Original Article

Microbial evaluation of the effectiveness of different methods for cleansing clear orthodontic retainers: *A randomized clinical trial*

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ABSTRACT

Objective: To compare effectiveness of brushing an Essix retainer with water only and brushing followed by soaking in one of three commercial cleansing tablets.

Materials and Methods: A double-blind, parallel, randomized clinical trial with a split-mouth design was completed with 60 volunteers with specific eligibility criteria assigned to three groups to compare the cleansing effectiveness of brushing an Essix retainer with water only, followed by soaking in one of three alkaline peroxide–based commercial cleansing tablets (Retainer Brite®, Kukis®, and Corega®). Each participant wore an upper Essix retainer (with an electronic microsensor) on a full-time basis for 14 days and used one of the three products on one side of the retainer for 15 min/d. The effectiveness of the cleansing tablets was tested by the scanning electron microscopy, bacterial identification, bacterial quantification, and disk diffusion methods.

Results: Bacterial quantification tests demonstrated nonsignificant statistical differences between the control and test sides of the three cleansing tablets: Retainer Brite®, Kukis®, and Corega® (Mann-Whitney test *P*-values were .6, .37, and .5, respectively). A Kruskal-Wallis test also showed nonstatistical difference in the bacterial counts between the three groups (*P*-value = .5). In vitro tests showed a minimal inhibition zone of *Staphylococcus epidermidis* only with Corega® tablets.

Conclusions: Using chemical cleansing tablets after mechanical cleansing did not significantly reduce the bacterial count in Essix retainers when compared to use of mechanical cleansing alone. However, the tablets seem to be effective against "*cocci*" bacterial species. (*Angle Orthod.* 2017;87:460–465)

KEY WORDS: Clear orthodontic retainers; Essix retainer; Cleaning retainer; Cleansing tablets

INTRODUCTION

A study based on clinical observations reported that patients who used Essix retainers were more susceptible to caries because the retainer prevented salivary flow over the tooth surface and provided a protective cover for bacteria.[1,2] Currently, clear clinical protocols for cleaning orthodontic retainers have not been established. In the literature,[3] brushing retainers is recommended for hygiene maintenance of acrylic orthodontic appliances.

In addition, various studies[4–10] have evaluated different cleaning systems with the aim of establishing a protocol for cleaning removable dentures. Survey studies[11,12] showed that brushing and soaking in chemical agents were the most popular methods for cleaning removable appliances. Guidelines for the care and maintenance of dentures published by the American College of Prosthodontists[13] advised cleaning dentures by soaking and brushing with an effective nonabrasive cleansing agent. Webb et al.[14] and Chan et al.[10] stressed the need to use chemical agents in conjunction with mechanical cleaning methods.

An in vivo study[15] of 17 children reported that spraying an 0.12% chlorhexidine gluconate solution on orthodontic appliances significantly reduced the bacterial count. A randomized changeover clinical trial[16] of 15 dental students compared the effect of

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brushing and spraying tap water on the acrylic baseplate of a removable orthodontic appliance (control) and brushing and spraying 0.12% chlorhexidine gluconate once a week and twice a week on the growth of *Streptococcus mutans* (test). The results revealed a significant difference between the control and test groups.

As a result of the shortage of studies of different methods of cleaning clear orthodontic retainers, this clinical trial was designed to compare the cleansing effectiveness of the following: brushing Essix retainers with water only and brushing followed by soaking in one of three types of commercial cleansing tablets, two of which (Retainer Brite® and Kukis®) are produced for removable orthodontic retainers. The third (Corega®) is manufactured for removable dentures.

MATERIALS AND METHODS

This double-blind, parallel, randomized clinical trial was conducted in the orthodontic clinic after obtaining the ethical approval from the ethics committee of the research center in the college. The inclusion criteria for the study participants are described as follows:

- Participant avoidance of steroid-based or antibacterial mouthwash or broad-spectrum antibiotics for 2 weeks prior to study participation[6,7,10,17,18];
- Nonsmoker[17];
- No history of sensitivity to persulfate;
- Non–mouth breather;
- Free from any medication that reduces salivary flow;
- Caries free;
- Well-aligned teeth or mild crowding;
- Normal pH of saliva (ranging from 6.5 to 7.5);
- Nonpregnant female; and
- Presence of upper permanent dentition, full anterior teeth.

