morse Taper (MT) platform of 4.3 mm in diameter. Conical abutments (with 3.0 mm in height) for screw-retained prosthetic restorations were selected for each connection. Forty-eight conventional titanium alloy abutments (Pross, Dabi Atlante) were used for both iodoform and control groups. Twenty-four silver-coated abutments (Pross, Dabi Atlante) were used for the remaining group. The implants and the prosthetic components were randomly divided into the 6 groups using a random number generator software, according to the connection type and antimicrobial agent.

2.2. Contamination test

Human saliva was used as a contaminant medium for the incubation of the implant-abutment assemblies. Three milliliters of non-stimulated saliva were collected from 5 periodontally healthy individuals aged between 27 and 30 years (mean age of 29.40 ± 1.14) and mixed together into the same 50 mL Falcon tube. The inclusion criteria were no clinical signs of oral diseases or gingivitis, no active carious lesions, and a probing depth ≤3 mm. Additional exclusion criteria were pregnancy, lactation, periodontal or antibiotic treatment in the last 3 months, smoking, or any systemic disease which could influence the microbiological periodontal status. The study was approved by the research ethics review committee of the Faculty of Dentistry of Ribeirão Preto, University of São Paulo (CAAE 0055.0.138.000-11). The participants were requested to sign a consent form and were given the right to leave the research project at any stage of the investigation. The study was performed in compliance with the Declaration of Helsinki.

The implants and their respective abutments were sterilized from the manufacturer. All the contamination tests were performed under aseptic conditions, in a laminar flux hood and using sterile instrumental and gloves. Before implant-abutment attachment, the contents of the inner parts of all the implants were collected with sterile microbrushes (KG Brush, KG Sorensen, São Paulo, SP, Brazil) to be used as negative control. They were used to assess possible external contamination by handling manipulation. In the instances of contamination, the samples would be excluded. The negative samples were transferred to microtubes containing 150 µL of TE (10 Mm Tris-HCl, 1 Mm EDTA pH 7.6) followed by the addition of 150 µL of 0.5 M NaOH. The tubes were stored at −20 °C until the laboratorial processing.

The abutments were torqued either with 20 Ncm for the internal hexagon implants or with 30 Ncm for the morse taper implants according to the manufacturer's instructions. In the iodoform group, the antimicrobial paste Proheal containing 15.5% iodoform and 5% calendula oil (Biomac Med, Juiz de Fora, MG, Brazil) was used to seal the implant-abutment interface before attachment. The internal chamber of the implants were completely filled with the paste. During connection and torquing of the abutments, the implants were held with pliers allowing a firm gripping. After attachment, the assemblies of all groups were immersed into individual microtubes containing 200 µL of human saliva and incubated at 37 °C ± 1 °C during 7 days. This volume was sufficient enough to cover the implant-abutment interface while preventing contaminant medium leakage through the abutment screw channel on the upper part of the assemblies. After incubation, the assemblies were removed from the microtubes and rinsed with 0.9% of saline solution followed by 70% ethanol. After drying, the assemblies were opened and the contents of the inner parts of the implants as well as screw threads were collected with sterile microbrushes. All the collected samples were individually transferred to microtubes containing 150 µL of TE followed by the addition of 150 µL of 0.5 M NaOH and stored at −20 °C until the laboratorial processing.

Table 1
Microbial species (and respective ATCC Number) used for preparation of the target-specific DNA probes.

