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Effect of sonic vibration of an ultrasonic toothbrush on the removal of *Streptococcus mutans* biofilm from enamel surface

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Abstract: Purpose: To evaluate in vitro the effect of sonic vibration of an ultrasonic toothbrush in the removal of *Streptococcus mutans* (*S. mutans*) biofilm from human enamel. **Methods:** *S. mutans* dental biofilm was formed in vitro on human enamel blocks coated by salivary pellicle. The blocks were incubated with a suspension of *S. mutans* at 37ºC for 24 or 72 hours. The blocks were divided to one of three conditions according to the different toothbrush action modes: ultrasound plus sonic vibration (U+SV), ultrasound-only (U) and no ultrasound and no sonic vibration (control). Samples were exposed to each mode for 3 minutes with the toothbrush bristles placed 5 mm away from the enamel block surface. The samples were observed by scanning electron microscopy (SEM) and quantification of *S. mutans* was performed. **Results:** U+SV showed lower bacterial counts compared to U and control on the 72 hour-biofilm ($P < 0.05$). The SEM analysis revealed that U+SV and U disrupted the *S. mutans* chains in the 24- and 72-hour biofilm. (*Am J Dent* 2015;28:347-350).

Clinical significance: Sonic vibration improved the effect of an ultrasonic toothbrush in reducing bacterial viability and in disrupting *S. mutans* biofilm in this in vitro model. The ultrasonic toothbrush plus sonic vibration may be an innovative alternative for the removal of dental biofilm.

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Introduction

For the manifestation of caries, it is essential that the tooth surface be covered with biofilm. For dental biofilm to be considered cariogenic, it must contain cariogenic microorganisms. Of the species implicated in dental caries, a large body of epidemiologic evidence links *Streptococcus mutans* to the initiation of dental caries.[1,2] These are acidogenic bacteria, that besides having the ability to produce extracellular polysaccharides that form the structure of the biofilm, have intracellular polysaccharides which are used as an energy source when sugar is no longer obtained through diet.[3] Several authors suggest that the risk of developing caries is directly proportional to the increase in the number of *Streptococcus mutans*.[4-6]

For there to be success in anti-caries therapy, it is essential to control dental biofilm. The mechanical method is often viewed as the best alternative to achieve this goal,[7] although many patients have difficulty with achieving correct mechanical control of this process.[8] This problem arises mainly in areas of difficult access, such as in the interproximal regions and around prostheses, where toothbrush bristles cannot reach. Thus, alternative methods to control dental biofilm are currently being studied.[9]

In order to facilitate and/or supplement the mechanical removal of biofilm, electric toothbrushes that use acoustic energy are becoming increasingly popular in the market, offering an alternative to remove biofilm when manual toothbrushes cannot be used. Taking this idea one step further, one such alternative is the ultrasonic toothbrush, which aims to offer an alternative method of biofilm removal and/or act as an adjunct to mechanical biofilm removal.

The ultrasonic toothbrush, in liquid media, causes two effects: cavitation and microstreaming. Cavitation is the effect of bubble formation caused by ultrasound. This phenomenon occurs because ultrasound is a form of sound, which means that it consists of acoustic pressure waves. Microstreaming is another phenomenon caused by ultrasound, which refers to the unidirectional movement that occurs in fluids due to the growth and retraction of cavitation bubbles during cycles of compression and rarefaction.[10-12] The interaction of these effects (cavitation and microstreaming) would suggest that the ultrasonic toothbrush may be able to remove biofilm, even without contact with the tooth surface, in an effective manner.

The ultrasonic toothbrush could be an effective and safe alternative for the control of biofilm in patients who for any given reason have difficulty in using or controlling these conventional methods. In 1992, the US FDA approved the first ultrasonic toothbrush for daily home use with a frequency of 1.6 MHz.[13]

In addition to offering ultrasound, the particular brush used in this study augments this mode by introducing mechanical action of the bristles at sonic frequencies, comparable to those in traditional sonic toothbrushes. Thus, this study evaluated in vitro the effect of sonic vibration on the functionality of the ultrasonic toothbrush in the removal of *Streptococcus mutans* biofilm.

