

Efficacy of a triclosan formula in controlling early subgingival biofilm formation: a randomized trial

Ernesto ANDRADE^(a)
Patricia WEIDLICH^(b)
Patrícia Daniela Melchioris ANGST^(b)
Sabrina Carvalho GOMES^(b)
Rui Vicente OPPERMANN^(b)

^(a)Universidade de La República - UDELAR, School of Dentistry, Department of Periodontology, Montevideo, Uruguay.

^(b)Universidade Federal do Rio Grande do Sul – UFRGS, School of Dentistry, Department of Periodontology, Porto Alegre, RS, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:

Patricia Weidlich
E-mail: patricia.weidlich@ufrgs.br

DOI: 10.1590/1807-3107BOR-2015.vol29.0065

Submitted: Out 29, 2014
Accepted for publication: Jan 28, 2015
Last revision: Apr 30, 2015

Abstract: The aim of this study was to determine the efficacy of rinses with slurries of a dentifrice containing triclosan (TCS), as compared with rinses with slurries from a control dentifrice, in controlling early subgingival biofilm formation. A double-blind, randomized and cross-over clinical trial was designed, and 26 dental students were included. In the first period, participants were randomized to rinse with a TCS slurry or a control slurry, in a 12 h interval, and to refrain from mechanical cleaning. A Plaque Free Zone Index was assessed at 24 h, 48 h, 72 h and 96 h. After a washout period of 10 days, the second experimental period was conducted, following the same protocol as the first period, except that the slurry groups were switched. Use of the TCS slurry resulted in a significantly higher percentage of plaque-free surfaces, both at 24 h and at 72 h ($p < 0.01$). In the of 48-72 h interval, the triclosan slurry showed a lower percentage of sites converted to a score of 2 (38.1% for the test *versus* 40% for the control product, $p = 0.015$). In conclusion, rinsing with slurries of dentifrice containing TCS retards the down growth of bacterial biofilms from the supra- to the subgingival environment.

Keywords: Biofilms; Growth and Development; Triclosan; Randomized Controlled Trial.

Introduction

The onset and progression of destructive forms of periodontal disease are closely related to the subgingival presence of microbial biofilms.¹ These biofilms are derived from and have a close relationship with the supragingival environment.^{2,3} The transition from the supra- to the subgingival environment is a critical step, because a new ecological environment is established in a location not readily accessible to daily oral hygiene procedures.^{4,5} Recent data has been presented identifying supragingival biofilm as a risk factor for the re-infection of the subgingival area, and consequent recurrence of periodontitis in treated patients.⁶ Accordingly, adequate control of supragingival deposits should be an essential measure to prevent the establishment of bacterial biofilms subgingivally.

The transition of the dental biofilms from a supra- to a subgingival location was followed in a 96 h period without oral hygiene.⁷ During the initial biofilm formation period, a Plaque Free Zone (PFZ) was observed between the cervical borders of the biofilm and the gingival margin. This

PFZ disappeared with time in most of the examined surfaces, indicating that part of the supragingival biofilm was now covered by the gingival soft tissues.

Using a similar approach, a study analyzed the effect of chlorhexidine (CHX) on the initial formation of subgingival deposits. Daily rinses with 0.12% CHX resulted in significant inhibition of the subgingival extension of the biofilm in a 96 h period of observation, as compared with placebo rinses.⁸ However, the recommendation of daily use of CHX is hindered by adverse effects associated with this antiseptic.⁹ Several other antiseptics have been suggested in the literature.^{10,11,12} Among them, triclosan (TCS) can be found in different formulations of dentifrices and rinsing solutions. Daily brushing with dentifrices containing TCS is associated with significant reductions in supragingival biofilm and gingivitis levels. Systematic reviews of the literatures and meta-analyses have shown that these effects are significant, even under different methodological conditions.^{12,13,14}

Short-term experimental studies have demonstrated significant reductions in supragingival biofilm, when rinsing with slurries of dentifrices containing TCS.^{15,16} These studies have not examined the possible effect of TCS in subgingival biofilm formation. TCS has antimicrobial and anti-inflammatory properties that may also be important for inhibiting subgingival biofilm formation.^{17,18} The purpose of the present study was to determine the efficacy of rinses with slurries of a dentifrice containing TCS in the initial formation of subgingival biofilm, as compared with rinses containing slurries of a control dentifrice.

