Antimicrobial effects of the combination of chlorhexidine and xylitol

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IN BRIEF

- The xylitol + chlorhexidine combination was efficient and superior to single treatments in controlling biofilm and suppressing *Streptococcus mutans*.
- Chlorhexidine, as varnish, is a good vehicle for use in children.
- Recommends that the use of chewing gum as a means of administering xylitol is important for achieving co-operation among children.

RESEARCH

Aims To assess the effect of combining 1% chlorhexidine varnish (CHX) with xylitol chewing gum (XYL) on *Streptococcus mutans* and biofilm levels in 6-8-year-old children. **Design** Randomised controlled study. **Subjects and methods** Eighty-two 6-8-year-old children were randomly divided into groups as follows: G1 (n = 20): xylitol chewing gum twice a day after breakfast and lunch; G2 (n = 20): xylitol gum as G1 plus chlorhexidine varnish application at the start of the study and after one and two months; G3 (n = 20): chlorhexidine varnish as G2; and G4 (n = 22): fluoride gel application at the start of the study and after one and two months. Microbiological tests were performed to assess *Streptococcus mutans* colony forming units (CFU) and the teeth of those children with moderate or higher CFU scores were examined for visible biofilm. CFU scores were categorised as follows: 0 = absence of *S. mutans*, 1 = low level (1-10 CFU), 2 = moderate level (11-100 CFU), 3 = high level (101-250 CFU), 4 = very high level (>250 CFU). Biofilm scores based on a scale from 0 (absence of biofilm) to 5 (thick biofilm firmly adhered to posterior and anterior teeth) were obtained. **Results** The biofilm reduction was greater in G2 and G3, with mean values of 3.38 and 3.17 to 1.79 and 1.88, respectively ($p < 0.05$). All groups presented a reduction in the *S. mutans* levels. XYL + CHX showed the largest reduction throughout the study period, with 58.3% in the first month, 84.2% in the second and 92.9% at the end of the study. **Conclusions** The XYL + CHX combination was efficient and superior to single treatments in controlling biofilm and suppressing *S. mutans*.

INTRODUCTION

Streptococcus mutans has been considered the main pathogen associated with dental caries. It induces mineral loss due to its strong adhesion to the tooth surface and to the acid production resulting from fermentable carbohydrates, which keeps local pH low.[1] The biofilm produced by this pathogen accounts for *S. mutans* adhesion, and is therefore considered to be cariogenic as well.[2] Chlorhexidine (CHX)[3,4] and xylitol (XYL)[5] have been used as strategies to prevent and reduce carious lesions.[6,7]

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Chlorhexidine is a bis-biguanide with antibacterial, anticariogenic and remineralising actions and few toxic effects.[4,8,9] Chlorhexidine acts by damaging the cell membrane of prokaryotes and by disrupting the cytoplasmatic constituents. Cell death occurs due to the rapid accumulation of metal ions inside the cells as they become more permeable.[10]

S. mutans counts have been found to be reduced in the saliva following the use of xylitol chewing gum[11–14] because cariogenic micro-organisms do not metabolise this substance.[15] Xylitol inhibits bacterial growth[15–17] through two mechanisms: direct inhibition of the glycolytic route resulting from the xylitol 5-phosphate derivative and/or indirect inhibition resulting from the competition for the HPr-P carrier between glucose and xylitol.

An *in vitro* combination of chlorhexidine and xylitol has been studied by Modesto and Drake[10] and by Deng *et al.*,[18] who observed that the combined use of such substances drastically inhibits both growth of *S. mutans* and formation of biofilm. Fluoride also has antimicrobial action when used in high concentrations, usually higher than those in fluoride-containing products for clinical purposes.[19–21] The present study assessed the effect of combining 1% chlorhexidine varnish (CERVITEC®) with xylitol chewing gum (Valda®) on *S. mutans* and biofilm levels in 6-8-year-old children.

METHODS

A randomised, blind experimental study was performed at a public school in Niterói, Rio de Janeiro, Brazil. This study obtained ethics approval from the Institute for Studies in Public Health – IESC/UFRJ. Initially, 1,385 children from the whole school were examined. Of these, only 200 children were aged 6-8 years and only 82 children presented with early mixed dentition without active carious lesions (selection criteria).

The children must not have taken antimicrobial medication for at least three months before the study and their caregivers had to sign an informed consent. Dental examination was performed in their classrooms with the use of a flashlight and wooden spatula.