Species	ATCC Number
Bacteria	
Aggregatibacter actinomycetemcomitans a	29523
Aggregatibacter actinomycetemcomitans b	29522
Bacteroides fragilis	25285
Capnocytophaga gingivalis	33624
Campylobacter rectus	33238
Escherichia coli	2193
Eikenella corrodens	10798
Enterococcus faecalis	23834
Fusobacterium nucleatum	51299
Fusobacterium periodonticum	25586
Klebsiella pneumoniae	33693
Lactobacilos casei	334
Mycoplasma salivarium	23064
Neisseria mucosa	49233
Pseudomonas aeruginosa	27853
Peptostreptococcus anaerobius	27337
Porphyromonas endodontalis	35406
Porphyromonas gingivalis	33277
Prevotella intermedia	25611
Prevotella melaninogenica	25845
Parvimonas micra	33270
Prevotella nigrescens	25261
Pseudomonas putida	12633
Staphylococcus aureus	25923
Staphylococcus pasteuri	51128
Streptocuccus constellatus	27823
Streptocuccus gordonii	10558
Streptococcus mitis	49456
Solobacterium moorei	CCUG39336
Streptococcus mutans	25175
Streptococcus oralis	35037
Streptococcus parasanguinis	15911
Streptococcus salivarius	25975
Streptococcus sanguinis	10556
Streptococcus sobrinus	27352
Treponema denticola	35405
Tanerella forsythia	43037
Veillonella parvula	10790
Candida	
Candida albicans	10231
Candida dubliniensis	44508
Candida glabrata	15545
Candida krusei	2159
Candida tropicalis	66029

2.3. Microbiological assessment

The identification and quantification of the microbial species colonizing the assemblies were performed by the Checkerboard DNA-DNA hybridization method. Thirty-eight bacteria, including pathogenic and non-pathogenic species, and 5 *Candida* spp. were set as target species (Table 1). Microbiological processing was carried out as described by do Nascimento, Albuquerque, Monesi, and Candido-Silva (2010). Briefly, after thawing, the microtubes containing harvested samples were vortexed during two minutes for contents disaggregation. The samples were boiled during 5 min until DNA denaturation. After cooling, 800 µL of 5 M ammonium acetate were added microtubes and the contents were applied into the extended slots of the MiniSlot apparatus (Immunetics, Cambridge MA) over the nylon membranes (Hybond N + Amershan Biosciences) and followed by baking at 80 °C during 2 h. As standard references, mixtures of genomic DNA corresponding to either 10^5 or 10^6 microbial cells of each target species were assembled, denatured, precipitated, and applied into the two standard lanes of the MiniSlot. The membranes were pre-hybridized and then hybridized in the checkerboard format. The hybridization signals were detected by exposing the membrane to ECL Hyperfilm-MP (GE Healthcare, UK) during 30 min. The number of the target species found in each sample were estimated in terms of genome counts by comparing the chemiluminescent intensity signals of samples against the signals emitted from the standard references. The software CLIQS – Core Laboratory Image Quantification (TotalLab Ltd, Newcastle upon Tyne, UK) was used for the identification and quantification of the microbial species.

2.4. Data analysis

The data were summarized as medians and quartile values. Since the data did not fit the model assumptions, the response variables were compared by nonparametric mixed model of regression analysis. The Wald-Type Statistic (ATS) and the ANOVA-Type Statistic (ATS) were used to evaluate the relative effects of the implant connection (morse taper and internal hexagon), the antimicrobial agents (iodoform paste, silver-coated abutments, and control), target species, and their interaction. The calculated effects resulting from the regressions were adjusted for multiple comparisons using Friedman-Conover corrected by Benjamini-Hochberg of False Discovery Rate (FDR). The statistical significance was set at $p < 0.05$ level and the analyses were performed using the R Statistical Software Package with the Stats library 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

The total microbial count was selected as primary response variable in determining the post hoc power of the study. Comparing independent connections (morse taper or internal hexagon) with repeated measures performed for the two proposed antimicrobial sealing (iodoform paste or silver-coated abutments), and considering a standard deviation of 1.15, the sample size (n = 12) provided a statistical power equal to 94% for the factor “connection” and 98% for the factor “antimicrobial sealing”, with a significance level of 5% and effect size of 1.73 for the factor “connection” and 2.09 for the factor “antimicrobial sealing”.

Regarding the external microbial contamination test, no positive signals of hybridization were detected in the negative control samples (data not shown). Thus, all the implant-abutment assemblies were used in the statistical analysis.

