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Effect of electric, ultrasonic and manual toothbrushes on biofilm removal and gingivitis control: *in vitro* and parallel randomized controlled clinical trial study

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Aim: To evaluate the effect of manual (M), electric (E) and ultrasonic (US) toothbrushes on the removal of oral biofilm and control of gingivitis. Also, the roughness and tooth wear production were evaluated in vitro. **Methods:** For the *in vitro* analyses, thirty bovine dentin specimens were submitted to a 3-month brushing simulation (9 minutes) with the three types of toothbrushes (n = 10). Subsequently, a randomized controlled clinical trial was performed with 36 patients divided into 3 groups according to the toothbrushes used (n = 12). Gingival index, visible plaque index and the volume of crevicular fluid were evaluated at baseline and 3 months after the beginning of the toothbrush use. Furthermore, the performance of the biofilm removal per brushing cycle of 1 and 3 minutes with each toothbrush was made monthly until the end of the experiment. **Results:** The US group had the highest dentin wear. Clinically, the US group had a lower plaque index at 3 months than the M group. The M group also showed less biofilm removal efficiency from the second month of follow-up and more worn bristles at the end of the 3 month period than the E and US groups. **Conclusion:** The ultrasonic, electric and manual toothbrushes showed no differences in gingivitis control in the present study. The ultrasonic and electric toothbrushes had a more significant effect on biofilm removal than a manual toothbrush, but the ultrasonic toothbrush promoted greater dentin tissue wear.

Key Words: Gingivitis. Oral hygiene. Toothbrushing.

Introduction

The dental biofilm represents a true complex biofilm that forms on the non-descamative surface of the teeth[1]. It is considered the major etiological factor of the most prevalent human oral diseases: dental caries and periodontal disease. The periodic removal of dental biofilm plays a key role in the prevention of these diseases[2]. As such, personal daily oral hygiene by brushing and using other hygiene aids is an accessible, effective, and economical method to maintain oral health[3]. It has been extensively demonstrated in clinical trials conducted in different geographical regions that the effective removal of dental biofilm is essential to maintain dental and periodontal health[4]. There are currently several toothbrush options on the market to increase motivation and facilitate brushing techniques, such as electric and ultrasonic brushes that have emerged as an alternative to conventional ones[5].

The ideal brushing technique is the one that allows for complete plaque removal in a shorter time, without causing any damage to tissues. Also, a safe toothbrush should not contribute to the formation of gingival recession and excessive tooth wear, which may lead to the formation of non-carious cervical lesions[6]. In this context, a comparison of the safety of manual and electric toothbrushes has been little explored.

Systematic reviews[7,8] studies have demonstrated the superiority of the electric and ultrasonic brushes in biofilm removal and gingivitis control when compared to manual brushes. However, other studies did not confirm these results[9]. Besides, the degree of heterogeneity of included studies in the systematic reviews makes these results have only moderate evidence, which indicates the need for further studies.

Therefore, the objective of this study was to compare the efficiency of manual (M), electric (E), and ultrasonic (US) toothbrushes in the control of dental biofilm and gingival inflammation after 3 months of use. Also, an *in vitro* analysis evaluated the effect of these toothbrushes on the wear and roughness of dentin samples after a 3 month-brushing simulation test. The study hypothesis is that the US and E brushes have a greater effect on biofilm removal and gingivitis control and induce less wear and roughness on dentin samples than manual brushes.

Material and methods

In vitro

Preparation of the samples

Thirty intact bovine incisor teeth were selected, cleaned with the aid of McCall curettes (nº 13/14, Golgran, São Paulo, Brazil) to remove the remaining periodontal tissues and immersed in physiological saline until the samples preparation. The sectioning of the teeth was performed using a diamond disk mounted in a low rotation driller. Two cuts were made: one transversally, for the exclusion of the crown, and one longitudinally, dividing the root into two equal parts. The samples were planned in a polishing machine to obtain 60 samples with 10x4 mm^2 containing only dentin.