Methodology

Study design

The study was developed as a double-blind, randomized clinical trial with a cross-over design; the primary outcome was the formation of subgingival biofilm, defined as the percentage of surfaces with a score of 2, according to the PFZ Index.⁸ The trial was registered at www.clinicaltrials.gov (NCT02192060). The sample size was calculated estimating that 10% (+10) of the dental surfaces would show the formation of subgingival biofilm (criterion 2 of the

PFZ Index) using the dentifrice containing TCS, versus 21.6% of surfaces of similar condition using the control dentifrice.⁸ Considering an α error of 5% and a study power of 80%, the number of participants calculated was 24; therefore, a total number of 30 participants would be required to allow for an attrition rate of 20%. The study was approved by the University Human Research Ethics Committee (CAAE 11657613.3.0000.5347).

Sample

Thirty-two volunteers among students of the *Universidade Federal do Rio Grande do Sul - UFRGS* Dental School were examined, 5 did not meet the inclusion criteria, and 1 was lost during the study, comprising a total of 26 participants (Figure 1). The mean age was 22.96±3.51 years old, and 18 participants (69.23%) were females.

Eligible volunteers were included as participants if they were non-smokers, had good general health, had at least 20 natural teeth, not including 3rd molars, but including upper anterior teeth, pre-molars without restorations, and teeth with orthodontic appliances, anatomic irregularities, gingival recession, gingivitis¹⁹ and /or periodontitis, and were not taking antibiotics or undergoing anti-inflammatory therapy for over 30 days prior to the study, were not pregnant or breast-feeding, and had a negative history of allergy to the experimental products.

Randomization was performed by a software program (available at www.randomization.com) after the baseline examination. Allocation of each rinse solution to the sequence test/control or control/test was concealed in opaque, sealed and serially numbered envelopes. The researcher in charge of the distribution of the participants was not involved in the clinical exams (SCG). The study was developed between May-June 2013, at the Periodontology Clinic of the *Universidade Federal do Rio Grande do Sul - UFRGS*.

Training and Calibration Procedures

The clinical examiner (EA) was previously trained in the evaluation of the PFZ, aided by clinical pictures. Subsequently, he was calibrated with a gold standard examiner (PW). The kappa for the interexaminer calibration was 0.72. The intraexaminer calibration

