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Original Article

Effect of preoperative chlorhexidine, essential oil, and cetylpyridinium chloride mouthwashes on bacterial contamination during dental implant surgery: A randomized controlled clinical trial

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ABSTRACT

Background: Although the role of chlorhexidine and other mouthwashes in periodontal therapy has been elucidated, little information is available on their use as routine preoperative mouth rinses before surgery, especially in periodontal procedures such as dental implant surgery.
Objective: This study aimed to compare the efficacy of preoperative chlorhexidine, essential oil, and cetylpyridinium chloride mouthwashes in reducing bacterial contamination at the time of implant placement.
Materials and Methods: Eligible patients who underwent dental implant surgery were randomly divided into four groups based on the mouthwash used: (1) 0.12 % chlorhexidine, (2) essential oil, (3) cetylpyridinium chloride, and (4) saline (served as the control group). All the patients of each group rinsed preoperatively with 15 mL of the respective mouthwash for 60 s. Saliva samples before (pre) and immediately after rinsing with the mouthwash (post) and after suturing the flap (end) were collected on the day of the implant placement. Real-time quantitative polymerase chain reaction (qPCR) was performed to analyze the samples and quantify the targeted periodontal pathogens using a propidium monoazide (PMA) dye.
Results: Forty patients were included in the study. Real-time qPCR demonstrated a significant reduction in the number of pathogens in the saliva samples of the mouthwash groups compared to that of the control group. A statistically significant difference was observed between the groups for the pre–post and pre–end samples ($p < 0.001$) but not for the post–end samples ($p = 0.203$). A statistically significant difference was observed between the chlorhexidine, essential oil, and cetylpyridinium chloride mouthwash groups and the saline group ($P < 0.001$). The bacterial counts significantly differed with and without the use of the PMA dye.
Conclusions: Preoperative chlorhexidine, essential oil, and cetylpyridinium chloride mouthwashes can reduce the bacterial load at the time of implant placement, thereby reducing the incidence of implant-related complications.

1. Introduction

Failure to achieve and maintain successful osseointegration around dental implants can be attributed to several factors, including microbial infection (Tabanella et al., 2009; Heitz-Mayfield and Lang, 2010). Several studies have reported the presence of *peri*-implantitis microflora, including *Fusobacterium species, Campylobacter rectus, Prevotella intermedia, Candida albicans, Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans,* and *Treponema denticola*. Rapidly progressing *peri*-implantitis may clinically resemble aggressive periodontitis, while slowly progressive *peri*-implantitis may resemble chronic periodontitis (Shibli et al., 2007; Tabanella et al., 2009; Heitz-

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Mayfield and Lang, 2010; Casado et al., 2011). Longitudinal studies have demonstrated the transmission of periodontal pathogens from periodontally affected sites to implant sites in the oral cavity. The same pathogens identified in the periodontal pockets are reportedly responsible for colonizing implants at 3- and 6-months following implant placement. Thus, oral biofilms, which are potential reservoirs of these pathogens, should be eradicated prior to implant placement (Gunsolley, 2010; Van Strydonck et al., 2012).

Few studies have evaluated the effects of different mouthwashes over time (Summers et al., 2000; Ribeiro et al., 2007; Kosutic et al., 2009). Kosutic et al. (2009) reported a statistically significant reduction in the oral bacterial counts in saliva samples collected 5 min after rinsing and at the end of an oral surgical procedure. Chlorhexidine demonstrated a stronger antibacterial effect with longer duration of action compared to povidone-iodine and saline solutions. Although rinsing with saline solution resulted in a small, transient, statistically significant reduction in the oral bacterial counts 5 min after rinsing, a rapid increase in the bacterial count was observed shortly after rinsing compared to that preoperatively (Kosutic et al., 2009). Another study evaluated the effects of different solutions on bacterial decontamination in the oral cavity during various oral surgical procedures; however, the duration of the procedure was not noted. Although the bacterial load was reduced immediately following saline treatment, the difference was not statistically significant compared to that preoperatively. Thus, saline solution cannot be used as a standard preoperative mouthwash during oral surgical procedures (Summers et al., 2000). Another study evaluating the long-term effects of cetylpyridinium chloride (CPC) and essential oil mouthwashes on the bacterial counts reported no statistically significant difference in the bacterial counts between the two groups upon assessment at the 3- and 6-month evaluations; at baseline, 49 % of the sites were positive for *P. gingivalis*, which reduced to 43.9 % at the 6-month evaluation (Ribeiro et al., 2007).

