Effects of ultrasonic, electric, and manual toothbrushes on subgingival plaque composition in orthodontically banded molars

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Introduction: Orthodontic appliances hinder mechanical plaque control. In this study, we evaluated the effect of self-performed supragingival plaque removal with ultrasonic, electric, and manual toothbrushes on subgingival plaque composition in orthodontically banded molars. Methods: Twenty-one patients wearing fixed orthodontic appliances were assigned to this single-blind crossover study. Samples of subgingival plaque were collected from banded molars, before and after each toothbrush usage period, for quantification of 22 bacterial species by the checkerboard DNA-DNA hybridization method. For each crossover, patients used a toothbrush for 30 days, followed by a washout period of 14 days. Results: The prevalence of Tannerella forsythia decreased significantly after a month of electric brush usage. In the manual brush group, the prevalences of Selenomonas noxia, Streptococcus sanguinis, and Prevotella melaninogenica also decreased significantly. However, there were no significant differences in the prevalences and levels of bacteria after usage of the ultrasonic brush. Intergroup comparisons showed no statistical differences among the 3 brushes for the microbiologic parameters. Conclusions: All 3 brushes generally reduced bacterial prevalences, and, although electric and manual toothbrushes showed some isolated significant variations, we found no superiority with any toothbrush type when used three times daily for 2 minutes on microbiologic parameters in orthodontically banded molars. (Am J Orthod Dentofacial Orthop 2010;137:229-35)

The contribution of dental plaque control to prevent caries and periodontal diseases is well established. 1 Experimental gingivitis studies have clearly shown that gingival inflammation is treated by the removal of accumulated dental plaque. Plaque removal by manual toothbrushing is the most common method of oral hygiene in the world. Because so much effort is directed at controlling organisms by this means, it is surprising that few studies have examined the changes in plaque composition from this procedure. 2 The few published studies have used culture media, 3 DNA probes, 4 or, more recently, checkerboard DNA-DNA hybridization. 2 Patients undergoing orthodontic therapy face greater difficulties maintaining good oral hygiene than other patients. Orthodontic appliances with bands, brackets, and arches are barriers for brush bristles and dental floss, leading to greater accumulations of plaque and impairment of gingival health. Orthodontic treatment with fixed appliances is highly associated with gingival inflammation and bleeding, gingival enlargement, and increased probing pocket depths. 5-7

Microbiologic subgingival changes have also been associated with the placement of fixed orthodontic appliances. Studies have shown decreases in gram-positive cocci, which are associated with periodontal health, and statistically significant increases in suspected periodontal pathogens such as spirochetes, motile rods, and other gram-negative organisms. 5-8

Data examining the effects of various toothbrushes on subgingival plaque composition of patients with orthodontic appliances are sparse. No study has reported the efficacy of an ultrasonic toothbrush used by orthodontic patients on subgingival plaque. Thus, the purpose of this investigation was to determine the effect of self-performed supragingival plaque removal with ultrasonic, electric, and manual toothbrushing on subgingival
plaque composition in these patients. The clinical changes in these subjects were described elsewhere.9

MATERIAL AND METHODS

Twenty-one adolescent patients (11 boys, 10 girls; age range, 12-18 years; average, 15.2 ± 1.7 years) undergoing orthodontic treatment at the School of Dentistry at São Paulo State University, Araraquara, Brazil, were consecutively selected for this study.

Each had at least 20 teeth and had been in orthodontic treatment with fixed appliances for a minimum of a year. All were nonsmokers with no obvious periodontal disease or attachment loss. They had taken no medications in the last 3 months and had no systemic or local disease affecting the periodontium. According to the protocol of the orthodontic clinic, all patients received plaque control and instructions in oral hygiene before treatment, and periodontal conditions were regularly evaluated during treatment.

This study was a single-blind crossover clinical trial. It was approved by the Ethics and Research Committee of the School of Dentistry of São Paulo State University.