With the aid of cylindrical diamond drills mounted in a high rotation driller, a groove was made in the center of each sample dividing the sample into two areas. One of the

areas was enveloped by insulating tape, thus constituting the control region, which was not subjected to the brushing simulation. The other region was exposed to the brushing simulation procedure[10]. The samples were then placed in a metal matrix prepared for this study and embedded in self-polymerizable acrylic resin (VIP Cril, Pirassununga, São Paulo, Brazil). A random distribution of these specimens was performed in three Experimental Groups (n=10/group), according to the toothbrush used for the brushing simulation: 1) Ultrasonic toothbrush (US) (Ultrasonex Ultima Toothbrush®, Sonex International Corp, Brewster, New York, EUA); 2) Electric toothbrush (E) (Braun Oral B 3D Plaque Remover, Braun GmbH, Kronberg, Alemanha); 3) Manual toothbrush (M) (Oral B Model 30, Gilette do Brasil, Manaus, Brazil). The same dentifrice was used for all groups during the simulation of brushing (Colgate Anticarie, Colgate do Brasil, São Bernardo do Campo, SP, Brazil).

Brushing simulation

The specimens of the M group were submitted to the brushing simulation test in a brushing machine designed in the Department of Prosthesis from the School of Dentistry at Araraquara - Unesp (Araraquara, São Paulo, Brazil). In this device, the samples were arranged in horizontally framed metal bases, which provided, in addition to the fixation, the immersion of the specimens in distilled water or distilled water/dentifrice solutions in a ratio of 1: 1. The active part of the toothbrushes was fixed in metallic arms, which made horizontal movements of constant amplitude over the sample. The simulation of three months of brushing was obtained with 300 cycles, with a vertical force of 200N and frequency of 8rpm[11].

For the simulation of brushing with the electric and ultrasonic brushes, a previously described[12] device was adapted. The heads of these brushes were positioned directly under the specimens with a constant force of 200N. With a metal rod's aid, the specimens were fixed and raised slightly to allow maximum contact with the brushes. The samples were brushed for 9 minutes to simulate 3 months of brushing, considering patients with an average of 20 teeth and the use 2 minutes per brushing section: 120/20=6 seconds per tooth. Six seconds x 2 times a day x 90 days=18 minutes. As only one face was brushed, the brushing time was approximately 9 min.

Roughness analysis

The surface roughness measurement (Ra) was recorded on each sample's surface with the aid of a profilometer (Surftest SJ-401, Mitutoyo Sul Americana Ltda, Santo Amaro, SP) with an accuracy of 0.01mm. Three readings were performed per region of each specimen, at distinct locations within a predetermined area and similar for all specimens. For each reading, the needle of the device scanned 1.5 mm always in a single direction with a cutoff of 0.8 mm. After registering the roughness values, an average of the three readings of each area of the sample was determined.

Analysis of the tooth wear

The specimens were decalcified in Morse solution (formic acid and sodium citrate) for 30 days, followed by paraffin embedment. Serial 5 µm-thick histological sections were obtained and stained by Hematoxylin-Eosin. The relative wear of the experimen-

tal surfaces was analyzed in 3 equidistant sections (72µm) about a control surface[10]. Histological images with 25X magnification were obtained using an optical microscope (Leica-Reichert Diastar Products & Jung, Wetzlar, Germany). Image J software (Image J, Jandel Scientific, San Rafael, USA) was used for image analysis performed by a blinded and trained examiner (IJP).

In Vivo

Patient Selection

Thirty-six patients participated in this randomized clinical trial, after having read and signed the term of Free and Informed Consent, approved by the Human Ethics Committee of the School of Dentistry at Araraquara – UNESP (CEP. 03/11) and registered at the Brazilian clinical trials records (U1111-1204-1231). This study was performed following the Helsinki Declaration of 1975, revised in 2015. The inclusion criteria for this study were: 1) Presence of at least 20 teeth in the mouth; 2) Clinical diagnosis of biofilm-induced gingivitis[13]; 3) Presence of marginal bleeding and visible dental biofilm in more than 50% of the sites, 4)Probing pocket depth (PPD) ≤ 3mm, 5) 10% or more of sites with bleeding on probing and 6) absence of loss of attachment. After this evaluation, the patients were not included in the study if they had the following exclusion criteria: 1) Periodontal treatment in the last 12 months; 2) Systemic diseases that may affect the treatment outcome; (3) Pregnancy; 4) Use of systemic antibiotics in the last 6 months; 5) Use of anti-inflammatory drugs in the last 3 months; 6) Smokers and ex-smokers; 7) Use of oral contraceptives; 8) Patients with orthodontic appliances; 9) Patients with total dentures, partial removable or fixed prosthesis with more than 2 elements and implant prosthesis.