Although the role of chlorhexidine and other mouthwashes in periodontal therapy has been elucidated, little information is available on their use as routine preoperative mouth rinses before surgery, especially in dental implant surgery (de Albuquerque et al., 2004). Therefore, the primary aim of this study was to evaluate and compare the efficacy of a 60-second rinse with 0.12 % chlorhexidine, essential oil, CPC, and saline mouthwashes on bacterial count reduction when used preoperatively.

2. Material and methods

2.1. Study design

This was a single-blind, randomized controlled clinical trial. The study protocol was approved by the Tufts University Health Sciences Campus Institutional Review Board (IRB: 10951) and registered at ClinicalTrials.gov (NCT02002442). Informed consent was provided by all the participants of the study.

2.2. Inclusion and exclusion criteria of the study participants

Patients who were referred to the postgraduate periodontal clinics at Tufts University School of Dental Medicine for dental implant treatment were included in the study. Patients who (i) were prescribed antibiotics within 2 weeks of implant placement, (ii) were allergic to any of the agents used in the study, (iii) were pregnant, (iv) currently have/previously had a systemic disease that could impair the immune response, (v) were fully edentulous, or (vi) regularly used mouthwashes were excluded from the study.

2.3. Group allocation and randomization

The study participants were randomly divided into four groups based on the mouthwash used: (1) 0.12 % chlorhexidine (Paroex® Chlorhexidine Gluconate Oral Rinse USP, 0.12 %. Schaumburg, IL, USA), (2) Essential oil (Listerine Zero ®, Johnson & Johnson Healthcare Products, Division of McNeil-PPC, Inc. Skillman, NJ, USA), (3) CPC (Crest Pro-Health ®, Procter & Gamble, Cincinnati, OH, USA), and (4) saline, which served as the control group. All the participants of each group rinsed preoperatively with 15 mL of the respective mouthwash for 60 s.

2.4. Saliva sample collection

Three saliva samples were collected from eligible patients according to a modified previously reported method (Casado et al., 2011). Briefly, the participants were asked to keep their head facing downward and let the saliva drop passively into a 50-mL centrifuge tube while avoiding contact with the tube. The first 5-mL sample (pre) of whole saliva was collected before rinsing with mouthwash. The participant was then asked to rinse with 15 mL of the allocated mouthwash for 60 s. The second saliva sample (post) was similarly collected immediately after rinsing with the mouthwash, while the third sample (end) was collected after the completion of the implant surgery. The duration of the procedure was recorded for each participant. All the saliva samples were stored at −20 °C until the microbiological analysis.

2.5. Experimental procedure

2.5.1. Saliva preparation and DNA extraction

All the saliva samples were centrifuged for 4 min at 4 °C at 1,500 rpm, the obtained supernatant was discarded, and the pellet was mixed with phosphate-buffered saline (PBS). DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN, Inc. Valencia, CA, USA) was used according to the manufacturer's instructions. Centrifugation was performed at room temperature (15–25 °C) yielding 3–12 μg DNA. All the extracted DNA from the samples was stored at −20 °C for further quantification using qPCR.

2.5.2. Propidium monoazide treatment

Some samples were mixed with propidium monoazide (PMA; PMA^{TM}, Biotium Inc., Hayword, CA, USA) to differentiate between viable and non-viable bacteria. In these cases, the three samples were collected in the same manner as described previously; however, two different samples were collected at each time point (i.e., pre, post, and end). One sample was treated in a similar manner as that for DNA extraction, whereas the other sample was mixed with the PMA dye following the manufacturer's recommendations. All the samples were stored at −20 °C until further analysis using qPCR.