The children were randomly divided into four groups. in Group 1 (XYL) (n = 20), xylitol chewing gum (VALDA XILITOL, VALDA®, São Paulo, Brazil) was used by children twice a day, after breakfast and after lunch; in Group 2 (CHX + XYL) (n = 20), chlorhexidine varnish at 1% (CERVITEC – Ivoclar Vivadent®, Schann-Liechtenstein-Austria) was applied on each child's dentition using a micro-brush at the beginning of the study, one month later and in the second month, totalling three applications throughout the study. Furthermore, these children also used xylitol chewing gum during the study period as did their peers in Group 1, except on those days of chlorhexidine varnish application. In Group 3 (CHX) (n = 20), chlorhexidine varnish at 1% was applied as described for Group 2. In Group 4 (F) (n = 22), acidulated phosphate fluoride gel (DFL®, Petrópolis, RJ, Brazil) was applied at the beginning of the study, one month later and in the second month, thus totalling three applications. The gel was used for ease of application. A Q-tip was used to apply the gel and after one minute[22] the child was asked to expectorate the excess fluoride in order to avoid any ingestion, following instructions of the manufacturer. The child was also told not to eat, not to drink and not to rinse the mouth for 30 minutes following fluoride application (Fig. 1).

Saliva samples were taken after one, two and six months. A microbiological test was performed according to a method where the colony forming-units (CFU) existing on a tongue blade (180 mm × 18 mm) were counted.[19] A sterile tongue blade was inserted into the child's oral cavity and was then moved around up to ten times, with both sides being then pressed on a Rodac® plate (Kracjeler Scientific, Inc) containing 12 ml of Mitis salivarius agar base (Becton, Dickinson & Company, Sparks, MD, USA) containing 0.2 g/ml sorbitol, 0.01 mg/ml potassium tellurite, 1.66 µg/ml bacitracin and 1.275 µg/ml kanamycin sulphate.[20]

The plates were incubated at 37°C for 72 hours in an anaerobiosis jar (BBL Gás Pak, Becton Dickinson and Co., Cockeysville, MD) with an atmosphere of 80% N_2, 10% H_2 and 10% CO_2. The period of time lapsed between inoculation and anaerobic incubation did not exceed 4 hours. Colony forming unit scores (CFU) were counted in the spatula impression

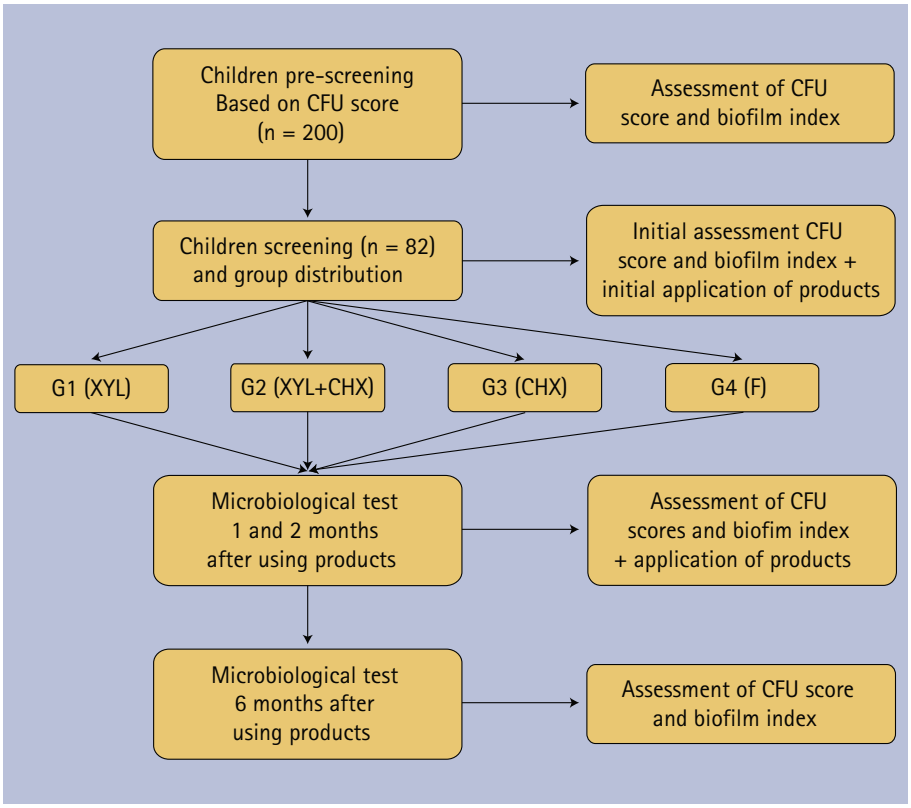

Fig. 1 Procedures followed in the study.