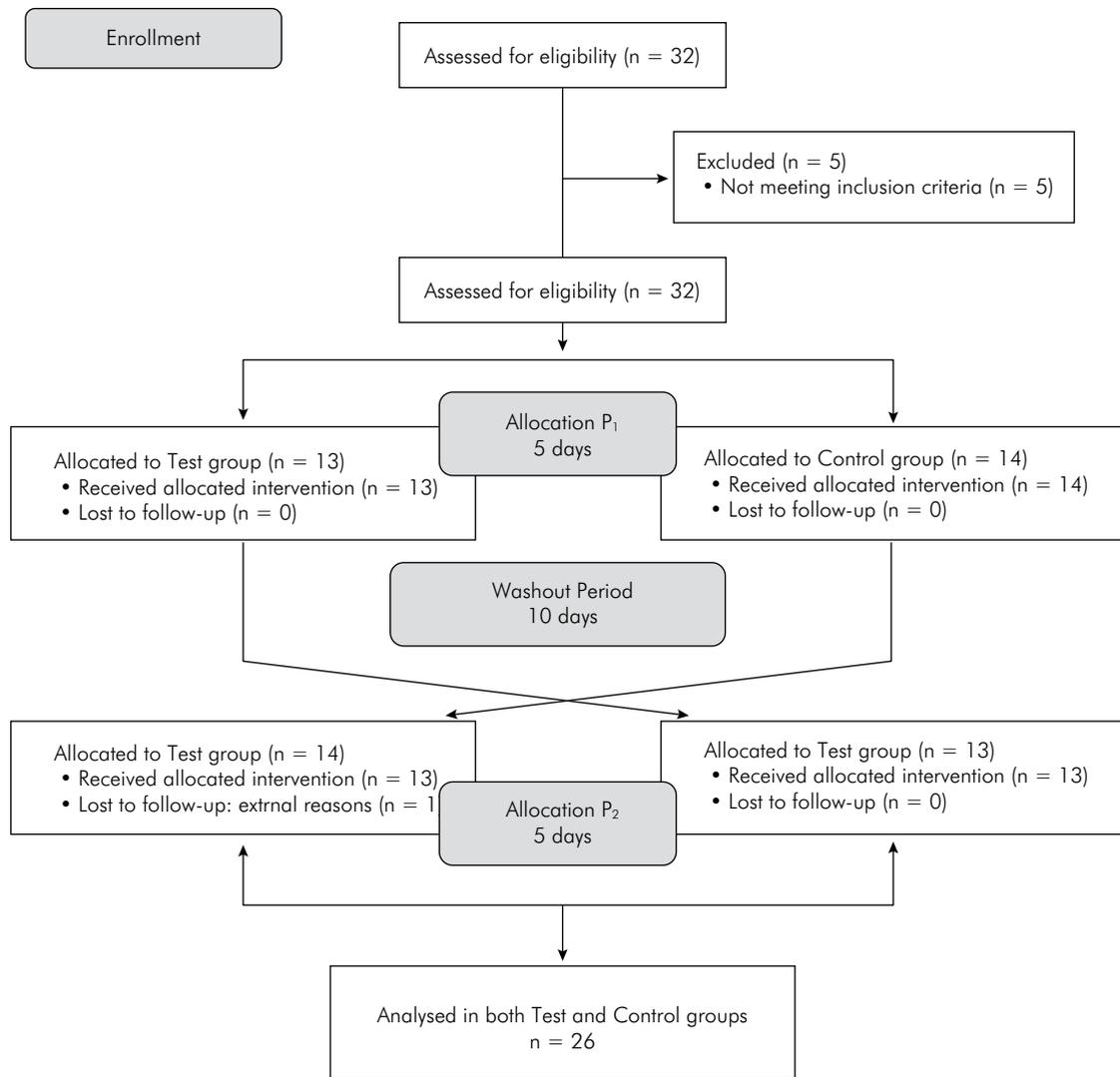


Figure 1. Flowchart of the study.

consisted of repeating the PFZ exams 1 hour after the first exam, in 15% of the sample ($\kappa = 0.70$).

Preparation of the Slurries

The slurries were prepared by adding 1 gram of the dentifrice to 10 ml of distilled water. The slurries were conditioned in single-dose plastic vials, coded and distributed to the participants. Slurries of the experimental dentifrices were prepared by a member of the team who was not involved in the clinical examinations (SCG). The test dentifrice (Colgate® Total 12, Colgate-Palmolive Comercial Ltda, São Paulo, Brazil) and the control (Colgate® Máxima Proteção Anti-cáries, Colgate-Palmolive Comercial Ltda., São

Paulo, Brazil) were purchased by the researchers in the local market. The test and control slurries were identical in color and taste, and were identified by an alphanumeric code. Both the examiner and the participants were blinded to the slurry allocation in each experimental period.

