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LETTER TO THE EDITOR

ANTI-PLAQUE AND ANTIMICROBIAL EFFICIENCY OF DIFFERENT ORAL RINSES IN A 3-DAY PLAQUE ACCUMULATION MODEL

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The idea of incorporating a mouthrinse with normal tooth brushing could be a useful adjunct to oral hygiene. Despite the principle nature of the toothpaste vehicle, most alcohol-based chemical plaque-control agents have been evaluated and later formulated in the mouthrinse vehicle. The current study was aimed to investigate the persistence of antimicrobial action and plaque inhibitory properties of a new alcohol-free mouthrinse when compared with positive control, chlorhexidine 0.12%, and placebo control, physiologic saline solution mouthrinses. The evaluation of the antimicrobial activity was performed by saliva samples collected during the 3 days of usage. The results of this study indicate that this new oral rinse has an equivalent plaque inhibitory action to chlorhexidine, and the plaque inhibitory action of the rinse appears to be derived from a persistence of antimicrobial action in the mouth. Furthermore, no side effects were reported during the study, and the additional benefit of no alcohol presence in the rinse solution.

To the Editor,

Dental plaque (DP) is a biofilm that slowly accumulates on the surface of teeth. If not removed regularly, it can lead to dental cavities (caries) or periodontal problems (such as gingivitis) (1, 2). Colonization of enamel surfaces by the cariogenic bacterium *Streptococcus mutans (S. mutans)* is thought to be initiated by attachment to a saliva-derived conditioning film, the acquired enamel pellicle (2-5).

Mechanical control of dental biofilm has had a somewhat limited success in part because it is regarded as time-consuming and technically difficult by most patients. This fact, coupled with an increase in the information available on the microbiology of periodontal diseases, has stimulated a great interest in developing topical antimicrobial agents to control dental biofilm (1).

In fact, periodontitis is initiated by oral biofilm formation which, if untreated, progresses to gingivitis further leading to periodontal disease. (6) According to the Evidence Based Dentistry, mouthwashes have been particularly well accepted by patients due to their ease of use, considering also their adverse effect if containing ethanol (7-9).

Key words: chlorhexidine, gingivitis, gingival index, plaque index

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

On the one hand, chlorhexidine (CHX) is considered to be the gold standard for oral antiseptics (10), on the other hand, according to some reporters, organisms may develop resistance against long-term effects of 0.2% CHX (up to two years) after continuous use (11).

Tetrasodium pyrophosphate, disodium dihydrogen pyrophosphate, and sodium tripolyphosphate are all tartar control ingredients (7). Studies have shown that these not only serve to disrupt or retard the formation of calcium phosphate crystals, but also inhibit some bacterial growth (12).

The purpose of the present study was to evaluate the antimicrobial and anti-plaque efficiency of two different mouthwashes and a placebo through a standard 3-day plaque accumulation model in patients affected by peri-implantitis or gingivitis and periodontally healthy subjects.

MATERIALS AND METHODS

Twenty-two healthy volunteers participated in the study. All subjects were screened for suitability by the research team. Selection criteria were a dentition with ≥20 evaluable teeth (minimum of five teeth per quadrant), no oral lesions, no severe periodontal problems (no probing depth ≥5 mm), and no removable prostheses or orthodontic bands or appliances. Persons allergic to several mouthwash components were excluded from the study. All the selected subjects were informed about the products and the purpose of the study and informed consent was given. The study was conducted in accordance with the ethical principles originating in the Declaration of Helsinki and consistent with good clinical practices.

Prior to the study period, the volunteers received a soft and hard tissue examination of the oral cavity, followed by a professional scaling and polishing to remove all calculus, plaque and extrinsic tooth stain. Professional scaling and polishing was performed using hand instruments, mechanical scalers, rotating brushes with polishing paste, and dental floss in the interproximal areas. Randomization of subjects into a control or a test group was performed using computer-generated random numbers. A person not directly involved in the research project carried out the allocation of tests or control products.