Table 1 Criteria for evaluating the biofilm level according to Ribeiro & Souza[22]

Scores	Description
0	Absence of biofilm
1	Thin biofilm on anterior teeth only
2	Thin, diffuse, easily removable biofilm on anterior and/or posterior teeth
3	Thick biofilm adhered to anterior/posterior teeth only
4	Thick biofilm firmly adhered to anterior teeth and thin biofilm on posterior teeth, or thick biofilm firmly adhered to posterior teeth and thin biofilm on anterior teeth
5	Thick biofilm firmly adhered to posterior and anterior teeth

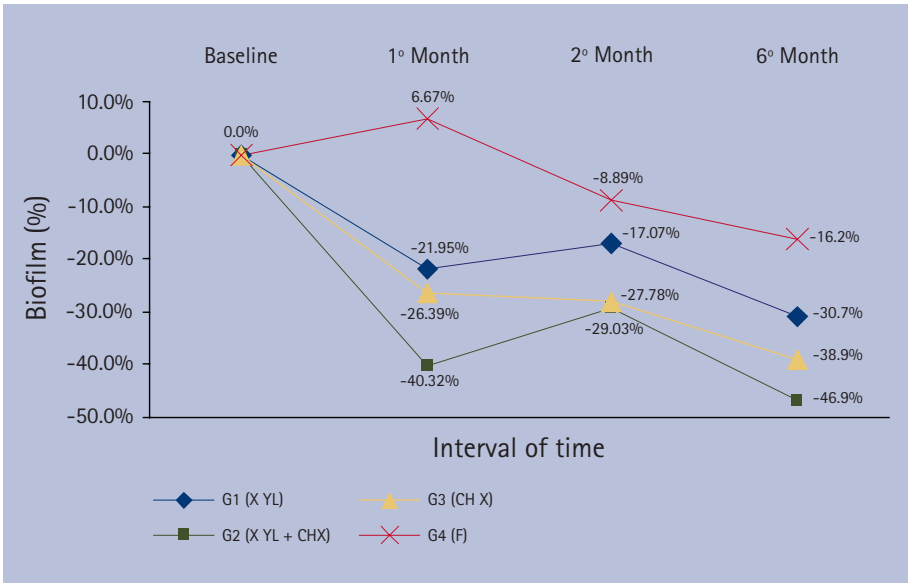

Fig. 2 Influence of different treatment types on the biofilm levels in 6–8-year-old children

area by the same operator using a stereoscopic microscope.

The CFU scores for *S. mutans* were expressed according to the criteria described by Weber[21] as follows: 0 = absence of *S. mutans*, 1 = low level (1-10 CFU), 2 = moderate level (11-100 CFU), 3 = high level (101-250 CFU), 4 = very high level (>250 CFU). Only those children with CFU scores equal to or above moderate CFU level were included in the study. These children were then examined by the same operator in order to investigate the amount of visible biofilm on the tooth surface. This procedure followed the criteria established by Ribeiro and Souza,[22] as can be seen in Table 1.

Data were analysed by using the statistical program SPSS 11.0. Kruskall-Wallis, Mann-Whitney and ANOVA statistical tests were performed with a 5% significance level.

RESULTS

Eighty-two children were selected for this study and 52.4% were girls, who also represented 65% of the children in group G3 (CHX). The mean age was 7.27 years old, and the 8-year-old children were mostly (75%) seen in groups G2 (CHX + XYL) and G3 (CHX).

Five of the 82 children who had been previously selected for the study were excluded because four had to move to another school and one could not follow the treatment adequately because of nausea, emesis and diarrhoea between the first and second months of the study.

A decrease of biofilm levels was observed in all groups during the study period, except in G4 (F) (Fig. 2). Group G2 (CHX + XYL) had the lowest biofilm levels. The overall reductions compared to the baseline values were 40% in the first month, 29% in the second month, and 46% in the last month, with statistical significance for the three intervals of time ($p < 0.05$). The largest variations in biofilm levels were observed in groups G2 (CHX + XYL) and G3 (CHX), whose initial mean values decreased from 3.38 and 3.17 to 1.79 and 1.88, respectively, which were found to be statistically significant ($p < 0.05$) (Table 2). In groups G1 (XYL) and G4 (F) a decrease in biofilm levels was not significant, ie the levels decreased from 3.00 to 2.00 and from 2.76 to 2.24, respectively. Absence of biofilm (biofilm level = 0) was the least common condition observed among the children, representing only 2 (2%) at the baseline, whereas 28 (34%) of all participants had biofilm levels equal to 2 at the end of the study.