The results of the WTS and ATS for all the main effects and interactions for the whole-plot levels are summarized in the Table 2. Both WTS and ATS have indicated a significant interaction between all the levels at the 1% with the p values of 5.55×10^{-14} and 6.97×10^{-8},

Table 2
The results of the analyses of variance of the relative effects and their interaction.

	Statistic	Df	p value
Wald-Type Statistic			
Platform Connection	89.91	1.00	2.48×10^{-21}
Antimicrobial Agent	24.03	2	6.03×10^{-6}
Species	237.88	42	3.49×10^{-29}
Platform : Antimicrobial Agent	191.94	2.00	2.08×10^{-42}
Antimicrobial Agent:Species	345.91	84	1.70×10^{-33}
Platform Connection:Species	144.34	42	3.76×10^{-13}
Platform:Antimicrobial Agent:Species	218.94	84	5.55×10^{-14}
ANOVA-Type Statistic			
Platform Connection	89.91	1.00	2.48×10^{-21}
Antimicrobial Agent	11.45	1.96	1.24×10^{-5}
Species	4.74	38.1	1.66×10^{-20}
Platform : Antimicrobial Agent	96.55	1.99	1.28×10^{-42}
Antimicrobial Agent:Species	3.37	65.10	1.09×10^{-18}
Platform Connection:Species	2.72	31.17	7.21×10^{-7}
Platform:Antimicrobial Agent:Species	2.22	63.93	6.97×10^{-8}

Df- Degree of freedom.
Significant at $p < 0.05$.

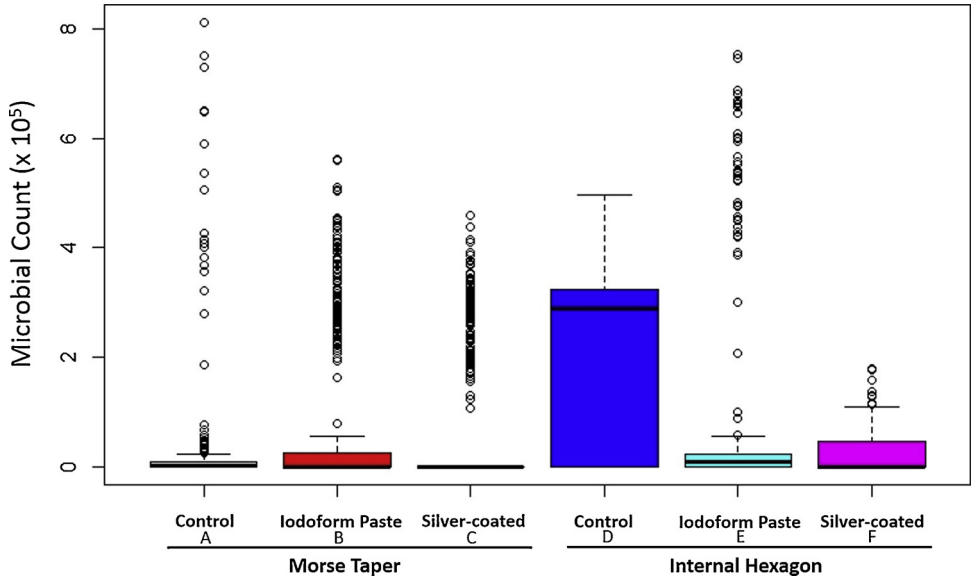

Fig. 1. Scatter plot of the total microbial counts with median and interquartile range for all the tested groups. Different uppercase letters mean significant differences detected by Friedman-Conover adjusted by Benjamini-Hockberg-FDR multiple comparisons post-tests ($D < E < F < B < A < C$; $p < 2.20 \times 10^{-16}$).

respectively. The analyses of variance point out that there exists a significant difference in the microbial counts between the two connections and antimicrobial treatments. Both a significant connection (p value of 2.48×10^{-21} for WTS and ATS) and treatment effect (p value of 6.03×10^{-6} and 1.24×10^{-5}, respectively for WTS and ATS) were observed from the analyses. The species factor was also extremely significant, with p values of 3.49×10^{-29} and 1.66×10^{-20}, respectively for WTS and ATS.