The ultrasonic brush tested was the Ultrasonex Ultima Toothbrush (Sonex International, Brewster, NY), which has a removable center head and operates at a frequency of 1.6 MHz. Comparisons were made with an electric brush (Braun Oral B 3D Plaque Remover, Braun GmbH, Kronberg, Germany) and a manual brush (Oral B Model 30, Gillette do Brasil, Manaus, Brazil).

The participants were randomly divided into 3 sequences of brush use: (1) ultrasonic, electric, and manual; (2) manual, ultrasonic, and electric; and (3) electric, manual, and ultrasonic.

The sequence distribution of the patients was done to eliminate the influence of the Hawthorne effect on the results, after each type of brush was used by a group of patients in the 3 periods of the study.10

The subjects used each assigned brush for 30 days followed by 14 days when they returned to the toothbrush and dental floss usage according to the instructions from their orthodontist before the study. The patients were evaluated at the end of morning or afternoon periods with 3 to 5 hours of plaque accumulation both at baseline and after every 30-day period. During baseline visits, the subjects were instructed in oral-hygiene techniques. For those receiving a manual brush, the Bass technique was demonstrated; those receiving the electric and ultrasonic versions were given audiovisual presentations on the correct use according to the manufacturers followed by a session for personal instruction and answering questions. The subjects were asked to use their assigned toothbrush 3 times daily for 2 minutes with a designated toothpaste (Sorriso, Colgate-Palmolive Indústria e Comércio Ltda, São Bernardo do Campo, São Paulo, Brazil) and to avoid other oral health products or techniques.

Clinical measurements were presented previously.9 Briefly, plaque and gingival inflammation were assessed on all teeth except the second and third molars by using the plaque index of Silness and Löe7 and the gingival index of Löe and Silness,12 respectively. The orthodontic modification of the plaque index8 was used on the buccal surfaces of the teeth because of the brackets.13 Probing depth was measured at 6 sites per tooth. All measurements were done by a calibrated examiner (M.R.C.).

Clinical and microbiologic monitoring was done at baseline and 4 weeks after the use of each brush.

The presence and levels of 22 species were determined by a modification14 of the checkerboard DNA-DNA hybridization method described by Socransky et al.15 Individual subgingival plaque samples were obtained from the 4 first molars (banded teeth) in each subject at baseline and after the period of use of each brush. After removal of supragingival plaque, subgingival plaque samples were taken from the mesiobuccal line angle area of each tooth by using sterile Gracey curettes. Each sample was placed in a tube containing 0.15 mL TE (10 mmol/L Tris-hydrochloric acid, 1 mmol/L EDTA, pH 7.6). Then, 0.10 mL of 0.5 M sodium hydroxide was added to each sample, and the tubes were frozen for future analysis. After this, the cells were lysed, and denatured DNA was fixed in individual lanes on a nylon membrane by using the checkerboard slot blot device.

Twenty-two digoxigenin-labeled whole genomic DNA probes were hybridized at 90°C to the lanes of the plaque samples. Bound probes were detected by using phosphatase-conjugated antibody to digoxigenin and chemiluminescence. Signals were evaluated visually by comparison with the standards at 105 and 106 bacterial cells for the test species on the same membrane. They were recorded as 0, not detected; 1, <105 cells; 2, about 105 cells; 3, 105 to 106 cells; 4, about 106 cells; and 5, >106 cells. The sensitivity of this assay was adjusted to permit the detection of 104 cells of a species by adjusting the concentration of each DNA probe. This procedure was carried out to provide the same sensitivity of detection for each species.

Failure to detect a signal was recorded as zero, although counts in the 1 to 10,000 range could conceivably have been present. A total of 504 plaque samples were evaluated.