2.5.3. Quantification of the targeted bacteria using real-time qPCR

The samples were analyzed microbiologically using real-time qPCR by a blinded examiner at the Immunology Department of the Forsyth Institute. For the monoplex reaction, in a 1.5-mL microcentrifuge tube, 1 μL of the saliva sample was mixed with 2 μL of 10 μM of the bacterium-specific primers (Table 1) (Integrated DNA Technologies, Inc. Skokie, IL, USA), 10 μL of 2X Sybre Green qPCR reagent (Bio-Rad Laboratories, Inc. Hercules, CA, USA), and 7 μL of distilled water. The reaction mix, standards, and negative controls were placed in a LightCycler ® 480 Instrument II (Roche Diagnostic Corporation, Indianapolis, IN, USA). The thermal cycler was programmed as follows: 1 cycle at 95 °C for 4 min; 35 cycles at 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 33 s; 1 cycle at 95 °C for 5 s and 60 °C for 60 s; 1 cycle at 60–97 °C (in 5 °C increments) for 30 s, followed by a final cycle at 40 °C for 30 s. The qPCR data were analyzed using absolute quantification with cycle threshold (C_T).

2.6. Statistical analysis

Descriptive statistics were used for all the groups; values are expressed as medians and interquartile ranges. Friedman's test was used to compare the bacterial counts in each group in terms of the overall

Table 1
Primers used for quantifying genomic DNA from the targeted bacteria.

Bacteria	3′ primer	5′ primer	Length (bp)
Fn	ATGACGGTACCAACAGAAGAAGTGACGGCTAA	CCAATAAATCCGGATAACGCTCGTGACATA	32
Td	GGTATCCGGCCTGAGAGGGTGAACGGACA	TTCTTAGCTGCTGCCTCCCGTAGGAGTTT	29
Pg	TTGAATGTACCGTAAGAATAAGCATCGGCTAAC	CTCGCATCCTCCGTATTACC	33
Tf	AATGCATAGAGATCACGCAGAACTCCGATT	TTGATACCCACGCTTTCGTGCTTCAGTGT	30
Pi	CCACATATGGCATCTGACGTGGACCAAAGA	GGGCCGTTACCCGCACCAACAAGCTAATC	30
Aa	GCACAAATCGTTGGCATTCTCGGCGAA	AAAGTGCGGGAAACTTCTTGTTTAGCT	27

Fn: Fusobacterium nucleatum. Td: Treponema denticola, Pg: Porphyromonas gingivalis. Tf: Tannerella forsythia, Pi: Prevotella intermedia, Aa: Aggregatibacter actinomycetemcomitans, bp: base pair.

bacterial count and each bacterium individually for the different saliva samples (i.e., pre, post, and end). The Wilcoxon signed-rank test with Bonferroni correction was used in case of significant findings. The Kruskal–Wallis test was used to evaluate the change and percentage reduction of the overall bacterial count and each bacterium individually in the different samples (i.e., pre, post, and end). The Mann–Whitney *U* test with Bonferroni correction was used in case of significant findings. The Wilcoxon signed-rank test was used to compare the bacterial counts in samples with and without the PMA dye. Statistical significance was set at $p > 0.05$, except when Bonferroni correction was used. SPSS Version 24 (IBM Corp., Armonk, NY, USA) was used for all the analyses.

3. Results

3.1. Study participants

A total of 40 participants with 150 saliva samples were included in the study. The saliva samples of 30 of the 40 participants (i.e., 90 samples) were analyzed using qPCR without the PMA dye, whereas the saliva samples of the other 10 participants (i.e., 60 samples) were examined using qPCR with and without the PMA dye.

Each group contained 10 participants. The age range was 25 to 80 years with a mean + SD of 56.5 + 14.4 years. This study included 21 (52.5 %) men and 19 (47.5 %) women; 36 (90 %), 2 (5 %), and 2 (5 %) participants were classified as Whites, Asians, and other, respectively according to the race (Table 2).

3.2. Comparison of the overall bacterial count and that of each bacterium separately in each group

3.2.1. Chlorhexidine group

The overall bacterial counts demonstrated a statistically significant difference between the saliva samples of the participants ($p < 0.001$). Pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni correction revealed a statistically significant difference between the groups for the pre–post and pre–end samples ($p = 0.005$) but not for the post–end samples ($p = 0.169$) (Tables 3 and 4).