The microbial leakage through the implant-abutment interface was detected in all the tested groups. However, the frequency of contamination was very low. The Fig. 1 illustrates the medians and interquartile range of the total microbial counts for all the tested groups, irrespectively the distinction between species. The Friedman-Conover posttests adjusted by Benjamini-Hockberg - False Discovery Rate have found significant differences between all the medians, including comparisons within the same type of connection ($p < 2.20 \times 10^{-16}$). In the Morse Taper connection, idoform and silver-coated abutments presented median as 0 (zero), which means that at least 50% of samples from these groups did not present signals of contamination; the control group presented median as 0.01 of microbial count. The values of third quartile (75% percentile) were 0.09, 0.26, and 0 (zero), respectively for control, iodoform, and silver-coated. In the internal hexagon group, similar results were found only for implants sealed with silver-coated abutments, which presented median as 0 (zero). Conversely, the samples from the control of the internal hexagon group presented the high frequency of microbial detection (median of 2.89). The median recorded for idoform sealing was 0.08. The values of third quartile (75% percentile) were 3.24, 0.22, and 0.45, respectively for control, iodoform, and silver-coated. The highest mean counts (± SD) were recorded for the internal hexagon control group. The means were recorded as follow: Control: 1.90 ± 1.65 < Idoform: 0.50 ± 1.44 < Silver-coated: 0.24 ± 0.33, for internal hexagon; and Control: 0.23 ± 0.97 < Silver-coated: 0.60 ± 1.17 < Idoform: 0.69 ± 1.35, for morse taper.

The median, lower, and, upper quartiles of the microbial counts ($\times 10^5$ cells) of the 43 target species are displayed in Table 3. Friedman chi-squared test showed significant differences comparing all the medians ($p < 2.20 \times 10^{-16}$). However, the pairwise comparisons using Conover's test for a two-way balanced complete block design (p adjusted by False Discovery Rate) revealed no significant differences comparing the medians of each target species ($p < 0.05$).

4. Discussion

The late implant failures are usually related to the colonization of the implant-abutment assemblies by periodontal pathogenic microorganisms harboring the oral cavity (Koo et al., 2010; Sridhar et al., 2018). The implant systems characteristics, such as type of platform connection, seems to play an important role in the etiology and progression of the peri-implantitis (Mishra et al., 2017). Several studies have focused in reducing the microbial leakage through the implant-abutment interface but it has proven difficult to completely eliminate bacterial colonization and leakage at the interface (Ozdiler, Bakir-Topcuoglu, Kulekci, & Isik-Ozkol, 2018; Pita et al., 2017). Components with antimicrobial properties and interface sealing materials may be a good alternative to minimize this leakage. In this *in vitro* study, we investigated the effectiveness of the iodoform antimicrobial paste and silver-coated abutments in the prevention of microbial colonization and leakage through the implant-abutment interface. We have tested the proposed antimicrobial treatments in two different platform connections, morse taper and internal hexagon.

Our results revealed that both treatments were not effective in preventing the microbial colonization and leakage through the implant-abutment interface in both morse taper and internal hexagon connections. These findings were expected and are in accordance with the current literature, in which has been stated that even a good marginal fit of implant components seemed not to be able to prevent bacterial leakage (Khorshidi, Raoofi, Moattari, Bagheri, & Kalantari, 2016; Mishra et al., 2017). However, the null hypothesis of the present study was confirmed since both iodoform paste and silver-coated abutments significantly reduced the colonization and leakage through the implant-abutment interface. The frequency of implant-abutment assemblies that tested positive for the microbial colonization was quite low in groups submitted to the proposed treatments.