Materials and Methods

Enamel blocks and S. mutans biofilm preparation - Seventy-eight enamel blocks (3×3×2 mm) were prepared from human molar teeth, being sterilized through storage in 2% formaldehyde solution, pH 7.0, for at least 1 month.[14] Of these, 60 were used for microbiological analysis and 18 for analysis by scanning electron microscopy. This study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (protocol number 18678) and the teeth used in this study were donated willingly by patients who had third molars extracted. All donors signed an informed consent form.

Table 1. Bacterial viable counts (log_{10} CFU) of 24 hours *Streptococcus mutans* biofilm after different treatments (n = 10).

	Percentiles		
Treatment	Median	25	75
Control	4.98	4.70	5.37
Ultrasound-only	5.00	4.33	5.18
Ultrasound plus sonic vibration	4.44	3.53	5.13

Kruskall-Wallis test (P= 0.179).

Table 2. Bacterial viable counts (log_{10} CFU) of 72 hours *Streptococcus mutans* biofilm after different treatments (n = 10).

	Percentiles		
Treatment	Median	25	75
Control	8.07[a]	7.84	8.27
Ultrasound-only	7.84[ab]	7.19	7.97
Ultrasound plus sonic vibration	7.38[bc]	6.67	7.90

Medians followed by different letters differ significantly, Kruskall-Wallis test followed by Dunn test with Bonferroni adjustment (P< 0.05).

The enamel blocks were immersed in a salivary solution to form salivary pellicle on the surfaces and to create a suitable environment for the development of biofilm.[15] *Streptococcus mutans* (UA 0159) was grown in Brain Heart Infusion[a] (BHI) with 1% sucrose, for 12 hours at 37°C. This bacterial suspension was transferred to BHI with 5% sucrose and the microbial concentration was adjusted to an optical density of 0.6 at 550 nm corresponding to 1.0×10^8 CFU/mL. Enamel blocks attached onto sterile stainless wires were then incubated with that suspension containing *Streptococcus mutans* at 37°C for 24 or 72 hours, to promote the formation of a young biofilm and a mature biofilm, respectively.

Experiment with ultrasonic toothbrush - An ultrasonic toothbrush (Megasonex[b]) was used in the present study. This toothbrush employs a 3×17 mm piezoceramic crystal, located under the brush head which emits ultrasound at a frequency of 1.6 MHz. It has several modes that can be selected by the user. For this experiment, a pure ultrasound mode with no sonic vibration was compared to a mode with the same ultrasound continuously and simultaneously accompanied by sonic vibration at 150 Hz (18,000 movements per minute). The control was the same toothbrush with both vibration and ultrasound turned off (off mode). Each biofilm sample was fixed onto a holder where the biofilm on the enamel surface was situated 5 mm from the tips of the ultrasonic toothbrush bristles. This apparatus was then submerged into a sterile saline solution. Each sample was then exposed to the toothbrush for 3 minutes (following the manufacturer's instructions), with an identical setup for each condition (the ultrasound only mode, ultrasound plus sonic vibration mode and the control condition without ultrasound or sonic vibration).

Streptococcus mutans count - Saline solution samples were serially diluted in sterile saline, and 25 µl samples from each dilution were placed in duplicate in Mitis-Salivarius bacitracin agar[c] for the quantification of *Streptococcus mutans*. Cultures were incubated for 48 hours at 37°C under microaerophilic conditions. *Streptococcus mutans* were identified by morphological criteria, and microbia l counts were expressed as colony-forming units per milliliter of solution (CFU/mL). All counts were performed by the same blinded investigator.