Experimental Design

The participants' upper premolars, canines and incisors comprised the experimental teeth. The teeth received a thorough scaling, and polishing for the complete removal of biofilm until no deposits could be identified, following the use of basic fuchsin (Replamic T, Iodontec, São Paulo, Brazil) as a disclosing

agent. Next, the first rinse with the given slurry was performed for 1 minute. Oral and written instructions advised participants to refrain from mechanical cleaning of their experimental teeth. As for the remaining teeth, brushing and flossing were allowed, but the participants were asked to refrain from using dentifrices and from rinsing with oral antiseptics. They were also asked to repeat their rinsing with the slurry after 12 h, and further instructed not to eat or drink up to 30 min after rinsing. Daily doses were distributed every 24 h, at which time the used plastic vials were also collected.

During the first experimental period (P1), clinical exams were repeated at 24 h, 48 h, 72 h and 96 h, according to the following scheme. The experimental teeth were washed thoroughly with a water spray, and then dried. Next, the disclosing solution was applied for 30 sec. The PFZ Index was recorded according to Maliska *et al.*⁸ The buccal surfaces were divided into three areas: two proximal and one facial. Score 0 was given to the absence of biofilm, score 1, to the presence of biofilm and the presence of a PFZ, and score 2, to the presence of biofilm and the absence of a PFZ.

Once P1 was concluded, the participants received a new dental prophylaxis and were allowed to return to their usual homecare procedures for a washout period of 10 days. The second experimental period (P2) followed the same protocol as P1, except that the experimental slurry groups were switched.

Data Analysis

Codified results were collected on a spread sheet and analyzed with SPSS 18.0 software (SPSS Inc., Chicago, USA). The mean percentages for each score of each group in the different experimental periods were calculated. The average percentages of each score were also calculated for the proximal and facial thirds, individually. The conversion of scores 0 and 1 to score 2 in each experimental period was assessed to evaluate the initial formation of the subgingival biofilm. Comparisons were performed with the Friedman and the Wilcoxon tests. The Bonferroni corrections were applied in the case of multiple comparisons. The entire analysis took into consideration the cross-over design of the study. The level of significance was established at 5%.

Results

When all the experimental surfaces were observed, the test product was found to have a significantly higher proportion of plaque free surfaces, as shown by the score of 0 for the test, compared with the control product ($p < 0.001$), at 24 h. For all the other experimental time periods, there were no statistically significant differences in plaque formation (Table 1).

When the facial surfaces were analyzed separately, significant differences were observed in the mean percentage of the scores between the test and the control group after 24 h and after 96 h. The control product showed significantly higher percentages of scores 2 at 24 h (0.5% for the control *versus* 0% for the test product) and at 96 h (61.7% for the control *versus* 60.2% for the test product). No significant differences were observed between test and control at 48 h and at 72 h (Figure 2).

Regarding the proximal surfaces, significant differences were observed in the percentage of scores between test and control groups for the experimental periods of 24 h and 72 h. The use of the test product resulted in a significantly higher percentage of

Table 1. Mean percentage of surfaces with Plaque Free Zone Index scores of 0, 1 and 2, considering all the surfaces at each experimental period.

	Control	Test	p*
24 h			
0	63.3	67.6	
1	35.4	31.2	
2	1.3	1.1	0.001
48 h			
0	21.4	24.6	
1	65.2	61.3	
2	13.4	14.1	0.082
72 h			
0	3.6	6.5	
1	48.2	46.8	
2	48.2	46.8	0.209
96 h			
0	1.9	0.3	
1	22.3	20.7	
2	75.6	79	0.143

* Comparison between control and test products – Friedman test.

plaque-free surfaces, borne out by score 0, both at 24 h (53.4% for the control *versus* 60.4% for the test product) and at 72 h (2.9% for the control *versus* 6.6% for the test product). In relation to the 48 h and 96 h time periods, there were no significant differences between both products (Figure 3).