Sample size calculation

The sample size calculation was based on data published in a previous clinical study comparing mechanical plaque control in patients using manual and ultrasonic brushes[14]. A relevant clinical difference was determined for the reduction in the variable plaque index before and after treatments of 0.5 with a standard deviation of 0.35. Therefore, the standardized difference at 0.85 ($1-\beta = 0.85$) and $\alpha = 0.05$ determined a sample size of at least 12 patients to perform the mechanical control with each brush.

Study design

This study was a randomized, single-blind, controlled clinical trial with a parallel model in which each patient used one type of toothbrush. The 36 patients were randomly divided into 3 groups according to the type of toothbrush used for 3 months: 1) Ultrasonic toothbrush (US); 2) Electric toothbrush (E); 3) Manual toothbrush (M). During that period, patients were oriented to perform only mechanical oral hygiene with the selected toothbrush, avoiding other mechanical or chemical plaque control methods. Patients receive guidance on adequate oral hygiene using the Modified Bass brushing technique[15]. At each return appointment, the brushing instructions were redone. Besides, patients were asked about adverse effects of the use of toothbrushes, such as sensitivity, discomfort, or pain. The flow of the study is depicted in Figure 1. The same dentifrice was used for all patients during all the study period (Colgate Anticarie, Colgate do Brasil, São Bernardo do Campo, SP, Brazil). The demographic data of the patients are exposed in Table 1.

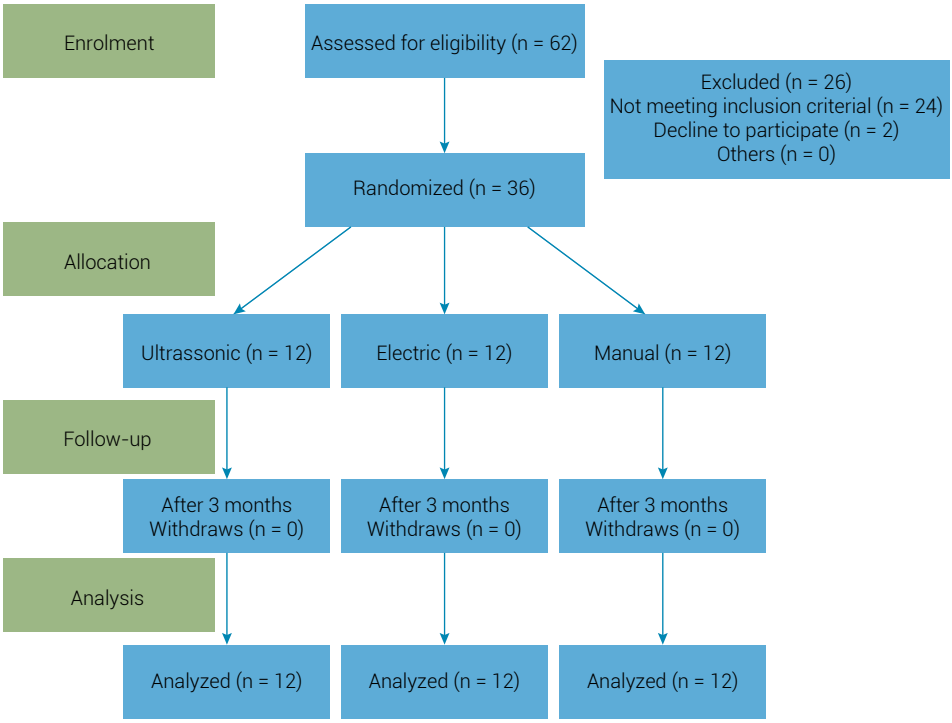

Figure 1. Study Flowchart

Table 1. Demographic characteristics of the patients at baseline.