All groups had statistically significant reductions in *S. mutans* levels ($p < 0.001$) (Table 2). Groups G2 and G3 were found to have the largest reductions, on average 1.97. The mean scores (0 to 4) for *S. mutans* ranged from 3.79 and 3.67 at the baseline to 2.06 and 1.30 in the sixth month for groups G2 (CHX + XYL) and G3 (CHX), respectively. At the baseline, 70% of the children were evaluated as having moderate *S. mutans* levels (11-100 CFU), whereas 17% had high levels and 12% the highest. In the sixth month (last saliva collection), however, the levels of *S. mutans* had gradually decreased and were found to be lower than those at the baseline: 70%

Table 2 Levels of biofilm and Streptococcus mutans in the different groups during the four time intervals

Groups	Time interval	S. mutans CFU (mean scores)[a]	Biofilm (mean scores)[b]
Group 1 (XYL)	Baseline	3.53	3.00
	1st month	3.13[c]	2.42[c]
	2nd month	1.79[c]	2.58[c]
	6th month	1.55[c]	2.00[c]
Group 2 (XYL + CHX)	Baseline	3.79	3.38
	1st month	2.29[c]	2.26[c]
	2nd month	1.85[c]	2.56[c]
	6th month	2.06[c]	1.79[c]
Group 3 (CHX)	Baseline	3.67	3.17
	1st month	3.08[c]	2.55[c]
	2nd month	1.95[c]	2.40[c]
	6th month	1.30[c]	1.88[c]
Group 4 (F)	Baseline	3.71	2.76
	1st month	2.55[c]	2.64
	2nd month	1.98[c]	2.36[c]
	6th month	1.76[c]	2.24[c]

[a] According to criteria by Weber[21]
[b] According to criteria by Ribeiro and Souza[22]
[c] Statistically significant

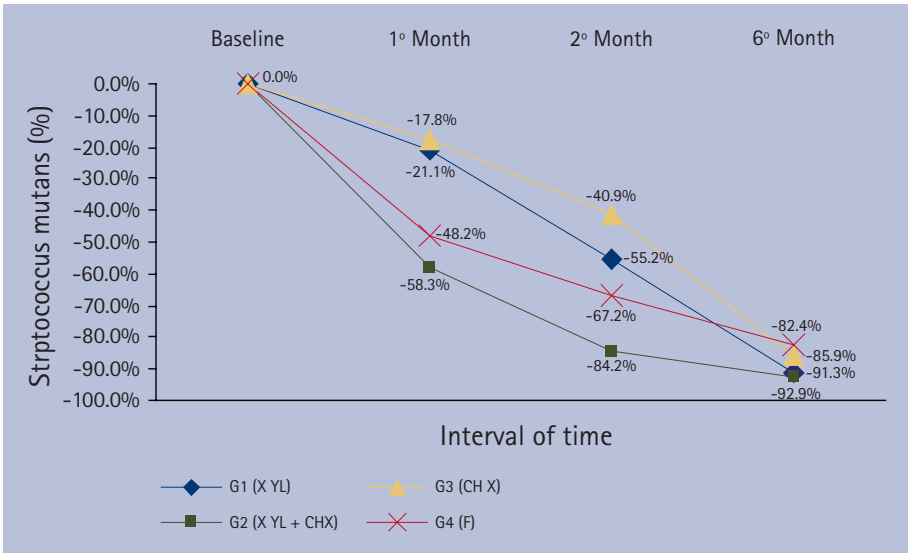

Fig. 3 Influence of different treatment types on the Streptococcus mutans levels in 6–8-year-old children

of the children had low levels, 22% had moderate levels and 1% had no *S. mutans*. None of the children had high or very high levels of this micro-organism at the end of the study. *S. mutans* was gradually suppressed in all groups during the study and all the comparisons were statistically significant ($p < 0.05$) (Fig. 3). In the group G2 (CHX + XYL), however, it should be highlighted that there were reductions of 58%, 84%, and 92% in the *S. mutans* levels in the first, second and sixth months, respectively.