Finally, the conversion from scores 0 and 1, representing the absence of subgingival plaque, to score 2, representing the presence of subgingival plaque, was analyzed. Between 48 h-72 h, subjects who used the test product presented a lower percentage of sites converted to score 2, *i.e.*, use

of a TCS slurry resulted in a lower percentage of subgingival plaque sites than use of the control slurry (38.1% for the test *versus* 40% for the control product, $p = 0.015$). Subjects who used the test product between 72 h-96 h presented a significantly higher percentage of sites converted to score 2 ($p = 0.002$) (Figure 4). The same analysis performed with the facial surfaces showed no statistically significant differences in the conversion from scores 0 and 1 to score 2, regardless of the time period (Figure 5). No adverse effects were reported during the study.

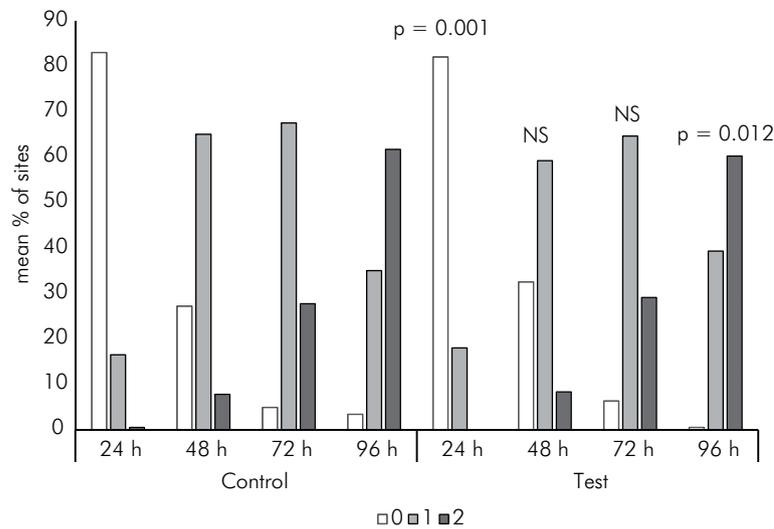


Figure 2. Mean percentage of scores 0, 1 and 2, in relation to facial surfaces in each experimental period.

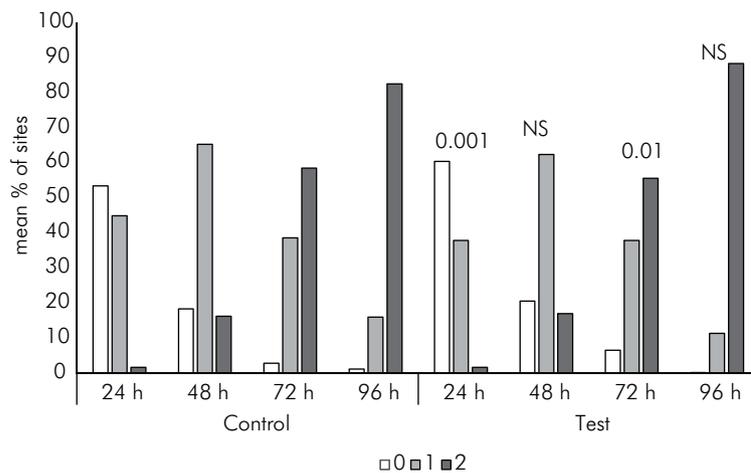


Figure 3. Mean percentage of scores 0, 1 and 2 in relation to proximal surfaces in each experimental period.

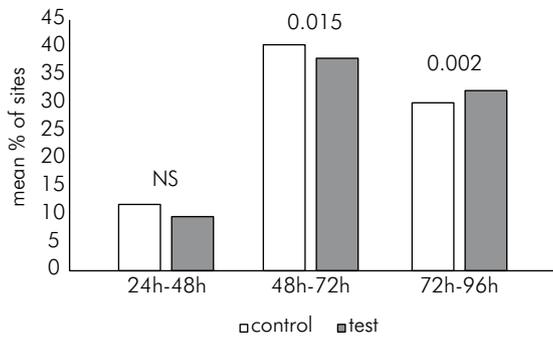


Figure 4. Percentage of conversion from scores 0 and 1 to score 2 (presence of subgingival biofilm) between time periods, for proximal surfaces.