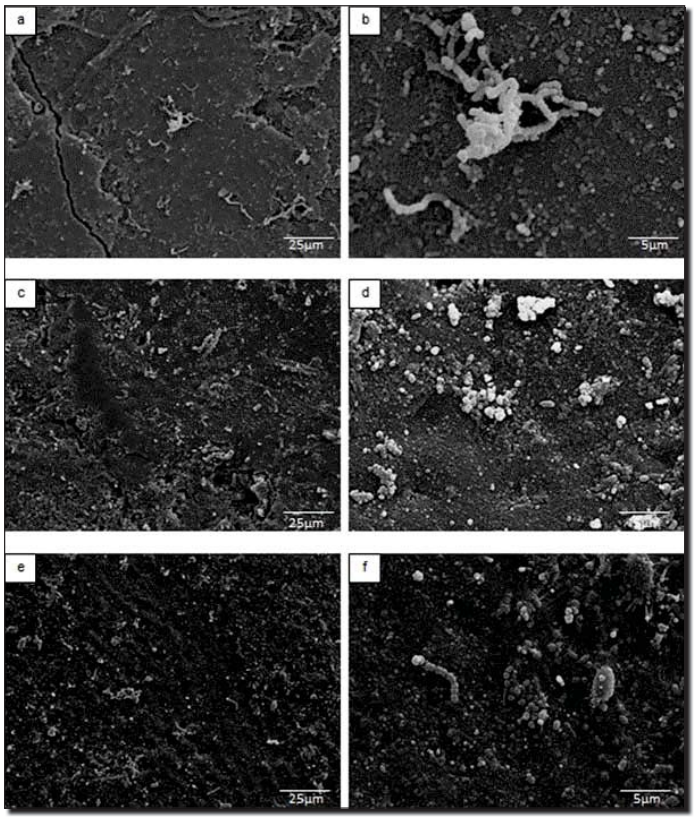

Fig. 1. Scanning electron micrographs of 24 hours *Streptococcus mutans* biofilm formed on enamel surface after different treatments with ultrasonic toothbrush: control (a,b), ultrasound-only (c,d) and ultrasound plus sonic vibration (e,f).

Statistical analysis - After treatments each sample was analyzed following one of two methods: bacterial counts or scanning electron microscopy. Prior to statistical analysis, the bacterial counts were transformed to log_{10}. A Kruskal-Wallis test followed by Dunn test with Bonferroni adjustment was performed on these log transformed bacterial counts at a significance level of 5%. Statistical analysis was performed with SPSS[d] version 17.0 for Windows. For the scanning electron microscopic results a descriptive analysis was performed.

Results

The results obtained for the counts for 24-hour *Streptococcus mutans* biofilm are shown in Table 1 and for 72 hours in Table 2. No significant differences among the three conditions were observed for the microbial counts of the samples where biofilm was grown for 24 hours (P> 0.05) (Table 1). However when comparing the microbial counts in the biofilm specimens grown for 72 hours (Table 2), the control differed significantly from the condition with ultrasound plus sonic vibration (P< 0.05). No significant difference was observed between the control and the ultrasound-only condition, or between the ultrasound-only condition and the ultrasound plus sonic vibration condition for these samples.

Figures 1 and 2 show scanning electron micrographs of the biofilms grown for 24 and 72 hours, respectively, after being exposed to each of the three different modalities of the ultrasonic toothbrush. In the 24 hours biofilm, the micrographs