Parameter/Groups	Ultrasonic (n = 12)	Electric (n = 12)	Manual (n = 12)
Age(y)	38.83 ± 12.40	35.58 ± 13.51	36.08 ± 12.75
Females (n)	8	7	6
Males (n)	4	5	6
Teeth	27.00 ± 3.27	27.42 ± 2.19	27.33 ± 3.33

Clinical analysis

The patients were analyzed at baseline and after 3 months for the following clinical parameters: 1) Gingival Index (GI)[16]; 2) Visible Plaque Index[16]. These clinical parameters were analyzed by a blinded, trained, and calibrated examiner (TC). In addition, the following parameters were analyzed at baseline for patient selection: 1) Marginal gingival bleeding; 2) Probing depth- Measured from the gingival margin to the bottom of the gingival sulcus, 3) Gingival margin level- Measured from the Cement-enamel junction to the gingival margin; 4) Clinical attachment level – Measured from the Cement-enamel junction to the bottom of the gingival sulcus.

Analysis of biofilm removal efficiency per brushing cycle

After the clinical analysis at the baseline, the patients were instructed about using the different toothbrushes. The bacterial biofilm was stained, and each patient underwent dental brushing for 1 and 3 minutes. During these periods, the Quingley & Hein modification of the Turesky plaque index[17] was evaluated to analyze plaque removal efficiency

in each brushing cycle. This analysis was repeated at 1, 2, and 3 months after the baseline. These exams were performed by a blind, trained, and calibrated examiner (TC).

Analysis of the volume of the crevicular fluid

To collect crevicular fluid, a strip of paper - periopaper (PerioPaper - Oralflow Inc. New York - USA) was introduced to the base of the gingival sulcus at the mesiobuccal site of the first molars until some resistance was felt, remaining in place for 30 seconds. Soon after, the crevicular fluid volume was measured through the Periotron 6000 equipment (Oraflow Inc. New York-USA). In the absence of a first molar, the sample was collected at the mesiobuccal site of the 2nd premolar at the same quadrant. This analysis was performed by a blinded to group allocation and trained examiner (TC) at baseline and after 3 months.

Analysis of the toothbrushes bristles

In the baseline period and 3 months after being used by the patients, the toothbrushes were evaluated for bristle wear and their tips' morphology. To analyze the wear of the bristles, five measurements were recorded for each brush using a digital caliper (Series 500-144B, Mitutoyo, Suzano, Brazil), according to the methodology used by Rawls et al. 1989[18]: FLL (Free-long- length): corresponds to the length of the brush head at the top of the larger side; BLL (Base-long- length): corresponds to the length of the brush head at the bottom (base) of the larger side; FFL (Front free length): corresponds to the length of the brush head measured at the top of the smaller side; BFL (Base free length): corresponds to the length of the brush head measured at the bottom (base) of the smaller side and BRL (Bristles length): a measure of the height of the bristles. The wear index was calculated using the formula: WI = FLL-BLL + FFL-BFL / BRL[18].

For the analysis of the bristle tips' morphology, four images with 20x magnification were reproduced from each brush using an optical microscope (Leica Microsystems, Wetzlar, Germany). Two of the images were taken from the top view to evaluate the central bristles, and two images were made in lateral view to evaluate lateral bristles. All the images were captured in aleatory fields. A blinded and trained examiner evaluated the images twice in different moments using the index proposed by Silverstone and Featherstone[19] (1988) (Figure 2). The analysis was performed by a blinded, trained, and calibrated examiner (EF).

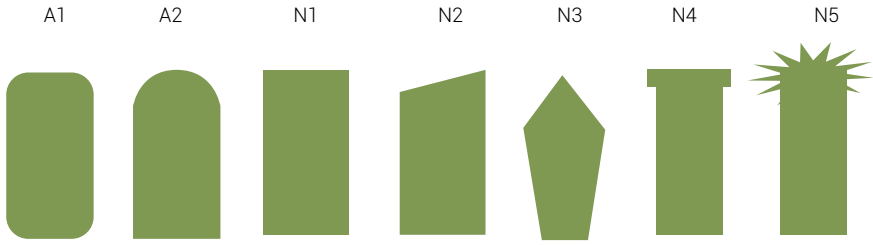

Figure 2. Classification of bristle tip morphology. Group A represents the acceptable rounding of the tip of the bristles, and group N represents the non-acceptable rounding of bristles tips.