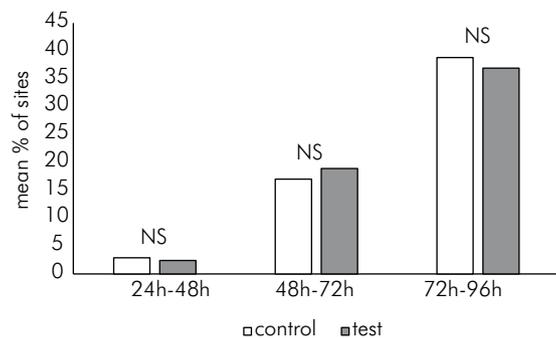


Figure 5. Percentage of conversion from scores 0 and 1 to score 2 (presence of subgingival biofilm) between time periods, for facial surfaces.

Discussion

The results of the present study have shown that rinsing with slurries from a dentifrice containing TCS significantly maintained a PFZ, as compared with the control slurry. The major consequence of this effect is that the formation of subgingival biofilm was also inhibited by the test formulation, as compared with the control.

The inhibitory effect of the TCS dentifrice on supragingival biofilm formation was observed in the first 24 h of undisturbed accumulation. When the facial third of the buccal surface was considered separately, this effect was observed in the 24 h and 96 h measurements. When the proximal thirds were examined, the inhibitory effect was observed in the 24 h and 72 h measurements. These results confirm previous data reporting the inhibitory effect of TCS on biofilm formation.¹³ They are also

in agreement with the observation that this effect is more pronounced in the proximal areas.²⁰ However, there are some important differences between this study and others, in regard to how the inhibitory effect of the TCS formulations was measured. Most of the previous studies used plaque indices that measured the distribution and/or quantity of biofilm formed on the dental surface. In the present study, the analysis introduced an additional component, which was the presence or absence of the PFZ. It has been previously shown that the transition between the presence and disappearance of the PFZ occurs when part of the supragingival bacterial deposits are covered by the gingival margin, thus favoring the subgingival location of the bacterial biofilm.⁷

The major difference observed between the test and the control product was in the percentage of 0 scores favoring the test formulation. This result per se is important, since it discloses the presence of the initial inhibitory effect attributed to TCS formulations.^{13,15,16} However, the conversion of scores 0 and 1 to score 2 are essential to the hypothesis of the present study, since the subgingival location of the biofilm is directly related to score 2. In this sense, in regard to the proximal aspects, significantly smaller conversions to scores 2 from 48 h to 72 h were observed in association with rinsing with slurries of the TCS dentifrice, as compared with the control. Conversions to score 2 observed from 72 h to 96 h were significantly higher with the TCS slurry, suggesting that TCS does not prevent subgingival colonization. However, the use of the TCS slurry retarded early subgingival formation. Some studies have reported that the inhibitory effect of TCS increases in direct proportion to the amount of existing biofilm.²¹ This observation could explain the different behavior observed for the proximal and facial aspects. The conversion to scores 1 and 2 occurred earlier and in greater proportion at the proximal thirds than at the facial aspect. On the other hand, it should be borne in mind that the teeth examined were incisors, canines and pre-molars. It is known that the formation of supragingival biofilm is different in different areas of the mouth, and is especially pronounced in the molar region.²² In the present study, molars were

not included because of the technical difficulties in performing a reproducible examination in these areas.

Dental students formed the test panels. Bear in mind that these students may give responses associated with their degree of knowledge and professional motivation.²³ The experimental period was 4 days, considered by previous studies to be enough time to observe the inhibitory properties of TCS for supragingival biofilm formation.^{7,15,16,24,25,26} A more prolonged period of observation would allow an analysis of a larger number of sites with scores 0 and 1, especially in the facial aspects. However, studies of this nature, requiring more than 4 days of no oral hygiene, have ethical restrictions. On the other hand, the results of the present study call for studying the process of subgingival biofilm formation in models that include regular oral hygiene procedures.