The results show that at least 75% of the assemblies from morse taper with silver-coated abutments, and 50% of the assemblies from both morse taper with iodoform paste and internal hexagon with silver-coated abutments did not present signals of microbial contamination in the inner parts of the implants. The antimicrobial treatments were also effective in reducing the microbial count levels on the assemblies, with the exception for the morse taper implants associated to iodoform paste that presented higher counts than their control. Probably, the paste could have negatively influenced the parallelism friction on the tapered

Table 3
Median, lower, and upper quartiles of microbial counts (x105) assessed by Checkerboard DNA-DNA hybridization method.

	MORSE TAPER									INTERNAL HEXAGON								
	Control			Iodoform			Silver-coated			Control			Iodoform			Silver-coated		
	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
C. tropicalis	0	0	0	0	0	0	0	0	0	0	0	2.13	0	5.54	6.61	0	0	0.16
C. krusei	0	0	0	0	0	0	0	0	0	0	0	0	0	2.14	5.69	0	0	0
C. glabrata	0	0	0	0	0	0.10	0	0	0	0	0	2.16	0	5.04	5.53	0	0	0
C. dubliniensis	0.002	0.03	0.08	0	0	0.17	0	0	0	0	2.84	3.01	0	4.38	5.53	0	0	0.07
C. albicans	0	0.60	0.10	0.01	0.15	0.25	0	0	0	0	2.95	3.22	0	0.15	0.27	0	0	0.31
V. parvula	0.02	0.03	0.12	0	0	2.54	0	0	2.07	0	0	3.00	0	0.07	0.23	0	0	0.44
T. denticola	0.01	0.02	0.10	0	0.17	0.25	0	0	0	0	2.98	3.14	0	0.07	0.16	0	0	0.28
T. foshytia	0.01	0.05	0.12	0	0.12	0.28	0	0	0	0	2.89	3.25	0	0.12	0.21	0	0	0.08
S. sobrinus	0.06	0.10	0.12	0	1.20	2.91	0	0	2.01	0	2.92	3.17	0	0.06	0.23	0	0	0.31
S. sanguinis	0.03	0.12	0.20	0	2.07	3.21	0	0	0	0	2.93	3.05	0	0	0.11	0	0	0.18
S. salivarius	3.90	3.75	4.07	0	0	1.59	0	0	0	0	2.93	3.42	0	0	0.16	0	0	0.25
S. pasteuri	0.02	0.11	0.28	0	0.03	0.16	0	2.47	3.11	0.74	3.08	3.21	0.01	0.08	0.20	0	0	0.24
S. parasanguinis	0.002	0.06	0.10	0	0	0.16	0	0	2.38	2.97	3.14	3.37	0.12	0.14	0.24	0	0	0.55
S. oralis	0	0.015	0.10	0	0.20	0.17	0	0.89	2.33	2.82	3.16	3.36	0.007	0.13	0.23	0	0.10	0.32
S. mutans	0	0.07	0.15	0	0.03	0.19	0	2.02	2.69	2.81	2.90	3.47	0	0.14	0.30	0	0.12	0.37