Statistical analysis

Data on tooth wear and roughness, clinical parameters, and brush bristles wear were submitted to the Shapiro-Wilk normality test. Data from tooth wear and dentin rough-

ness presented normal distribution, and the parametric test one-way Anova complemented by the Tukey's test was applied for inferential data analysis.

Data from clinical analysis and the bristle wear were performed using the non-parametric tests of Kruskall-Wallis supplemented by the Dunn for the comparison between groups in the same evaluation period. The comparison within each group at the baseline and 3-month periods was performed using the Wilcoxon test. Besides, the analysis within each group in the analysis of efficiency of removal of bacterial biofilm in different brushing cycles times (baseline, 1 minute and 3 minutes) was performed by the Friedman test complemented by the Dunn test. Data from the analysis of the morphology of the bristle tips were analyzed using the chi-square test. All tests of this study were applied using the software GraphPad Prism 6 (San Diego, CA, USA) at a significance level of 5%.

Results

In vitro

The in vitro analysis presented no differences among the groups concerning the degree of roughness obtained after the brushing simulation. However, it was observed that the US group presented higher dentin wear than the E and M groups (Figure 3).

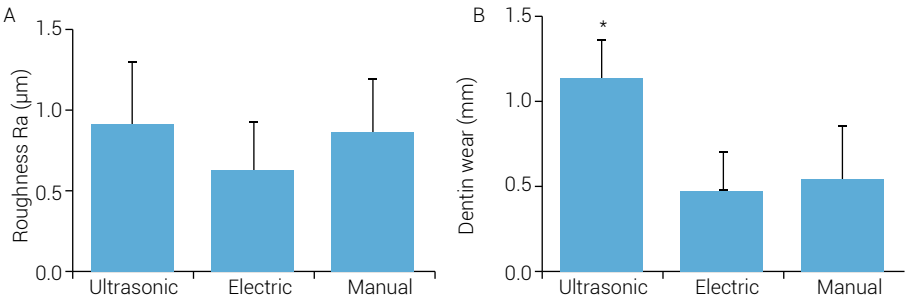

Figure 3. Representative graphs of the dentin A) roughness and B) wear data. It´s possible to note that the US presented higher dentin wear than the other groups. *p<0.05-Higher dentin wear than the other groups- One-way Anova complemented by Tukey.

Clinical Trial

There were no differences ($p > 0.05$) among the groups regarding the demographic data (Table 1). No patient in this study had clinical attachment loss at baseline. None of the patients mentioned adverse effects. US and E groups had a reduction in the plaque index levels at 3 months, a fact not observed in the M group. In the intergroups comparison, the US group presented lower plaque index values than the M group at the end of the experiment (Figure 4A). Regarding gingival inflammation analysis, the E and M groups presented a reduction in the gingival index at 3 months, a fact not observed in the US group (Figure 4B). However, no differences were identified between the groups at the end of the experiment about the gingival index (Figure 3B) and in the volume of the crevicular gingival fluid (Figure 4C). No clinical attachment level loss of enhance in probing depth was detected in this study.