In the present study, the intraexaminer kappa (0.70) was slightly lower than the interexaminer measure (0.72); this can be interpreted as a limitation of the study. Likewise, the long-term gingival response was not assessed.⁷ A greater period of observation would be needed to ascertain the inflammatory response

and its correlation with the presence/absence of a PFZ. This effect may be of particular interest, since it has been shown that TCS also has anti-inflammatory properties.^{17,27} Some studies have shown that the daily use of TCS-containing dentifrices reduces the progression of periodontitis,²⁸ and prevents periodontitis in young populations.²⁹ The colonization of the subgingival area by bacterial biofilms is essential to the establishment of periodontitis.³⁰ This colonization is promoted by supragingival biofilm and its progression towards the subgingival area.²⁸ In this context, measuring the PFZ clinically indicates the sequence of how this process occurs and what measures may be implemented to prevent it.

Conclusion

The results of the present study have shown that rinsing with slurries of dentifrice containing TCS retards the down growth of bacterial biofilms from the supra- to the subgingival environment. The impact of these results requires further testing to secure new avenues for the prevention of destructive periodontal diseases.

References

1. Marsh PD, Moter A, Devine DA. Dental plaque biofilms: communities, conflict and control. *Periodontol* 2000. 2011 Feb;55(1):16-35.
2. Jakubovics NS, Kolenbrander PE. The road to ruin: the formation of disease-associated oral biofilms. *Oral Dis*. 2010 Nov;16(8):729-39.
3. Listgarten M. Formation of dental plaque and other oral biofilms. In: Newman HN, Wilson M, Michigan University, editors. *Dental plaque revisited: oral biofilms in health and disease*. Cardiff: Boline; 1999. p. 187-210.
4. Claydon NC. Current concepts in toothbrushing and interdental cleaning. *Periodontol* 2000. 2008;48:10-22.
5. Rode SM, Gimenez X, Montoya VC, Gómez M, Blanc SL, Medina M, et al. Daily biofilm control and oral health: consensus on the epidemiological challenge--Latin American Advisory Panel. *Braz Oral Res*. 2012;26 Suppl 1:133-43.
6. Mdala I, Haffajee AD, Socransky SS, Blasio BF, Thoresen M, Olsen I, et al. Multilevel analysis of clinical parameters in chronic periodontitis after root planing/scaling, surgery, and systemic and local antibiotics: 2-year results. *J Oral Microbiol*. 2012;4.
7. Weidlich P, Souza MA, Oppermann RV. Evaluation of the dentogingival area during early plaque formation. *J Periodontol*. 2001 Jul;72(7):901-10.
8. Maliska AN, Weidlich P, Gomes SC, Oppermann RV. Measuring early plaque formation clinically. *Oral Health Prev Dent*. 2006;4(4):273-8.
9. Van Strydonck DA, Slot DE, Van der Velden U, Van der Weijden F. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. *J Clin Periodontol*. 2012 Nov;39(11):1042-55.
10. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *J Am Dent Assoc*. 2006 Dec;137(12):1649-57.
11. Oppermann RV, Haas AN, Villoria GE, Primo LG, Serra-Negra JM, Ferreira EF, et al. Proposal for the teaching of the chemical control of supragingival biofilm. *Braz Oral Res*. 2010;24 Suppl 1:33-6.
12. Hioe KP, van der Weijden GA. The effectiveness of self-performed mechanical plaque control with triclosan containing dentifrices. *Int J Dent Hyg*. 2005 Nov;3(4):192-204.
13. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. *J Dent*. 2010 Jun;38 Suppl 1:S6-10.