P. intermedia, *P. gingivalis*, *T. Denticola*, *T. forsythia*, and *F. nucleatum* showed similar patterns upon observing each bacterium individually. Moreover, each bacterium was statistically significant at each period (pre–post, pre–end, and post–end). *A. Actinomycetemcomitans* did not show any statistical significance ($p = 0.773$) (Tables 3 and 4).

3.2.2. Essential oil group

Friedman's test demonstrated a statistically significant difference between the samples ($p < 0.001$) for each period (pre–post, pre–end, and post–end) for the overall bacterial load. *P. gingivalis, T. Denticola, T. forsythia*, and *F. nucleatum* demonstrated similar patterns to those of *P. intermedia*, which were not significant for the pre–end samples when using the Bonferroni correction ($p = 0.022$). *A. Actinomycetemcomitans* did not show any statistical significance ($p = 0.148$) (Tables 3 and 4).

3.2.3. CPC group

The overall bacterial counts and between-group samples for all the periods were statistically significant. *P. intermedia*, *P. gingivalis,* and *T. forsythia* showed similar patterns. *A. Actinomycetemcomitans* did not show any statistical significance ($p = 0.539$) (Tables 3 and 4).

3.2.4. Control group

The overall bacterial load was significantly different between the saliva samples of the participants ($p < 0.001$). However, a statistically significant difference was not observed between the saliva samples for different periods when using the Bonferroni correction ($p = 0.114$, 0.047, and 0.285 for the pre–post, pre–end, post–end samples, respectively). *T. Denticola* and *F. nucleatum* showed similar patterns. *A. Actinomycetemcomitans* did not show any statistical significance ($p = 0.165$) (Tables 3 and 4).

3.3. Comparison of the bacterial reduction among different mouthwash groups over time in terms of the proportional difference

A statistically significant difference was observed in the change in the overall bacterial percentage between the groups for the pre–post and pre–end samples ($p < 0.001$) but not for the post–end samples ($p = 0.203$) (Table 5). The change in the overall bacterial percentage was significantly different between the chlorhexidine, essential oil, and CPC

Table 2
Participant demographics.

	Chlorhexidine	Essential oil	CPC	Saline	Total
Sex	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Women	6 (60)	5 (50)	4 (40)	4 (40)	19 (47.5)
Men	4 (40)	5 (50)	6 (60)	6 (60)	21 (52.5)
Total	10 (100)	10 (100)	10 (100)	10 (100)	40 (100)
Race	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
White	10 (100)	9 (90)	9 (90)	8 (80)	36 (90)
Asian	0	0	1 (10)	1 (10)	2 (5)
Other	0	1 (10)	0	1 (10)	2 (5)
Total	10 (100)	10 (100)	10 (100)	10 (100)	40 (100)
Age (years)	Mean + SD (Min–Max)	Mean + SD (Min–Max)	Mean + SD (Min–Max)	Mean + SD (Min–Max)	Mean + SD (Min–Max)
	55.6 + 11.6 (42–80)	54.8 + 20.8 (25–76)	60.2 + 11.9 (35–78)	55.4 + 12.9 (33–70)	56.5 + 14.4 (25–80)

CPC: cetylpyridinium chloride, No.: Number, SD: standard deviation, Min: minimum, Max: maximum.

Table 3
Descriptive statistics of the different mouthwash and control groups.