Two examiners were responsible for collecting the samples. All of the study steps were explained to the participants verbally and in writing. Written informed consent was obtained from each participant.

Fabrication of the Essix Retainer

An upper polyvinyl siloxane impression was taken for each participant, and the Essix retainer was fabricated using Essix material (Invisacryl A, 0.030-inch, round, 0.75 mm/125 mm; Great Lakes Orthodontics, Tonawanda, NY).[2,19,20] The retainer was then split between the central incisors. A microsensor (TheraMon®, IFT Handels-und Entwicklungsgesellschaft GmbH, Handelsagentur Gschladt, Hargelsberg, Austria) was then embedded on the lingual surface of the left first molar to document the actual wearing time. All of the retainers were disinfected, and the TheraMon® sensor was activated before the retainers were given to the participants.

Sample Size Calculation

The sample size was determined from a previous study.[16] Our standard difference = 0.6, and power = 80; 20 was the number of volunteers needed per group to determine the cleansing tablet effect.

Clinical Study Protocol

Out of 82 volunteers, six were smokers, five dropped out as a result of academic obligations, one had taken an antibiotic 4 days prior to the study, seven lost their retainers, and three failed to submit their retainers. Therefore, 60 subjects completed the study ($n = 20$ in each group). Across all three groups, 66.6% of the volunteers were females. The mean age of the volunteers was 24.22 ± 5.4 years.

Three commercial cleansing tablets were studied: Corega® (GlaxoSmithKline, Dublin, Ireland), Kukis® (Procter & Gamble Technical Centers Ltd, Egham, UK), and Retainer Brite® (DENTSPLY, Bradenton, Fla). Pharmacists repackaged the cleansing tablets under aseptic conditions and relabeled each product with the letter A, B, or C in packs of 14. Neither the examiner nor the volunteers knew the brands of the tablets. Volunteers were assigned into three groups through a simple, random technique. Each participant was provided with a soft toothbrush (Soft Micro Active Bristles, Sensodyne, Brentford, UK), toothpaste (Colgate Total, Colgate-Palmolive, Dammam, Saudi Arabia), a cup for soaking marked to 150 mL, and 14 tablets from the assigned tablet group.

The volunteers were asked to wear the Essix retainer 24 h/d for 14 days. They were instructed to brush both sides of the Essix retainer once a day for 1 minute before bedtime using only the toothbrush provided and water. They were then instructed to fill the cup to the marked line (150 mL) with tap water, dissolve one cleansing tablet, and soak only the left side of the retainer for 15 minutes (as instructed by the production company), after which they were to remove the retainer and wash it with tap water.

Microbiological Analysis

On day 14, the volunteers submitted the retainers after cleaning them as instructed; the area from the central incisor to the canine was immediately soaked in 5 mL of sterile normal saline and shaken for 20 seconds.[10,18] The buccal surfaces of the second premolars on both sides were subjected to scanning electron microscope (SEM) photography.

Table 1. Descriptive statistics of the three groups' bacterial count data.

	Retainer Brite®		Corega®		Kukis®	
	Control	Test	Control	Test	Control	Test
N	20	20	20	20	20	20
Mean	2522.4	2380.4	2214.6	1926.3	2424	2257.5
Mean Difference	142		288.3		166.5	
% of Bacterial Reduction	5.60%		13%		6.90%	
SD	1293.9	1274.4	1308.5	1017.2	1070.4	1252.6
Mann-Whitney P-Value	0.6		0.37		0.05	

Bacterial Identification

One hundred microliters of the sample suspension was used for the traditional plate culturing method. The samples were cultured twice, one plate in an aerobic condition and the other plate in an anaerobic incubator, at 37°C for 48 hours.[18] A Gram stain was then performed to identify the type of bacteria. A plastic test card containing 64 microwells was used for the identification of gram-positive (VITEK®2, GP kit, bioMérieux, France) and gram-negative (VITEK®2, GN kit, bioMérieux, France) bacteria. The results were obtained by VITEK2 machine (VITEK®2-compact 15, bioMérieux, Durham, NC, USA).