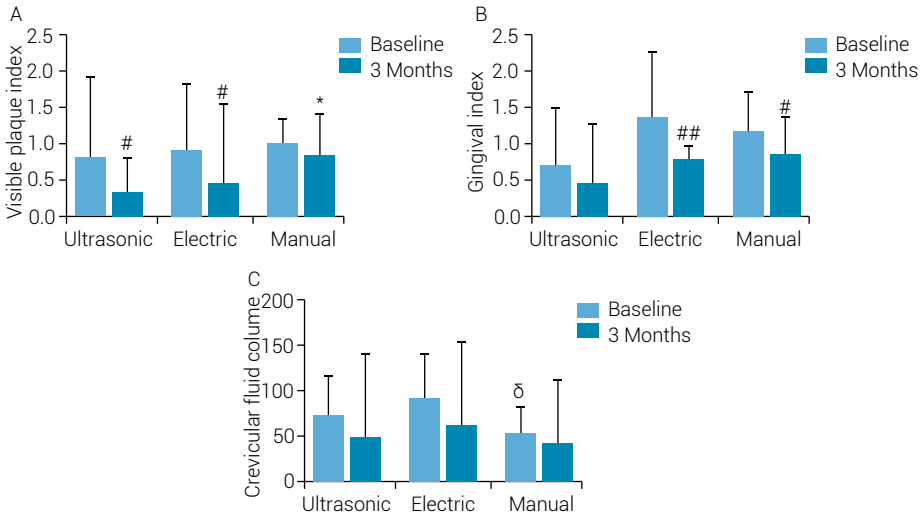

Figure 4. Representative graphs of the clinical analysis of the A) visible plaque index, B) gingival index, and C) crevicular fluid volume evaluated at the baseline and after 3 months of the toothbrushes use. *p<0.05-Higher visible plaque index than the US group at 3 months-Kruskall-Wallis complemented by Dunn; #p < 0.05; ##p < 0.01- Lower visible plaque index and gingival index than at baseline-Wilcoxon; δp < 0.05- Less crevicular fluid volume than the E group at baseline-Kruskall-Wallis complemented by Dunn.

The analysis of the brushing cycle performed monthly showed statistically significant removal of biofilm after 3 minutes of brushing in all groups. However, in the second month, the M group presented less biofilm removal after 1 minute of brushing than the US group. In the third month, less removal of biofilm than the E group at1 and 3 minutes of brushing (Figure 5).

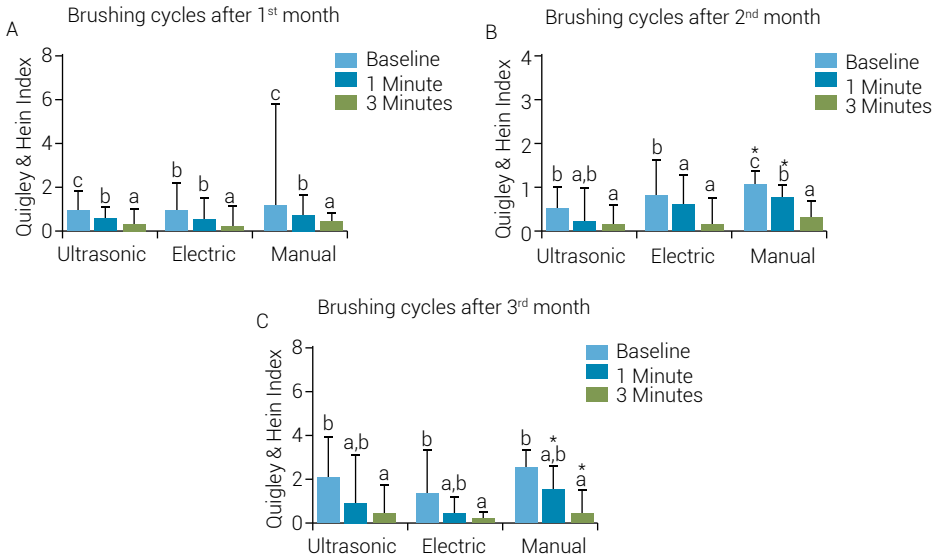

Figure 5. Representative graphs of the effect of plaque removal after the brushing cycles of 1 and 3 minutes performed at the A)1st month, B) 2nd month, and C)3rd month after the toothbrushes use. Different letters represent statistical differences within each group-Friedman complemented by Dunn; *p<0.05-Higher plaque index than the US group at the 2nd month-Kruskall-Wallis complemented by Dunn; #p<0.05-Higher plaque index than the E group at the 3rd month-Kruskall-Wallis complemented by Dunn.

The M and US toothbrushes were more worn at 3 months than in the baseline time-point. Also, the M toothbrushes presented more worn bristles than the other groups at 3 months. There were no differences between the groups concerning the bristle tips' morphology at the baseline and after 3 months of brushing (Figure 6). The bristle tip morphology showed an improvement in its pattern after the 3 months of use in the M and US groups (Figure 6B).