	Chlorhexidine Median (IQR) (Min–Max)	Essential oil Median (IQR) (Min–Max)	CPC Median (IQR) (Min–Max)	Control (Saline) Median (IQR) (Min–Max)
Overall				
Pre	1.02E12 (2.01E12) (6.07E9–5.50E12)	4.54E11 (2.16E12) (6.287E9–9.14E12)	4.66E11 (8.24E11) (7.35E9–4.12E12)	4.16E11 (5.59E11) (9.74E6–4.12E12)
Post	2.79E10 (4.7E10) (2.5E7–1.46E11)	7.68E10 (1.70E10) (2.48E7–1.05E10)	1.5E10 (4.37E10) (4.95E7–3.67E10)	2.62E11 (3.65E11) (1.29E8–1.08E12)
End	5.96E10 (1.49E10) (2.74E8–3.39E10)	8.75E10 (1.13E10) (8.82E8–1.32E10)	1.01E10 (3.92E10) (7.00E8–8.27E10)	3.49E11 (5.40E11) (4.67E9–1.33E12)
Pi				
Pre	9.84E6 (6.99E6) (2.34E5–1.21E9)	2.64E6 (2.52E8) (373–2.06E9)	1.12E7 (1.28E8) (5.27E5–1.35E10)	3.28E7 (6.53E7) (3.21E5–3.15E8)
Post	708 (2420) (116–2.78E6)	456.5 (434) (154–32400)	1395 (14239) (154–68000)	1.19E6 (3.99E6) (588–9.64E6)
End	3.58E5 (2.68E6) (2020–2.69E7)	2.28E5 (6.26E5) (766–3.16E7)	3.02E5 (1.17E6) (2200–9.11E6)	3.23E6 (4.13E6) (2450–1.75E7)
Pg				
Pre	2.39E8 (6.73E9) (1.21E6–6.07E10)	4.77E7 (3.17E8) (1.02E6–4.44E10)	2.42E7 (3.29E7) (2.82E6–7.41E9)	5.61E7 (3.00E7) (1.85E6–2.01E8)
Post	2.41E5 (2.09E5) (2680–5.53E5)	5000 (1.32E5) (1010–56000)	35,300 (48000) (1670–55300)	2.26E6 (4.48E6) (2.56E5–5.78E7)
End	7.27E5 (5.93E6) (2.03E5–9.15E6)	3.37E6 (3.43E6) (1.36E5–8.31E7)	1.08E6 (1.66E6) (2.45E5–4.75E6)	3.67E6 (6.70E6) (5.40E5–7.52E7)
Aa				
Pre	2.26E9 (2.61E10) (2.26E9–9.08E11)	5.15E9 (5.07E9) (2.26E9–6.90E10)	3.93E9 (4.97E9) (2.26E9–2.75E10)	4.58E9 (1.92E10) (2.26E9–3.20E11)
Post	2.83E9 (3.37E9) (2.26E7–1.44E11)	2.26E9 (3.47E9) (2.26E7–6.63E10)	2.93E9 (2.03E9) (2.26E7–4.35E10)	2.26E9 (2.06E9) (2.26E7–3.24E11)
End	2.26E9 (8.60E8) (2.26E8–9.64E9)	2.26E9 (5.4E8) (8.10E8–8.10E10)	2.26E9 (4.22E9) (6.48E8–2.04E10)	3.59E9 (3.03E10) (2.26E8–2.86E10)
Td				
Pre	7.94E11 (2.35E12) (6.04E8–5.49E12)	4.38E11 (2.11E12) (1.97E9–9.06E12)	4.42E11 (7.99E11) (3.36E8–4.05E12)	4.09E11 (5.62E11) (2.65E9–3.79E12)
Post	1.74E9 (4.88E9) (1.45E5–1.48E7)	7.63E8 (2.61E9) (1.04E6–1.19E10)	2.42E9 (1.45E10) (5.69E5–3.96E10)	1.54E11 (2.99E11) (3.35E7–1.07E12)
End	8.26E9(7.76E10) (1.53E6–9.35E10)	1.48E10 (6.48E10) (1.31E7–1.18E10)	1.58E (2.85E10) (2.31E6–7.63E10)	3.30E11 (5.37E11) (3.60E8–1.32E12)
Tf				
Pre	6.53E10 (1.14E10) (1.27E8–4.79E10)	4.98E10 (1.31E10) (3.01E8–6.80E10)	1.05E (3.40E10) (3.27E9–9.74E10)	7.66E9 (4.83E10) (2.72E8–9.9E10)
Post	1.17E9 (4.58E9) (1.81E–1.55E10)	4.47E7 (2.68E9) (1.27E5–2.21E10)	6.98E7 (4.86E8) (6.29E–2.21E10)	2.72E9 (1.84E10) (4.05E6–6.76E10)
End	3.36E9 (3.83E10) (5.04E6–1.73E10)	7.41E9 (2.12E10) (4.83E6–6.61E10)	2.61E9 (4.88E10) (3.24E6–3.74E10)	4.03E9 (4.34E10) (2.10E7–2.33E10)
Fn				
Pre	5.72E10 (3.21E10) (2.13E9–1.39E10)	7.73E10 (4.41E10) (1.21E9–2.98E10)	5.18E10 (2.85E10) (2.28E9–3.89E10)	2.75E10 (2.35E10) (1.65E9–8.68E10)
Post	1.01E10 (1.95E10) (1.22E5–3.04E10)	1.22E9 (1.34E10) (3.025–3.29E10)	351E9 (1.44E10) (4.45E6–1.04E10)	1.23E10 (30.0E10) (1.06E7–6.45E10)
End	2.50E10 (5.54E10) (2.35E6–8.13E10)	2.79E10 (3.83E10) (3.25E6–5.23E10)	4.05E10 (3.30E10) (1.56E7–1.53E10)	2.89E10 (3.26E10) (3.45E8–9.48E10)