Bacterial Quantification (AlamarBlue® Assay)

Ten microliters of AlamarBlue® stain (Alamar Blue®, Oxford, U.K.) were added to 100 μL of the remaining suspension in 96 wells on a flat-bottomed plate and incubated for 4 hours at 37°C. The incubation plate was then read using a Synergy 2 multimode microplate reader (BioTek, Winooski, VT, USA).

Scanning Electron Microscopy

Four samples in each group were randomly selected for the SEM study. The buccal surface of the first premolars on both sides was separated from the retainer and soaked in 10% paraformaldehyde in 0.2 M sodium cacodylate for 24 hours.[17] The sample was then washed with a buffer solution (sodium-cacodylate), followed by a fixation phase with 1% osmium tetroxide for 1 hour. The sample was dehydrated through washing with a series of graded ethyl alcohol, followed by sputter-coating with gold.[4,16,21] The following scale was used for scoring the bacterial distribution on the fitting surface of the retainers: score of 1: bacteria covered 0–25% of the examined area; score of 2: bacteria covered 25–50% of the examined area; score of 3: bacteria covered 50–75% of the examined area; and score of 4: bacteria covered 75–100% of the examined area.

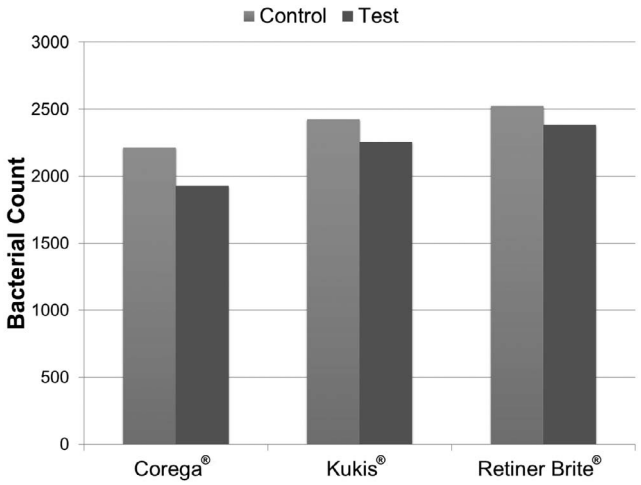

Figure 1. Comparison of the bacterial counts between the control and test sides of each of the three product groups.

In Vitro Study

The sensitivity of *S. mutans, Staphylococcus epidermidis,* and *Staphylococcus aureus* to each cleaning product was tested by measuring the diameter of the inhibition zone using the disk diffusion method.

Statistical Analysis

The Kruskal-Wallis test was used to compare the mean rank of the three groups, and the Mann-Whitney test was used to compare the mean bacterial count of the test and the control sides in each group. The significance level was set at a *P*-value of $\leq$.05, with a 95% confidence interval.

RESULTS

Statistical analysis revealed no significant differences in wear time between the groups ($P = .5$). Statistically nonsignificant differences were found in bacterial count between the control and the test sides in each group (Table 1; Figure 1) and between the three groups ($P = .5$) (Table 2).

Bacterial Identification

Gram-positive and gram-negative bacteria were present in all of the groups. In general, fewer bacterial species (gram positive and negative) were found on the test side than on the control side. The same gram-

Table 2. Statistical comparison of the bacterial count between the control sides and the test sides of the three groups.

	Retainer Brite®	Corega®	Kukis®	Kruskal Wallis P-Value
Control	2522.4	2214.6	2424	0.4
Test	2380.4	1926.3	2257.5	0.5

positive bacterial species were found on the control side in all three groups. *Streptococcus* and *Staphylococcus* species were predominant in the gram-positive group, and *Acinetobacter* and *Pseudomonas* were predominant in the gram-negative group.

Scanning Electron Microscopy

Corega® group. The control side was given a score of 2 (Figure 2A). The test side was heavily covered with bacilli bacteria and was given a score of 3 (Figure 3A).

Kukis® group. Heavy accumulation of *cocci* was observed on the control side (Figure 2B), with some bacilli bacteria. Thus, the control side was given a score of 3. A score of 1 was given to the test side. *Cocci*-shaped bacteria with residual debris are shown in Figure 3B.