S. moorei	0	0	0.02	0	0	0.09	0	0	2.16	2.88	3.13	3.65	0	0.11	0.22	0	0.16	0.30
S. mitis	0	0	0.03	0	0	0.19	0	2.23	3.08	0	2.97	3.56	0.11	0.17	0.25	0	0.18	0.45
S. gordonii	0.05	0.09	0.16	0	0	0.12	0	0	2.54	3.03	3.40	3.59	0	0.20	0.27	0	0.35	0.48
S. constellatus	0	0	0.08	0	0.06	0.22	0	1.80	2.76	2.82	3.03	3.91	0	0.20	0.31	0	0.15	0.49
S. aureus	0	5.21	7.10	0	0.02	0.15	0	1.96	2.89	2.83	3.05	3.72	0.96	4.31	5.15	0.06	0.34	0.57
P. putida	0	0.02	0.17	0	0	0	0	0.93	2.46	0	3.07	3.53	0	0.10	0.33	0	0.31	0.44
P. nigrescens	0	0	0.07	0	0.09	0.13	0	0	0.97	0.71	3.02	3.24	0	0.04	0.12	0	0	0.56
P. micra	0	0.02	0.13	0	0	0.06	0	0	0.80	0	3.00	3.14	0	0.11	0.16	0	0.78	1.04
P. melaninogenica	0	0.03	0.06	0	0	2.84	0	0.52	3.17	0.74	3.15	3.45	0	0.005	0.11	0	0.21	0.87
P. intermedia	0	0.01	0.08	0.52	2.56	3.03	0	0	0	0	2.94	3.38	0	0.13	0.24	0.05	0.40	0.75
P. gingivalis	0	0	0	0	1.11	2.74	0	0	0	0	2.90	3.18	0	0.14	0.23	0	0.48	0.82
P. endodontalis	0	0	0.01	0	0	0.08	0	0	0	0.70	3.06	3.44	0.04	0.10	0.18	0.09	0.49	0.65
P. anaerobius	0	0	0	0	0.11	0.21	0	0	0	0	3.07	3.26	0	0.01	0.09	0	0	0.49
P. aeruginosa	0	0	0.04	0	0	0.12	0	0	0	0	2.84	3.09	0	0.09	0.18	0	0	0.34
N. mucosa	0	0	0.03	0	0	0.16	0	0	0	0	0	3.00	0	0	0.07	0	0.13	0.39
M. salivarium	0	0.01	0.03	0	0	3.15	0	3.53	3.85	0	0	3.22	0	0.02	0.12	0	0	0.46
L. casei	0	0	0.06	0	2.61	3.17	0	0	0	0	0	3.39	0	0.08	0.14	0	0.09	0.61
K. pneumoniae	0	0	0	0	0	3.49	0	0	0	0	1.58	3.79	0	0.06	0.14	0	0	0.51
F. periodonticum	0	0.01	0.03	0	3.15	4.03	0	0	0	0	0	0	0	0.02	0.14	0	0.16	0.42
F. nucleatum	0	0	0.03	0	0	0	0	0	0	0	0	3.43	0	0.05	0.14	0	0.52	0.81
E. faecalis	0	0	0.02	0	1.68	3.97	0	0	0	0	0	3.60	0	0	0.10	0	0.54	0.78
E. corrodens	0.002	0.02	0.03	0	0	3.86	0	0	0	0	0	3.16	0	0.14	0.21	0	0.20	0.55
E. coli	0	0.005	0.03	0	0	3.66	0	0	0	0	0	2.15	0.01	0.14	0.15	0	0	0
C. rectus	0	0	0.01	0	0	0	0	0	0	0	0	3.36	0	0	0	0	0	0.31
C. gingivalis	0.01	0.02	0.03	0	3.43	3.72	0	0	0	0	0	3.42	0	0.02	0.16	0	0.19	0.64
B. fragilis	0.01	0.03	0.07	0	1.17	3.33	0	0	0	0	1.41	3.18	0	0	0.16	0	0.44	0.53
Aa b	0	0.04	0.12	0	0	2.07	0	0	0	2.92	2.99	3.01	0	0	0.16	0.10	0.63	0.76
Aa a	0.08	0.30	0.45	0	0	2.93	0	0	0	0	0	2.91	0	0	0	0	0.28	0.81