CPC: cetylpyridinium chloride, IQR: interquartile range, Min: minimum, Max: maximum, *Pi: Prevotella intermedia, Pg: Porphyromonas gingivalis, Aa: Aggregatibacter actinomycetemcomitans, Td: Treponema denticola, Tf: Tannerella forsythia, Fn: Fusobacterium nucleatum.*
Pre: Samples before rinsing, **Post**: Samples immediately after rinsing, **End**: Samples at the end of the procedure.

mouthwash groups and the saline group ($p < 0.001$).

The percentage change for each individual bacterium was significantly different between the groups for the pre–post samples for *P. gingivalis, T. denticola, T. forsythia*, and *F. Nucleatum* ($p < 0.001$, $p < 0.001$, $p = 0.003$, and $p = 0.044$, respectively); pre–end samples for *T. Denticola* and *T. Forsythia* ($p < 0.001$ and $p < 0.015$, respectively); and post–end samples for *P. intermedia* and *P. gingivalis* ($p = 0.007$ and $p = 0.003$, respectively) (Table 5).

3.4. Saliva samples with PMA dye

The bacterial counts in the saliva samples with and without PMA dye were compared using the samples of the control group participants owing to insufficient samples in each mouthwash group. Paired comparisons were performed for the overall and individual bacterial counts. The bacterial counts of the samples with and without the PMA dye were significantly different; lower bacterial counts were observed in the PMA samples. Only *P. intermedia* in the end samples did not show a statistically significant difference.

4. Discussion

A significant reduction in the overall bacterial count was observed in all the mouthwash groups. The study findings concerning the effect of chlorhexidine on the bacterial count were similar to those reported by de Albuquerque et al., 2008; however, their study evaluated the bacterial count of salivary *Staphylococcus aureus* and *Streptococcus mutans* using bacterial cultures.

Upon analyzing each bacterium individually, both the chlorhexidine and essential oil mouthwash groups demonstrated significant differences between the pre and end samples for all the six targeted periodontal pathogens, except for *A. actinomycetemcomitans*. This finding corresponds to that of previous studies, which demonstrate the prolonged antibacterial action of 0.12 % chlorhexidine against anaerobic bacteria resulting in significant bacterial count reduction during oral surgical procedures (Kosutic et al., 2009; Krayer et al., 2010). Moreover, several studies have demonstrated essential oil mouthwash to possess comparable substantivity and broad-spectrum and antimicrobial activity as chlorhexidine (Fine, 2010; Van Strydonck et al., 2012). Similarly, for the CPC group, there was a statistically significant reduction in all bacterial strains and across various sample times, with the exception of *A. actinomycetemcomtians*. This finding may be attributed to *A. actinomycetemcomtians*'s well-documented invasive nature, its ability to penetrate deeper into tissues, its persistence to mechanical treatment, and the need for systemic antibiotics to achieve successful eradication (Van Strydonck et al., 2012; Fine., 2010). The significant reduction in the targeted periodontal pathogens in the present study is indicative of successful dental implant treatment. The most common bacteria at *peri*-implantitis sites are *P. gingivalis, P. intermedia*, *T. forsythia,* and *A. actinomycetemcomitans*; thus, eliminating these periodontal pathogens from *peri*-implant sites is necessary (Young et al., 2002; Tabanella et al., 2009). A reduction in the bacterial load may significantly reduce the risk of postoperative infections (Summers et al., 2000; de Albuquerque et al., 2004).