Retainer Brite® group. The control side received a score of 3 (Figure 2C). It contained clusters of bacteria on the fitting surface of the retainers, most of which were *cocci* shaped. The test side had a score of 2, and the predominant bacteria were bacilli in shape, with some scattered *cocci* (Figure 3C).

In Vitro Study Results

Based on the disk diffusion test, none of the three products had any inhibition effect on the bacterial species tested (*S. mutans, S. epidermidis,* and *S. aureus*), except Corega®, which showed some ability (a very small inhibition zone of 2 mm) to inhibit the growth of *S. epidermidis* (Figure 4).

DISCUSSION

According to Nikawa et al.,[22] a cleanser can be considered effective when it reduces the bacterial cell count by 90%, or 500 bacterial cells. In the current study, the bacterial quantification was determined by the amount of dye reflection. The level of bacterial reduction indicated that the combination of the cleansing tablets and brushing, compared to brushing alone, did not significantly improve the hygiene level of the retainer.

This finding matches that of another study,[8] whose authors found that maintaining adequate brushing can successfully remove plaque and is even preferable to soaking in chemical agents. In the current study, the participants brushed both the control side and the test side, and this could have eliminated the effect of the cleansing tablets. As noted in a previous study,[23] orthodontic patients maintain excellent oral hygiene after orthodontic therapy as a result of the intensive hygiene instructions they receive; this hygiene also improves their level of care of their orthodontic appliances. Our study sample included a number of recently debonded orthodontic patients, which might explain the nonsignificant result. In the present study, most of the volunteers were dental/medical students who were familiar with the importance of maintaining good oral hygiene. Some previous studies[9,24] of dentures that compared a control and a test side soaked in a chemical agent reported significant effects in terms of bacterial reduction. However, these studies included older denture wearers who may not have been able to maintain good mechanical cleaning techniques as a result of impaired vision and manual ability. In orthodontics, the patients are generally young and healthy. This was reflected in the mean age of the volunteers (24.22 ± 5.4 years) in the present study. This study shows that brushing retainers is an effective cleaning method. This method is also easy and carries a lower cost. Soaking a retainer in a cleansing agent can be considered if the patient is young or lacks manual dexterity.

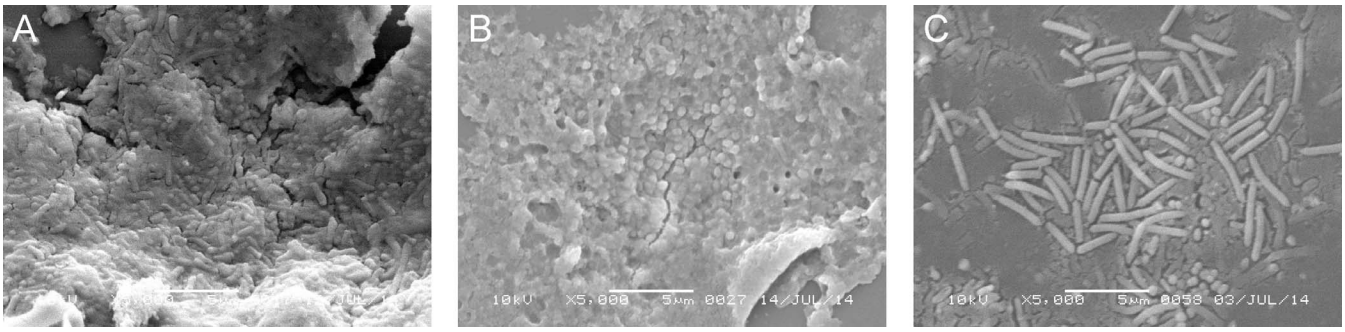

Figure 2. Scanning electron microscope images of the control side of (A) Corega®, (B) Kukis®, and (C) Retainer Brite®.