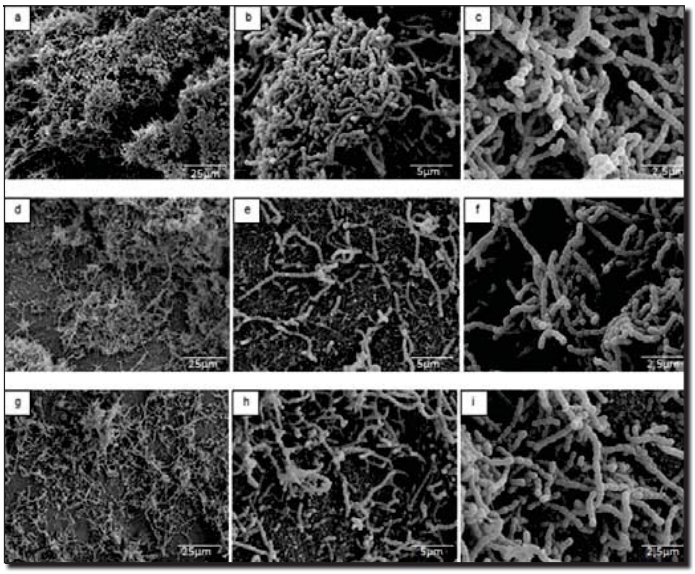

Fig. 2. Scanning electron micrographs of 72 hours *Streptococcus mutans* biofilm formed on enamel surface after different treatments with ultrasonic toothbrush: control (a-c), ultrasound-only (d-f) and ultrasound plus sonic vibration (g-i).

showed different aspects for the treatments. In the control condition, long chains of *Streptococcus mutans* formation was observed (Fig. 1 a,b). While in the other modes of the ultrasonic toothbrush (ultrasound-only and ultrasound plus sonic vibration), different structural aspects were observed where bacteria were dispersed on the enamel surface, resulting in shorter chains (Fig.1 c-f). Images of the 72-hour biofilm also showed different characteristics where the samples from the control condition had a biofilm that was thick and more structured with long chains of *Streptococcus mutans* (Fig. 2 a-c). While the images of samples from ultrasound-only and ultrasound plus sonic vibration conditions showed disruptions of these chains and when observed under higher magnifications these chains appear to be more dispersed across the enamel surface (Fig. 2 d-i).

Discussion

The present study aimed to demonstrate in vitro the synergistic effect of sonic vibration on ultrasound, as compared to pure ultrasound, as emitted by an ultrasonic toothbrush, on a *Streptococcus mutans* biofilm.

This in vitro study showed that the mode with ultrasound plus sonic vibration showed the best results, when compared to the other conditions, in a *Streptococcus mutans* biofilm. These results agree with the study of Roberts et al,[16] where an electric toothbrush with ultrasound and vibration also showed better in vitro results than the same toothbrush with sonic vibration only, a dedicated sonic toothbrush and a dedicated rotary toothbrush alike, in removing *Streptococcus mutans* biofilm. In that study, however, the authors did not include a condition with an ultrasonic toothbrush that was operated in the ultrasound mode alone. Therefore the present study is the first study showing that the best result achieved by a toothbrush with ultrasound plus sonic vibration mode may be due to the synergistic effect of sonic and ultrasonic energy instead of ultrasound alone.

To verify the effect of ultrasound emitted by an ultrasonic toothbrush in isolation (without any accompanying sonic vibration), this study included a condition with ultrasound alone. The study failed to demonstrate that ultrasound by itself would have a significant effect on decreasing the total number of bacteria, both in a biofilm grown for 24 hours and one grown for 72 hours. However, in micrographs of the biofilm grown for 24 hours, in the groups treated with ultrasound alone, bacteria were dispersed on the surface of enamel or formed short chains. In the micrographs of samples with biofilm grown for 72 hours, it was observed that for the groups treated with ultrasound alone, the chains of *Streptococcus mutans* showed greater disruption and these bacteria were also scattered over the surface of the enamel. This may indicate that despite the fact that ultrasound alone does not decrease the number of bacteria in a biofilm, it has the ability to disrupt this biofilm, which indicates that it may act as an adjuvant that can increase the efficacy of a toothbrush used for mechanical biofilm removal.