Statistical analysis

The mean frequency of levels (0-5) of each species was computed for each subject and then averaged across subjects in the 3 toothbrush groups (manual, electric, or
ultrasonic). In addition, the percentages of sites colonized by each species (prevalence) were also computed for each subject and averaged across subjects in the 3 groups. The significance of differences in mean percentages of sites colonized by subgingival species among the 3 groups was determined by using the Kruskal-Wallis test. Changes in plaque composition over time (baseline to 1 month) were evaluated by using the Wilcoxon test. Statistical significance was set at $P = 0.05$.

**RESULTS**

All 21 patients completed the study with no adverse effects reported by any of them or noted by the examiner.
(M.R.C.). Clinical results showed no significant differences among brush groups.\(^9\) However, the ultrasonic toothbrush showed significant improvement in the reduction of visible plaque on the buccal surfaces, with a tendency to remove more plaque around the brackets.\(^9\)

Figures 1, 2, and 3 summarize the prevalence of the colonized sites and the levels of the 22 subgingival species in the 3 brush groups. The most frequently detected species in all samples and subjects included *Fusobacterium nucleatum*, *Neisseria mucosa*, *Streptococcus oralis*, *S sanguinis*, and *Veillonella parvula*. The least prevalent species were *Actinobacillus actinomycetemcomitans*, *F periodonticum*, *Campylobacter rectus*, *Propionybacterium acnes*, and *S intermedius*. At
baseline, there were no statistical differences among the 3 brush groups for the 22 species evaluated ($P > 0.05$, Kruskal-Wallis test).

In general, most species, particularly suspected periodontal pathogens, showed decreasing trends in prevalence and levels after brushing in all groups. However, there were no significant differences in the prevalence and levels of bacteria after usage of the ultrasonic brush ($P > 0.05$, Wilcoxon test) (Fig 1). In the electric brush category, the counts of *Tannerella forsythia* decreased significantly after a month of usage ($P = 0.043$, Wilcoxon test) (Fig 2). Furthermore, in the manual brush

![Fig 3. Stacked bar chart of the prevalence and levels of subgingival species in 21 orthodontic patients. The total length of each bar indicates the percentage of sites colonized by the species. The shadings in each bar indicate the percentage of sites colonized by different levels of the species. The significance of differences in prevalence and levels of bacteria before (A) and after (B) the use of the manual toothbrush was determined with the Wilcoxon test.](image)
group, Selenomonas noxia, S sanguinis, and Prevotella melaninogenica counts also decreased significantly after a month (P = 0.01; P = 0.026; P = 0.012, respectively, Wilcoxon test) (Fig 3).

Although significant reductions were observed in the frequency of some species for the electric and manual brushes, no statistical differences were shown in intergroup comparisons for bacterial prevalence and levels among the 3 brushes (P >0.05, Kruskal-Wallis test).

DISCUSSION

In this investigation, we evaluated by the checkerboard DNA-DNA hybridization method the effect of self-performed supragingival plaque removal with ultrasonic, electric, and manual toothbrushes on subgingival plaque composition in orthodontic patients. This technique eliminates the need for culturing microorganisms and allows many samples to be screened for the presence of several species by using DNA probes in a short time. We evaluated 22 taxa including putative periodontal pathogens such as T forsythia, A actinomyctecomitans, Porphyromonas gingivalis, F nucleatum, and Treponema denticola, as well as host-compatible species normally residing in the gingival sulcus. For each patient, samples were collected from the 4 first molars, all with orthodontic bands. These sites were selected based on studies showing that banded teeth have greater changes in gingival parameters because of increased plaque accumulation.16,17 Gingival enlargement and increases in probing depth and bleeding on probing are the most common gingival alterations observed in banded teeth, and these changes tend to cause a shift to more pathogenic subgingival microbiota.8–10 Bacterial sampling also from teeth with brackets could improve the study results and should be evaluated in a future study.