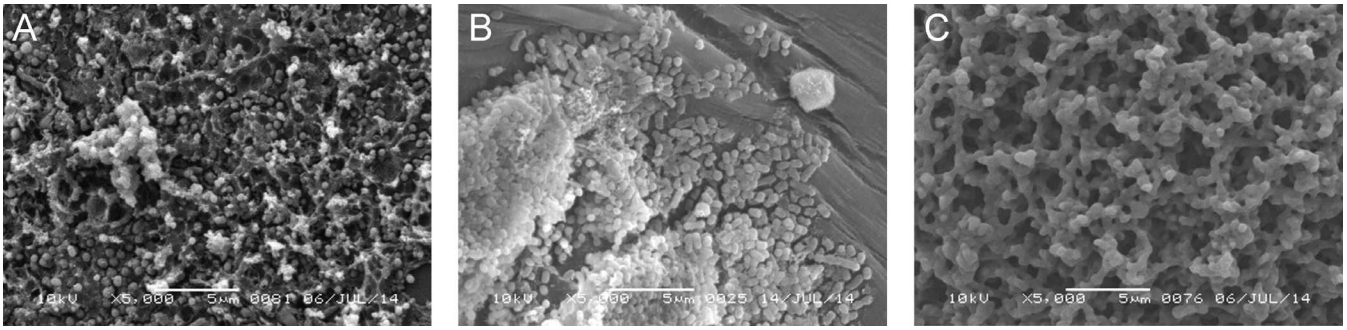

Figure 3. Scanning electron microscope images of the test side of (A) Corega®, (B) Kukis®, and (C) Retainer Brite®.

All three cleansing tablets in the present study had the same active ingredient: alkaline peroxide. This might explain the nonsignificant differences. In another study,[25] researchers attributed some of the effectiveness of a cleansing product to the presence of sodium lauryl sulfate, which is a detergent that targets proteins in microorganisms, causing conformational changes. In the current study, the three products contained sodium perborate, sodium carbonate, and sodium lauryl sulfoacetate. In addition, cleansing agents could be effective against some types of bacteria but not against others, and the total bacterial count may not differ as a result of the growth of resistant bacteria. The findings of the present study match those of a previous study,[25] which demonstrated that brushing and soaking in sodium perborate was not more effective than brushing alone.

S. mutans was selected for analysis in the present in vitro study because it plays a major role in the development of dental caries.[22,26] *S. epidermidis* and *S. aureus* were the most commonly found bacteria in the samples. The in vitro results showed that none of the three products were effective against *S. mutans, S. epidermidis,* or *S. aureus,* although Corega® showed a slight inhibitory effect against *S. epidermidis*. The in vitro findings of the current study supported the in vivo results, with the exception of Corega®. Although Corega® showed a score of 2 in the control group and a score of 3 in test group with the SEM test, we cannot depend on this result because this method was performed on one sample only and it may be subject to technical defect during the processing or choosing of the area of photography. A previous study[22] confirmed that the results of in vivo and in vitro studies do not necessarily match. The results of trials[22,27] performed on dentures in vitro as well as in vivo showed conflicting results. Furthermore, if a specific microorganism is shown to have a significant reduction with a disinfectant the findings cannot be generalized to the same bacteria while it is in dental plaque. That is because the level of metabolic activity of bacterial species differs depending on whether they are alone or present with other microorganisms.[16] The bacterial cells alone are more sensitive to the disinfectant when compared with those present in a microbial biofilm.[22] A previous study[28] also showed that even if a chemical cleansing solution is effective against specific types of microorganisms, it will not eliminate all microorganism growth. The aforementioned may explain the appearance of some types of microorganisms on the control and test sides in this study. The in vitro part of the study confirmed the ineffectiveness of the cleansing tablets against the tested bacterial species. However, the effectiveness of the tablets was tested only against specific bacterial species. This is one of the study's limitations. For future studies, testing cleansing tablets

Figure 4. Disk diffusion test showing the effect of the cleansing tablets on *Staphylococcus epidermidis*: (A) Corega®, (B) Kukis®, and (C) Retainer Brite®.

with different active ingredients on an expanded variety of bacterial species is recommended.

CONCLUSIONS

Within this study's limitations:

- Using chemical cleansing tablets after mechanical cleansing did not significantly reduce the bacterial count found on Essix retainers when compared to mechanical cleansing alone.
- Chemical cleansing tablets seem to be effective against *cocci* bacterial species.

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