The subjects in the test group received a bottle of non-alcoholic calculus dissolution-based oral rinse containing tetrapotassium pyrophosphate (TKPP), sodium tripolyphosphate (STPP), sodium bicarbonate, sodium fluoride and citric acid (Periogen®, USA). The positive control was a 0.12% CHX solution, while the negative control was a physiologic saline solution (PSS) (placebo - no mouthwash dilution added).

All bottles with mouthwash provided to subjects were pre-weighed. All participants were instructed to refrain from using any other means of oral hygiene during the experimental period. All subjects were instructed to rinse twice a day, in the morning and in the evening, with an applicator spoonful dissolved in one cup of warm water for 60 seconds, or 20 ml of solution for CHX and PSS. Subsequent rinsing with water was not allowed and the subjects were also asked not to brush their teeth during the study period. For plaque scores, the participants rinsed for 30 seconds using a disclosing solution (1% erythrosine), according to Quigley and Hein index, Turesky modification(13).

All measurements were carried out under similar clinical settings and by the same blinded investigator. The plaque and bleeding scoring were recorded prior to saliva collection. All returned mouthwash bottles were weighed to calculate the amount of mouthrinse used and to check for compliance.

In order to have microbial samples representative of health- and disease-associated conditions, the 22 volunteers selected included 11 periodontally healthy individuals (less than 10% of the sites with bleeding of gingival margin, plus a maximum of 2 sites with probing depth > 6.0 mm or attachment level > 5.0 mm); 11 individuals presenting gingivitis (bleeding of gingival margin in at least 10% of the sites), but also no more than 2 sites with probing depth > 6.0 mm or attachment level > 5.0 mm, and 2 with peri-implantitis.

The volunteers were asked to collect 2–3 mL of unstimulated saliva in the morning as a baseline sample and at 15 minutes after swishing the solution around inside the mouth. The saliva collection was repeated on the first, second and third day.

Preparation of bacterial samples

Samples were put in a sterile vial and then stored at 4°C until they were processed on the same day. Brain-heart infusion agar was used as selective medium for *S.*

mutans. Purity of growth was checked by examining them under an optical microscope (Carl Zeiss ICS/KF2, Oberkachen, Germany).

For the assessment of the *in-vivo* antimicrobial activity of the solutions, a sensitivity test was carried out according to the agar dilution method described by the National Committee for Clinical Laboratory Standards (14). The plates were incubated at 37°C in an incubation chamber for 24 h and then colony forming units (CFUs) were counted (Fig. 1, A-C). The results were presented as colony-forming units (CFU)/mL, in qualitative form only, following the study design.

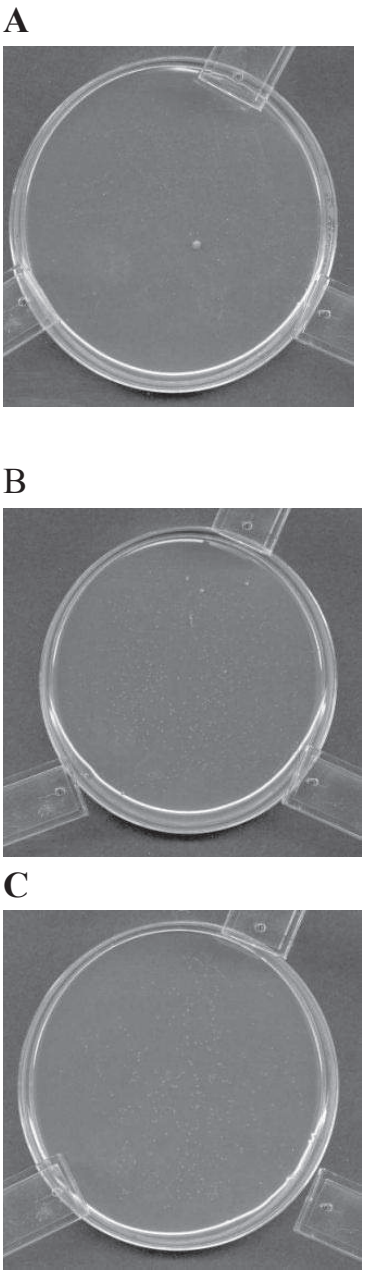

Fig. 1. *CFUs on agar plates of physiologic saline solution group (**A**), of Periogen® group (**B**), and of chlorhexidine group (**C**).*