14. Davies RM, Ellwood RP, Davies GM. The effectiveness of a toothpaste containing triclosan and polyvinyl-methyl ether maleic acid copolymer in improving plaque control and gingival health: a systematic review. *J Clin Periodontol*. 2004 Dec;31(12):1029-33.
15. Addy M, Jenkins S, Newcombe R. The effect of triclosan, stannous fluoride and chlorhexidine products on: (I) Plaque regrowth over a 4-day period. *J Clin Periodontol*. 1990 Nov;17(10):693-7.
16. Arweiler NB, Auschill TM, Reich E, Netuschil L. Substantivity of toothpaste slurries and their effect on reestablishment of the dental biofilm. *J Clin Periodontol*. 2002 Jul;29(7):615-21.
17. Arancibia R, Cáceres M, Martínez J, Smith PC. Triclosan inhibits tumor necrosis factor-alpha-stimulated urokinase production in human gingival fibroblasts. *J Periodontol Res*. 2009 Dec;44(6):726-35.
18. Wallet MA, Calderon NI, Alonso TR, Choe CS, Catalfamo DI, Lalane CJ, et al. Triclosan alters antimicrobial and inflammatory responses of epithelial cells. *Oral Dis*. 2013 Apr;19(3):296-302.
19. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975 Dec;25(4):229-35.
20. Mateu FA, Boneta AE, DeVizio W, Stewart B, Proskin HM. A clinical investigation of the efficacy of two dentifrices for controlling established supragingival plaque and gingivitis. *J Clin Dent*. 2008;19(3):85-94.
21. Mankodi S, Chaknis P, Panagakos FS, DeVizio W, Proskin HM. Comparative investigation of a dentifrice containing triclosan/copolymer/sodium fluoride and specially-designed silica and a dentifrice containing 0.243% sodium fluoride in a silica base for the control of established supra-gingival plaque and gingivitis: a 6-month clinical study. *Am J Dent*. 2011 Jul;24 Spec No A:21A-7A.
22. Ramberg P, Axelsson P, Lindhe J. Plaque formation at healthy and inflamed gingival sites in young individuals. *J Clin Periodontol*. 1995 Jan;22(1):85-8.
23. Espinoza Santander I, Muñoz Poblete C, Lara Molina M, Uribe Cifuentes JP. Hábitos de higiene oral en estudiantes de odontología de la Universidad de Chile. *Rev Clin Periodoncia Implantol Rehabil Oral*. 2010;3(1):11-8.
24. Addy M, Willis L, Moran J. Effect of toothpaste rinses compared with chlorhexidine on plaque formation during a 4-day period. *J Clin Periodontol*. 1983 Jan;10(1):89-99.
25. Jenkins S, Addy M, Newcombe R. Toothpastes containing 0.3% and 0.5% triclosan. I. Effects on 4-day plaque regrowth. *Am J Dent*. 1989 Sep;2 Spec No:211-4.
26. Cao C, Sha Y, Meng H, Kang J, Yeh CH, Chyon HY, et al. A four-day study to evaluate the anti-plaque efficacy of an experimental triclosan-containing dentifrice. *J Clin Dent*. 2001;12(4):87-91.
27. Mustafa M, Wondimu B, Yucel-Lindberg T, Kats-Hallström AT, Jonsson AS, Modéer T. Triclosan reduces microsomal prostaglandin E synthase-1 expression in human gingival fibroblasts. *J Clin Periodontol*. 2005 Jan;32(1):6-11.
28. Rosling B, Wannfors B, Volpe AR, Furuichi Y, Ramberg P, Lindhe J. The use of a triclosan/copolymer dentifrice may retard the progression of periodontitis. *J Clin Periodontol*. 1997 Dec;24(12):873-80.
29. Ellwood RP, Worthington HV, Blinkhorn AS, Volpe AR, Davies RM. Effect of a triclosan/copolymer dentifrice on the incidence of periodontal attachment loss in adolescents. *J Clin Periodontol*. 1998 May;25(5):363-7.
30. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000*. 1997 Jun;14:12-32.