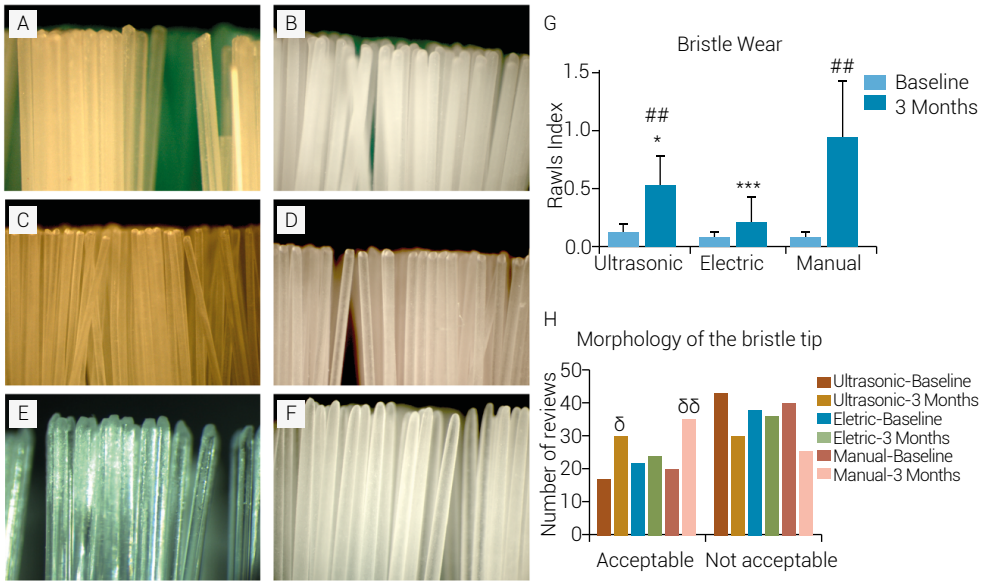

Figure 6. Figures A-F show the representative images of the tip of the bristles of the different types of toothbrushes used in this study. The images A, C, and E represent the conditions of the tip of the bristles of the US, E and M toothbrushes before the use, respectively. The images B, D and F represent the conditions of the tip of the bristles of the US, E and M toothbrushes after 3 months of use, respectively. Representative graphs of the G) bristle wear index and H) morphology of bristle tips index. The M group presented more worn bristles than the US and E groups after the 3 months of use. *p<0.05; ***p<0.001- Less bristle wear than the M group at 3 months-Kruskall-Wallis complemented by Dunn ##p<0.01-More bristle wear than the baseline period-Wilcoxon; δp<0.05; δδp<0.01- Morphological pattern more acceptable than the baseline period- Chi-square test.

Discussion

In the present study, *in vitro* and clinical analyses evaluated the performance of different toothbrushes in the removal of dental biofilm and the control of gingival inflammation in patients with gingivitis. The results showed that US and E toothbrushes had a greater effect on biofilm removal than the manual toothbrush. However, this observation was not followed by a significant reduction in gingival inflammation. Clinically, the three evaluated toothbrushes showed no differences between them in gingivitis control in the investigated period. This fact was demonstrated by the non-significance in the GI and the crevicular fluid volume analysis studied. Although the removal of the biofilm evaluated by the plaque index has shown a superior statistical result for the US compared to the M, these differences were not sufficient to result in a clinical improvement in gingival inflammation, as also observed in previous studies[20,21]. The greater variability of the results in the US group may explain, at least in part, the improvement in the results of gingivitis parameters without significant difference.

Regarding the plaque index, the present results corroborate with previous studies. A possible explanation for this result is that the ultrasonic waves could remove adhered bacteria and induce cell surface alterations, affecting biofilm attachment[22]. However, this is not a consensus in the literature since other short-term studies did not observe a significant difference between those toothbrushes[23]. In addition, it has been suggested that long-term studies provide a more accurate evaluation of the effect of brushing. This fact may explain why no differences were found in the studies mentioned above[24] for plaque index and why we did not find differences among brushes in gingivitis control.