Statistical analysis

The statistical analyses were performed by using Statistical Package for Social Sciences (SPSS for Windows, Version 11.5, Chicago, Ill, USA) software. The values of repeated bacterial count (CFU/mL) after PSS, CHX and Periogen® mouthrinsing were expressed as the minimum (min), maximum (max), and median. The recorded values were transferred to log values before statistical analyses. The bacterial count differences in median ranks for repeated measures among time intervals before mouth rinsing (T0), 15 minute effect (T1), second day effect (T2), and third day effect (T3), were compared by the non-parametric Friedman test, and significance levels were set at 0.05. Wilcoxon signed rank test was used to compare two dependent nonparametric values, and significance level was chosen as 0.01 according to Bonferroni adjustment. The Friedman test was used to determine the bacterial count difference at each time interval for different mouth-rinsing solutions, and the Wilcoxon signed rank test was used for two dependent non-parametric values. Significance level was chosen as 0.01 according to Bonferroni adjustment. Briefly, the general level of significance was 0.05, and the local *P* values were adjusted according to Bonferroni due to multiple testing.

RESULTS

All subjects (N = 22) completed the trial, and there were no missing values. The amounts of mouthwashes used indicated good compliance with the instructions. No adverse events or side effects were reported or observed. The plaque and bleeding scores for the test and control groups at the end of the experimental period are shown in Fig. 2 A-B.

The minimum, maximum, and median CFU of total *S. mutans* bacteria, in the saliva of subjects before mouth rinsing (T0), at 15 minutes after swishing the solution around inside the mouth, and on the second, and third days during treatment with antiseptics are