The ultrasonic toothbrush was installed at a distance of 5 mm from the surface of the tooth enamel containing *Streptococcus mutans* biofilm. The distance of 5 mm was chosen to conform with standards used in prior studies as identified through a literature review of in vitro studies that evaluated the effectiveness of acoustic toothbrushes (sonic and ultrasonic) in removing biofilm when physical contact with the biofilm is not provided.[9] The hypothesis that the ultrasonic toothbrush can help disrupt the biofilm even without mechanical contact between the brush bristles and the dental biofilm, may be of significant interest to the dental community because its confirmation means that an ultrasonic toothbrush serves as an innovative and effective alternative for patients who cannot perform their brushing effectively with a traditional manual, electric or sonic toothbrush.[17]

The choice of *Streptococcus mutans* as the test microorganism for this study was based on the importance of that species in the etiology of dental caries, which is due to its virulence factors, such as acid tolerance and acid production, as well as the ability to synthesize extra- and intra-cellular polysaccharides.[3,18,19] In addition, several authors[4-6] suggested that the risk of caries is directly proportional to the increase in the number of *Streptococcus mutans*, thus justifying the importance of quantification of this species in the present study.

Shinada et al[20] performed an in vitro study evaluating the effect of ultrasound (without accompanying sonic or mechanical vibration) emitted by an ultrasonic toothbrush on adherence of *Streptococcus mutans* on the surface of dental enamel, in addition to effects of this ultrasound on the cellular morphology of this microorganism. The authors reported satisfactory results.

In this study, the ultrasonic toothbrush, when used in the ultrasound-only mode, showed no significant difference compared to the control in reducing the counts of *Streptococcus mutans* biofilm grown for 72 hours. There was also no significant difference between the ultrasound-only condition and the ultrasound plus sonic vibration condition, which were observed to be statistically different from the control condition ($P < 0.05$). In addition, the values for the 25th and 75th percentiles of ultrasound-only treatment fell consistently in between the values of the other two treatments. This could indicate that there is a trend that a reduction of the bacterial count may have been statistically significant if the samples were to have had a greater exposure to ultrasound or repeated exposures over time. In this study, the samples with bacterial biofilms received only a single 3-minute exposure to the ultrasound from the ultra-

sonic toothbrush, which does not accurately simulate the repeated exposure to ultrasound emitted by an ultrasonic toothbrush that a patient would receive when using the toothbrush on a regular basis. Greater frequency in the use of an ultrasonic toothbrush could have a cumulative effect on disruption of the biofilm, enhancing its effects.

The ultrasonic toothbrush tested in this study was not capable of emitting sonic vibration without also emitting ultrasound, thus there was no sonic vibration-only condition in this study. Therefore, it was not possible to test the isolated effect of mechanical sonic vibration on biofilms grown for either 24 or 72 hours. It is suggested that further studies be conducted to evaluate sonic vibration emitted by an otherwise identical toothbrush (i.e. with ultrasound disabled) alone, so that it is possible to clarify the role of ultrasound in relation to sonic vibration in reducing bacterial count in biofilms of *Streptococcus mutans*.

One limitation of this study was to use a biofilm composed of only one bacterial species, *Streptococcus mutans*. In order to obtain preliminary results on the effect of ultrasound in a controlled laboratory environment with a minimum number of variables, this mono-species biofilm was used as a simple proxy to represent the complete ecosystem of cariogenic bacteria in the oral cavity.

In relation to the results obtained from the model used in this study and the clinical circumstances of toothbrush use, it is expected that similar results can be also found because when the ultrasonic toothbrush is used in the oral cavity, it will get wet or be bathed in saliva. Therefore, it is expected that clinical outcomes can be similar to those found in this study.

The study of the effects of ultrasonic toothbrushes on multi-species biofilms would be an interesting topic for future research, which would more accurately simulate actual conditions found in the oral cavity.