Another possible reason for this contrast is the Hawthorne effect[23], induced by the monthly appointments during the study period. In this kind of studies generally, we have a patient's positive contribution. In other words, patients pay more attention to their oral hygiene when they know that this will be evaluated[25]. Patients may have improved their brushing only previously to their visit to the clinic, which affected the biofilm index level but not the inflammatory parameters.

The advantage of powered toothbrushes in removing dental biofilm was confirmed by the brush cycles analysis from the second month of follow up. The toothbrushes of the US and E groups also showed less bristle wear than the toothbrushes of the M group. *In vivo*[26] and *in vitro*[27] studies showed that worn toothbrushes lose their effect on biofilm removal, so it is recommended that the toothbrush should be replaced whenever any signs of bristle wear are identified. However, the literature has shown no statistically significant differences in biofilm removal between used and new toothbrushes[28]. These studies indicate that other factors, such as brushing time, brushing strength, and patient motivation, are as important as the bristles' integrity for oral hygiene performance[29]. Another aspect of powered toothbrushes is their greater cost in comparison to the manual toothbrush[30].

Another effect observed in this kind of study is the Novelty effect, which hinders the effect of mechanical devices for plaque control[22]. This effect relates to the fact that a new brush attracts more attention while it is a novelty, resulting in more collaboration from the patient in controlling plaque[22]. In our study, it can be suggested that patients from the US and E groups became more susceptible to the Novelty effect than patients from the M group, affecting the patient motivation and the results of the study.

Besides, to analyze the effect on the removal of dental biofilm, another important aspect to be analyzed in toothbrushes is their risk of causing dental wear and gingival recession. According to a recent literature review, the factors most associated with oral injuries caused by brushing are the tooth brushing frequency, a horizontal or scrub tooth brushing method, bristle hardness, brushing duration, the morphology of the bristle tip, and the frequency of changing a toothbrush. The principal tooth brushing factors associated with non-carious cervical lesions were tooth brushing method and frequency[6]. Despite the greater dentin wear caused by the US toothbrushes in the present study, they were not associated with gingival recession, as confirmed by the literature[31]. It has been reported that US toothbrushes users apply less lateral force during the brushing procedures, which may explain the absence of side effects during the use of those toothbrushes, observed in this and other studies[32].

The different groups of toothbrushes presented similar morphology of bristle tips before the begging of the study. The majority of the bristles presented an inappropriate morphology, which reveals that this parameter does not present an adequate pattern in the studied brushes, as demonstrated in the literature[33]. The inadequate morphology of bristle tips has been related to the possibility of causing gingival lesions, which may induce gingival recessions[34]. The short-term follow-up of this study cannot provide if the use of these types of toothbrushes may induce a gingival recession, and long-term periods of evaluation will be necessary to test this hypothesis. Another important finding was that the morphology of the bristle tips in the M and US groups became more acceptable after 3 months of use. One study demonstrated that toothbrushes with hard bristles, with greater strength, improved in the morphological pattern of the tips of the bristles in comparison to toothbrushes with soft and extra-soft bristles in a 2-year brushing simulation[35]. Likely, the improvement observed in the morphological pattern of bristles tip of toothbrushes with hard bristles should be observed at earlier periods of use of toothbrushes with soft bristles. Perhaps this explains the findings of this study and the non-observance of gingival lesions induced by brushing.

An advantage of this study was that the monitoring during the brushing cycles provided a more realistic analysis of the potential for biofilm removal of each toothbrush since the supervision induces the maximum effect of the patient to perform oral hygiene[14]. Therefore, the differences found between the toothbrushes in this analysis were more related to their cleaning potential than to the patients' motivation. However, this study does not mimic what happens during the patients' daily oral hygiene practices, which may not reproduce the good clinical outcomes verified in this study. Besides, the toothbrushes here evaluated may have different ideal brushing times. Another aspect that limits the extrapolation of this study's findings was that the patients returned every month for the recall during three months, which is not a standard maintenance protocol for patients with gingivitis, usually called at longer intervals.

In conclusion, the ultrasonic, electric and manual toothbrushes showed no differences between them in gingivitis control in the present study. The ultrasonic toothbrush had a greater effect on biofilm removal than a manual toothbrush and promoted greater dentin tissue wear. Besides, the manual toothbrush presented greater bristle wear compared to the other toothbrushes.

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