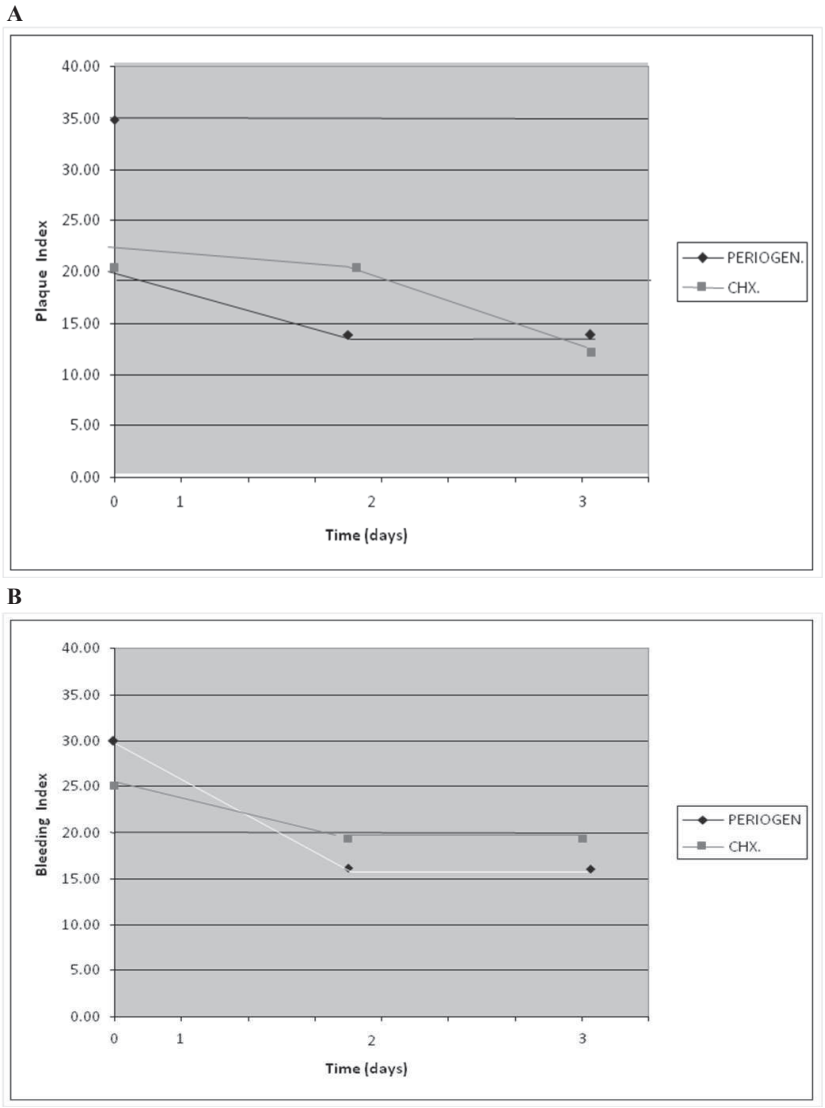

Fig. 2. *Plaque (**A**) and bleeding (**B**) scores for the test and control groups at the end of the study period.*

presented in Table I. The antiseptic efficacy of CHX 0.12% on *S. mutans*, was very similar to the efficacy observed with Periogen® mouth solution throughout the entire study period.

There was no significant difference between antibacterial effects of CHX and Periogen® during the study period, advocating the additional benefit of an alcohol-free solution.

DISCUSSION

In the present study, the efficacy of a new mouthwash (Periogen®) was compared with 0.12% chlorhexidine which was considered as a gold standard. Many studies use the 3-day *de novo* plaque accumulation and non-brushing model to assess the effect of various mouthwashes (15). The results from such studies indicate that the assessment was carried out under experimental conditions. On the basis of the results obtained in the present clinical study, it can be stated that Periogen® mouthwash had a promising plaque inhibitory potential. These findings are also consistent with those reported in a six-month clinical study by Saini R. (16), who found that Periogen®solution provides aclinically relevant reduction in calculus formation as a result

Table I. *Minimum, maximum, and median CFU (log^{10}) of Streptoccocus mutans in the saliva of subjects during treatment with different antiseptics.*

Solution[a] / Time[b]	*Streptococcus mutans* Min	Max	Median
PERIOGEN -T0	1.00	2.45	1.72
PERIOGEN -T1	NG	2.10	NG
PERIOGEN -T2	0.40	1.05	0.72
PERIOGEN -T3	0.50	1.10	0.80
* *P* value			<0.001
CHX-T0	1.30	2.40	1.35
CHX-T1	NG	1.70	NG
CHX-T2	0.30	1.66	0.98
CHX-T3	0.50	1.72	1.11
* *P* value			<0.001
PSS-T0	1.00	2.00	1.50
PSS-T1	1.00	2.85	1.93
PSS-T2	1.00	2.80	1.90
PSS-T3	1.00	2.75	1.87
* *P* value			<0.001

a PSS: physiologic saline solution; Periogen: sodium tripolyphosphate, tetrapotassium pyrophosphate, baking soda citric acid and fluoride 0.04%; CHX: chlorhexidine gluconate;
[b] T0: before mouth rinsing; T1: 15 min after swishing solution around inside the mouth; T2: second day effect; T3: third day effect.
[c] NG indicates no growth.
** Friedman test.*

of a reduced calcification of DP. However, studies of longer duration with cross-over study design and wash-out period would have been more authenticating as it eliminates the bias of viable host.

Further studies where safety and microbiological parameters on other bacteria species would be evaluated are essential to establish the true effectiveness of the mouthwash and its position among the other rinses that are used adjunctively to mechanical oral hygiene procedures (5).

Though CHX mouthwash is considered as a gold standard, it has been reported as having a number of local side effects including brown extrinsic tooth and tongue staining, taste disturbance, dryness of mouth, burning sensation (9). These side effects limit its acceptability to users and its long-term use, whereas Periogen® mouthwash had no reported side effects.

From the public health point of view, Periogen® mouthwash can serve as a good alternative to avoid alcohol-based products or those with any artificial preservatives. There is need to investigate other alternative mouthwashes (such as Periogen®) to prove their efficacy as equivalent to alcohol-based ones in reducing the plaque scores and gingival inflammation. As this is a short-term study, the results can be used as a baseline data for future studies with similar study design.

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