The prevalence and counts of all taxa were similar at baseline, without significant differences among the brush groups. Three putative periodontopathogens, F nucleatum, P gingivalis, and T denticola were among the 10 most prevalent species. Inversely, A actinomyctecomitans was among the least prevalent species. In a previous study, the subgingival microbiota of non-banded teeth of periodontally healthy Brazilian patients was evaluated by using the same DNA-DNA checkerboard technique.18 In general, the prevalence of species evaluated in both studies was higher in that study compared with our results. The mean age of the patients and methodologic variations might have influenced these differences. However, the 4 most prevalent species in that study 18 (V parvula, S sanguinis, S oralis, and N mucosa) also had high frequencies and levels in our study. Interesting differences between both studies were observed in the prevalence of the pathogens P gingivalis, T forsythia, F nucleatum, and A actinomyctecomitans. In the previous study, the authors reported similar or higher prevalences of these pathogens compared with our study.18 However, these species were among the least prevalent organisms of a total of 41 evaluated taxa in periodontally healthy patients in that study, a condition not observed in this study. In addition, T denticola had a higher prevalence in our study. These results suggest that the bands could have induced a shift in the subgingival microbiota, favoring some anaerobic species and changing the proportions of the species in our study.

At the reevaluation time, after each toothbrush usage, decreases in prevalence and counts of most taxa were observed. Statistically significant decreases in the prevalence of T forsythia for the electric brush group and S noxia, S sanguinis, and P melaninogenica for the manual group were observed after 1 month of usage. Among these species, T forsythia belongs to the “red complex” proposed by Socransky et al19 and shows strong correlation with clinical signs of periodontal disease. Reduction of periodontal pathogens in the subgingival microbiota is a consequence of improvement in clinical plaque control. However, the clinical relevance of this isolated finding is unknown.

Some host-compatible species had slight increases in their counts in the ultrasonic (V parvula) and manual (N mucosa and P acnes) groups. Increases in host-compatible species are associated with a shift to a healthier subgingival condition.20

Although some species had significant reductions in prevalence after a specific toothbrush, we did not find a statistically significant difference among the 3 analyzed brushes. However, methodologic factors, such as the small cohort size and the standardized limited time for brushing, cannot be discarded as influencing those results, since most bacterial species were not eliminated or significantly reduced. Among the few studies that evaluated changes in the microbiota induced by different toothbrushes, only Haffajee et al2 used the checkerboard DNA-DNA hybridization method to evaluate the effect of supragingival plaque removal with either manual or conventional power toothbrushing on its composition. Periodontal maintenance patients had markedly decreased counts and prevalences of most evaluated taxa in both toothbrushing groups after 6 months without differences between brush types.

Since the 1960s, electric brushes have been modified—eg, high-frequency movements. More recently, ultrasonic toothbrushes have been introduced. Even
though the results of various studies have not shown conclusive evidence for ultrasonic brushing effectiveness, the technology might have an advantage over manual or traditional mechanical toothbrushing.

The manufacturer suggests that the sonic waves, because they are transmitted subgingivally, can remove adherent bacterial plaque and disrupt bacterial growth through fluid pressures and shear forces.

As far as we know, the only study that examined the impact of sonic brushes on microbiologic parameters in orthodontic patients was that of Ho and Niederman. Using DNA probes, they found that the sum of 6 gram-negative species (A actinomycetemcomitans, P gingivalis, P intermedia, Eikenella corrodens, F nucleatum, and C rectus) decreased in subgingival plaque samples from 12 orthodontic patients who used a sonic toothbrush (Sonicare) after 4 weeks. This result reflects the superiority of sonic toothbrushes over manual brushes on supragingival plaque control. In our study, minimal differences were observed in the plaque and gingival indexes among toothbrushing groups, probably because of lower initial index values and the ability and motivation of the patients to use their brushes.

CONCLUSIONS

It can be surmised that, although counts and prevalences of some taxa examined decreased in the 3 groups, no toothbrush demonstrated superiority, when used three times daily for 2 minutes, on microbiologic parameters in banded molars of adolescent orthodontic patients. Furthermore, more comprehensive studies with other experimental designs are needed to determine whether these results can be sustained.

REFERENCES