DISCUSSION

The first micro-organisms described as being involved in the formation of a pellicle adhering to dental surfaces were *S. mitis, S. oralis,* and *S. sanguinis*. After pellicle formation, the growth of biofilm begins. During biofilm formation, *S. mutans* becomes an important factor in modification of the biofilm into a cariogenic form.[14] Biofilm development starts shortly after a tooth surface is cleaned and covered by a conditioning film of salivary proteins and glycoproteins, becoming complex and matured. *S. mutans* has been described as the most important bacteria related to the aetiology of dental caries. This bacterium has basic properties of cariogenicity necessary for dental caries processes.[23]

Furthermore, *S. mutans* is able to demineralise hydroxyapatite, thus demonstrating its relevance in the development of caries.[1,8,24] This key role played by *S. mutans* in the formation of cariogenic biofilm is a hypothesis already accepted in dentistry, since it is the best explanation for caries aetiology.[25] Therefore, reducing the levels of this micro-organism in the oral cavity seems to be crucial for controlling caries in general.

Chlorhexidine (CHX) is known to be an excellent antimicrobial agent, particularly in reducing the levels of *S. mutans*, which is very sensitive to this substance.[4,6,8,11,26] At low concentrations, chlorhexidine can be considered a bacteriostatic agent[3] because it affects the metabolic activity of micro-organisms by acting on cell membranes, causing intracellular disruption.[27] However, at high concentrations, this product seems to affect the cellular content, thus being bactericidal.[8,28] In our study, chlorhexidine was applied in groups G2 (CHX + XYL) and G3 (CHX), showing large reductions in the biofilm levels at different periods of time ($p < 0.05$). In the analysis performed at the end of the study (sixth month), such reductions were 46.9% and 38.9%, respectively. With regard to *S. mutans* levels, the largest reduction was also observed in group G2 (CHX + XYL) (91.3%), whereas group G3 (CHX) had 85.9%, a value close to that from G1 (XYL) (91.3%). These results support other previous studies[3,26,28] which showed that individuals using chlorhexidine had the best results regarding biofilm reduction compared to those using either fluoride or only xylitol (G4 and G1, respectively).

Chlorhexidine has the ability to remain inside the oral cavity for a long period of time, mainly as varnish, a finding already proved by other authors.[3,4,26,29] In our study, we observed that in those groups where chlorhexidine was one of the agents (G2 and G3), the levels of *S. mutans* continued to decrease even during the three month interval between the second and sixth month evaluations. This finding confirms its substantivity. With regard to the amount of chlorhexidine in the varnish, different concentrations can be used, although 1% chlorhexidine yields better results according to the literature.[3,26,29–31] The varnish vehicle was also justified due to the recommendation of not using gels or mouthwashes for young children.

With regard to the reduction of biofilm levels, group G2 (CHX + XYL) showed the best results at the first and sixth months with reductions of 40.32% and 46.9%, respectively. Some authors have reported that chlorhexidine plays a relevant role in this reducing process.[4,10,26–29,31] However, xylitol also reduces both biofilm and *S. mutans* levels when added to a patients' diet as a sweetener, thus preventing early development of caries.[11,13,32,33] The results found in the present study corroborate these findings, since groups G1 (XYL) and G2 (CHX + XYL) showed enhanced reduction in biofilm levels in the sixth month compared to other groups ($p < 0.05$).

The use of chewing gum as a means of administering xylitol was important for achieving co-operation among the children, in addition to remaining for a longer time in the oral cavity, thus prolonging its effect.[12,14,17] Nevertheless, the frequent use of xylitol chewing gum should be controlled to prevent the occurrence of microbial resistance.[15–17] This finding may be related to factors such as formation of acids resulting from glucose and/or polysaccharides resulting from sucrose.[33] The optimum length of time for using xylitol should be further evaluated in order to avoid or minimise the emergence of microbial resistance.[15,34]

Nausea and emesis during the period of study were observed in one child, who was immediately excluded from group G1 (XYL). This unique fact may be the result of inadequate use of xylitol chewing gum, since the child was given material to be used over 30 days. In spite of all recommendations, this child might have consumed more than 5-10 g/kg/day, which might explain the symptoms.[8] Future studies need to investigate the safety of using these combinations, since another possibility is that the subject may have consumed a dosage lower than 5-10 g/kg/day, but was more susceptible to present reactions to these drugs.

Although the results in group G4 (fluoride) were found to be inferior compared to other groups, this agent has antimicrobial action when used in high concentrations, usually higher than that in fluoride-containing products for clinical purposes. The good results observed may be related to the remineralisation provided by fluoride.[35,36]

CONCLUSION

Based on the results found, we can conclude that combining 1% chlorhexidine with xylitol chewing gum was efficient and superior to single treatments in controlling biofilm and suppressing *S. mutans*. Further investigation should be carried out in order to confirm the results and develop strategies for using such products to prevent dental caries.

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