A direct comparison with previous studies is challenging because the study duration and microbiological analyses performed to evaluate the bacterial count vary across literature. Furthermore, no standardized or generally accepted protocol exists for the use of preoperative mouthwashes for oral cavity decontamination. Some clinicians recommend the use of systemic antibiotic prophylaxis for preventing postoperative infections. However, its use is associated with potential allergic reactions, increased antimicrobial resistance, and increased expense. (Kosutic et al., 2009).

This study had certain limitations. The use of qPCR as a microbiological test for evaluating the bacterial count does not differentiate between viable and non-viable bacteria, which makes estimating the actual effect of mouthwashes challenging. Nevertheless, our study, albeit in a limited number of samples, demonstrated that the use of PMA

Table 4
Comparison of the overall bacterial count and each bacterium individually in each group (P–values).

	Chlorhexidine	Essential oil	CPC	Saline
Overall	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Pre–post	0.005^	0.005^	0.005^	0.114
Pre–end	0.005^	0.005^	0.005^	0.047
Post–end	0.169	0.005^	0.005^	0.285
Pi	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Pre–post	0.005^	0.005^	0.005^	0.005^
Pre–end	0.005^	0.022	0.005^	0.005^
Post–end	0.005^	0.005^	0.005^	0.202
Pg	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Pre–post	0.005^	0.005^	0.005^	0.005^
Pre–end	0.005^	0.005^	0.005^	0.009^
Post–end	0.011^	0.005^	0.013^	0.203
Aa	0.003*	0.004*	0.003*	0.005*
Pre–post	0.412	0.025	0.610	0.151
Pre–end	0.161	0.051	0.259	0.083
Post–end	0.508	0.795	0.837	0.608
Td	< 0.001*	< 0.001*	< 0.001*	0.002*
Pre–post	0.005^	0.005^	0.005^	0.114
Pre–end	0.005^	0.005^	0.005^	0.059
Post–end	0.005^	0.005^	0.037	0.241
Tf	< 0.001*	< 0.001*	< 0.001*	0.001*
Pre–post	0.005^	0.005^	0.005^	0.005^
Pre–end	0.005^	0.005^	0.005^	0.386
Post–end	0.005^	0.005^	0.005^	0.028
Fn	< 0.001*	< 0.001*	0.001*	0.002*
Pre–post	0.005^	0.005^	0.047	0.386
Pre–end	0.007^	0.005^	0.059	0.879
Post–end	0.005^	0.005^	0.005^	0.047

* Statistically significant with Friedman's test ($P < 0.05$).
^Statistically significant with Wilcoxon signed-rank test with Bonferroni correction ($P < 0.017$).
CPC: cetylpyridinium chloride, *Pi: Prevotella intermedia, Pg: Porphyromonas gingivalis. Aa: Aggregatibacter actinomycetemcomitans, Td: Treponema denticola, Tf: Tannerella forsythia, Fn: Fusobacterium nucleatum*.
Pre: Samples before rinsing, **Post**: Samples immediately after rinsing, **End**: Samples at the end of the procedure.

dye with qPCR can detect viable bacteria. This finding is consistent with that of a previous study that confirmed the ability of the PMA dye in detecting only viable DNA by successfully detecting viable *P. gingivalis, A. actinomycetemcomitans,* and *F. nucleatum* without affecting the efficiency of qPCR.

Future research should evaluate and correlate the incidence of implant-related complications, such as postoperative infections or implant failure, with the preoperative use of mouthwashes. Additionally, future studies should incorporate the use of the PMA dye with qPCR to attain the combined benefit of prompt detection of viable bacteria with high sensitivity.