Based on the results of this study it can be concluded that sonic vibration improves the effect of an ultrasonic toothbrush in reducing bacterial viability and in disrupting *Streptococcus mutans* biofilm.

a. Acumedia Manufacturers Inc., Lansing, MI, USA.
b. Goldspire Group Ltd., Hong Kong, China.
c. Difco, Becton Dickinson, Sparks, MD, USA.
d. SPSS, IBM, New York, NY, USA

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References

1. Van Houte J. Role of micro-organisms in caries etiology. *J Dent Res* 1994;73:672-681.
2. Loesche W. Role of Streptococcus mutans in human dental decay. *Microbial Rev* 1986;50:353-380.
3. Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation - new insight. *J Dent Res* 2006; 85:878-887.
4. Loesche WJ, Straffon LH. Longitudinal investigation of the role of Streptococcus mutans in human fissure decay. *Infect Immun* 1979; 26:498-507.
5. Zickert I, Emilson CG, Krasse B. Correlation of level and duration of Streptococcus mutans infection with incidence of dental caries. *Infect Immun* 1983;39:982-985.
6. Fujiwara T, Sasada E, Mima N, Ooshima T. Caries prevalence and salivary mutans streptococci in 0-2-year-old children of Japan. *Community Dent Oral Epidemiol* 1991;19:151-154.
7. Rode SM, Gimenez X, Montoya VC, Gómez M, Blanc SL, Medina M, Salinas E, Pedroza J, Zaldivar-Chiappa RM, Pannuti CM, Cortelli JR, Oppermann RV. Daily biofilm control and oral health: consensus on the epidemiological challenge--Latin American Advisory Panel. *Braz Oral Res* 2012;26:133-143.
8. Teitelbaum AP, Pochapski MT, Jansen JL, Sabbagh-Haddad A, Santos FA, Czlusniak GD. Evaluation of the mechanical and chemical control of dental biofilm in patients with Down syndrome. *Community Dent Oral Epidemiol* 2009;37:463-467.
9. Schmidt JC, Zaugg C, Weiger R, Walter C. Brushing without brushing? – a review of the efficacy of powered toothbrushes in noncontact biofilm removal. *Clin Oral Invest* 2013;17:687-709.
10. Sharma PK, Gibcus MJ, van der Mei HC, Busscher HJ. Influence of fluid shear and microbubbles on bacterial detachment from a surface. *Appl Environ Microbiol* 2005;71:3668–3673.
11. Lance M, Bataille J. Turbulence in the liquid phase of a uniform bubbly air–water flow. *J Fluid Mech* 1991;222:95–118.
12. Parini MR, Pitt WG. Removal of oral biofilms by bubbles: the effect of bubble impingement angle and sonic waves. *J Am Dent Assoc* 2005;136:1688–1693.
13. US Food and Drug Administration. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn_template.cfm?id=k913724 (accessed 24/10/2015).
14. White DJ. Reactivity of fluoride dentifrices with artificial caries. I. Effects on early lesions: F uptake, surface hardening and remineralization. *Caries Res* 1987;21:126-140.
15. Guggenheim B, Giertsen E, Schüpbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. *J Dent Res* 2001;80:363-370.
16. Roberts FA, Hacker BM, Oswald TK, Mourad PD, McInnes C. Evaluation of the use of ultrasound within a power toothbrush to dislodge oral bacteria using an in vitro Streptococcus mutans biofilm model. *Am J Dent* 2010;23:65-69.
17. Pizzo G, Licata ME, Pizzo I, D'Angelo M. Plaque removal efficacy of power and manual toothbrushes: a comparative study. *Clin Oral Investig* 2010;14:375-381.
18. Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, Kozai K. Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in pre-school children. *J Med Microbiol* 2005;54:661-665.
19. Mattos-Graner RO, Smith DJ, King WF, Mayer MP. Water-insoluble glucan synthesis by mutans streptococcal strains correlates with caries incidence in 12- to 30-month-old children. *J Dent Res* 2000;79:1371-1377.
20. Shinada K, Hashizume LN, Teraoka K, Kurosaki N. Effect of ultrasound toothbrush on Streptococcus mutans: an in vitro study. *Jpn J Conserv Dent* 1999;42:410-417.