Q1: Lower Quartile (25% Percentile)/Q3: Upper Quartile (75% Percentile).
No significant differences between species within groups after Conover's pairwise comparisons followed by Benjamini-Hockberg False Discovery Rate ($p > 0.05$).

tightening design (Schmitt et al., 2014). By contrast, the paste could have positively affected the internal hexagon joint acting as a lubricant. Some studies suggest that lubricants act increasing the preload of abutment screws (Lang, Kang, Wang, & Lang, 2003) and preventing loosening (Nigro, Sendyk, Francischone, & Francischone, 2010).

The silver-coated abutments were the most effective treatment in reducing the microbial colonization, with the low values of microbial counts. Overall, the implants with morse taper connection presented lower microbial counts when compared with those using internal hexagon. The current systematic reviews confirm that morse taper connection seems to be more effective concerning the long-term success of the dental implants, allowing lower bacterial leakage and reducing the bone resorption (Goiato, Pellizzer, da Silva, Bonatto, & dos Santos, 2015; Mishra et al., 2017). The morse taper design exhibits high mechanical stability resulting in reduced microgaps after implant-abutment attachment, which may difficult the microbial leakage and growth into the internal chambers (Liu & Wang, 2017). Both treatments were effective in reducing the total microbial counts of the internal hexagon implants, which are known to be more susceptible to micro-movements and leakage than morse taper connection. In this context, the results of our study showed that iodoform paste and silver-coated abutments can be used as an alternative method aiming to reduce the microbial colonization and the bacterial leakage in the internal hexagon implants.

Lower to moderate levels of all the target species were detected colonizing the implant assemblies from the different groups. The proposed treatments presented no effect on the microbial profile of implants since no significant differences were found comparing different species within the connections and treatments. As expected, the lower frequencies and levels of counts were found for *Candida* spp., while *Streptococcus* spp. were the most commonly found since contaminant media was human saliva from the healthy individuals. They are considered early colonizers and facilitate the biofilm development and maturation. Additionally, relevant levels of pathogenic species closely related to peri-implantitis, such as *P. gingivalis*, *T. forsythia*, *T. denticola*, and *Aggregatibacter actinomycetemcomitans* were mainly found in the assemblies from the control groups, with no antimicrobial treatment. Different of Streptococci, these are classified as late colonizers and are representative of species associated with the periodontal disease (Pérez-

Chaparro et al., 2016). Although no statistical significance was found comparing different target species between groups, these findings are interesting because suggest that the antimicrobial treatments may reduce the microbial charge of pathogenic microorganisms on the implant-abutment assemblies. It is of clinical relevance since a large panel of pathogenic species have been proved to act as a potential risk factor on the long-term success of dental implants (Koo et al., 2010; Pérez-Chaparro et al., 2016).

Despite of the striking significance of the relative effects (implant platform connection, antimicrobial agents, and target species) and their interaction, and considering the post-hoc power higher than 94% in the present study, further investigations based on a larger sample size are needed to elucidate the influence of the proposed treatments on the profile of specific microbial species. Also, our results are based on the short-term observations (7 days of saliva incubation) and do not reflect the possible influences of the long-term application of the proposed treatments. The use of human saliva in this study aimed to simulate the oral environment, providing the incidence of bacterial species commonly found in the healthy microbiota. The microbiological assessment (data not shown) showed no differences of the incidence for target species in the saliva used at baseline and after 7 days of specimens' incubation. Preliminary tests by conventional culture have confirmed the viability of the bacterial species in the saliva after 7 days of incubation under microaerofilic conditions. However, further long-term studies are needed to confirm these results.

The literature is still not conclusive on the bacterial sealing capability of alternative methods in the two-part dental implants. This *in vitro* investigation showed that both iodoform paste and silver-coated abutments may offer an alternative method on the prevention of the microbial colonization and leakage through the implant-abutment interface in the screw-retained implants.

5. Conclusions

Within the limitations of this study, we can conclude that: (1) the frequency of implant assemblies that tested positive for microbial colonization was significantly reduced in both connections (morse taper and internal hexagon) submitted to the antimicrobial treatments; (2) the iodoform paste and the silver-coated abutments significantly reduced the microbial counts of the internal hexagon implants; (3) the silver-coated abutments reduced the microbial counts of the morse taper implants; (4) the treatments were not selective in reducing the target microbial species.

Ethical approval

The study was approved by the local ethics committee and all the experiments were undertaken with the understanding and written consent of each subject and according to the ethical principles (CAAE 0055.0.138.000-11). The study was performed in compliance with the Declaration of Helsinki.

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