5. Conclusions

Preoperative mouthwashes can reduce the bacterial load at the time of implant placement, thereby reducing the incidence of implant-related complications. Additional randomized clinical trials with larger sample sizes are warranted to confirm the effect of the preoperative use of mouthwashes.

CRediT authorship contribution statement

Wael Yaghmoor: Investigation, Writing – original draft, Writing – review & editing. **Montserrat Ruiz-Torruella:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Yumi Ogata:** Supervision, Writing – review & editing. **Zuhair S. Natto:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Matthew Finkelman:** Supervision, Writing – review & editing. **Toshi Kawai:** Supervision, Writing – review & editing. **Yong Hur:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 5
Comparison of the bacterial count reduction among different mouthwash groups over time in terms of the proportional difference.

Overall	Chlorhexidine Median (IQR)	Essential oil Median (IQR)	CPC Median (IQR)	Saline Median (IQR)	P-value
Pre-post	0.995 + 0.011	0.997 + 0.012	0.996 + 0.007	0.636 + 1.988	< 0.001*
Pre-end	0.992 + 0.01	0.993 + 0.161	0.98 + 0.061	0.108 + 0.154	< 0.001*
Post-end	0.672 + 1.383	0.874 + 0.703	0.837 + 0.407	0.55 + 2.085	0.203
Pi					
Pre-post	0.991 + 0.085	0.99 + 0.412	0.999 + 0.005	0.95 + 0.07	0.107
Pre-end	0.897 + 0.563	0.9 + 1.783	0.986 + 0.29	0.918 + 0.071	0.628
Post-end	0.908 + 0.046	0.944 + 0.098	0.915 + 0.284	0.512 + 1.32	0.007*
Pg					
Pre-post	0.999 + 0.02	0.998 + 0.004	0.986 + 0.013	0.873 + 0.186	0.001*
Pre-end	0.977 + 0.341	0.867 + 0.244	0.943 + 0.072	0.876 + 0.214	0.125
Post-end	0.941 + 0.128	0.986 + 0.103	0.716 + 0.659	0.102 + 1.266	0.003*
Aa					
Pre-post	−0.053 + 0.535	0.039 + 0.994	−0.052 + 0.57	0.17 + 0.503	0.742
Pre-end	0 + 1.041	0.324 + 0.683	0.052 + 0.483	0.329 + 0.628	0.868
Post-end	0 + 1.041	0.324 + 0.683	0.052 + 0.483	0.329 + 0.628	0.916
Td					
Pre-post	1 + 0.001	1 + 0.001	0.999 + 0.002	0.637 + 2.05	< 0.001*
Pre-end	0.998 + 0.007	0.994 + 0.083	0.994 + 0.031	0.138 + 0.152	< 0.001*
Post-end	0.913 + 0.415	0.952 + 0.343	0.938 + 0.492	0.586 + 2.373	0.143
Tf					
Pre-post	0.924 + 0.251	0.98 + 0.064	0.991 + 0.079	0.643 + 0.399	0.003*
Pre-end	0.645 + 0.433	0.896 + 0.091	0.846 + 0.276	0.163 + 1.144	0.015*
Post-end	0.283 + 0.72	0.874 + 0.798	0.904 + 0.424	0.419 + 0.647	0.158
Fn					
Pre-post	0.764 + 0.286	0.948 + 0.132	0.894 + 0.35	0.498 + 1.633	0.044*
Pre-end	0.264 + 0.353	0.537 + 0.42	0.392 + 0.41	0.106 + 2.698	0.147
Post-end	0.571 + 0.737	0.784 + 0.855	0.879 + 0.538	0.467 + 0.383	0.398

* Statistical significant with Kruskal–Wallis test ($P < 0.05$).
Pre: Samples before rinsing, **Post**: Samples immediately after rinsing, **End**: Samples at the end of the procedure.
CPC: cetylpyridinium chloride, IQR: interquartile range, *Pi: Prevotella intermedia, Pg: Porphyromonas gingivalis, Aa: Aggregatibacter actinomycetemcomitans, Td: Treponema denticola, Tf: Tannerella forsythia, Fn: Fusobacterium nucleatum*.

the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sdentj.2023.12.011.

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