

Effect of different 1% chlorhexidine varnish regimens on biochemical composition of the dental biofilm

Efeito de diferentes posologias do verniz de clorexidina a 1% na composição bioquímica do biofilme dentário

Abstract

Purpose: This investigation evaluated in a randomized controlled study the effect of different 1% chlorhexidine varnish (1%CHX-V) regimens on biochemical composition of the dental biofilm.

Methods: Subjects with mutans streptococci $\geq 10^5$ CFU/mL saliva, were allocated into 4 groups: A - single 1% CHX-V application; B - 1% CHX-V was applied once daily on 3 consecutive days; C - 1% CHX-V was applied 3 times with an interval of 4 days between each application; and D - placebo varnish was applied once daily on 3 consecutive days. Dental biofilm samples were collected at baseline and 1, 4, and 8 weeks after the final varnish application and evaluated for inorganic phosphate and insoluble polysaccharide concentrations.

Results: No difference was observed between all groups during the different experimental periods in relation to biochemical composition. After 1 week, a significant increase was observed in inorganic phosphate concentration, returning to baseline values after 4 weeks. All groups showed a significant decrease in insoluble polysaccharide concentration for more than 8 weeks after treatment.

Conclusion: Varnish application resulted in modification of the dental biofilm toward a less cariogenic one.

Key words: Chlorhexidine; dental biofilm; inorganic phosphate; insoluble polysaccharide

Resumo

Objetivo: Este estudo avaliou o efeito de diferentes posologias do verniz de clorexidina a 1% (V-CHX1%) na composição bioquímica do biofilme dental.

Metodologia: Voluntários com níveis de *Streptococcus mutans* $\geq 10^5$ UFC/mL saliva, foram divididos em 4 grupos: A - uma única aplicação do V-CHX1%; B - aplicações de 1x/dia do V-CHX1% durante 3 dias consecutivos; C - 3 aplicações de 1x/dia do V-CHX1% com intervalos de 4 dias entre elas; D - aplicações de 1x/dia do verniz placebo durante 3 dias consecutivos. Amostras de biofilme dental foram coletadas, no início e após 1, 4 e 8 semanas das aplicações dos vernizes, e analisadas quanto às concentrações de fosfato inorgânico (P_i) e polissacarídeos insolúveis (PI).

Resultados: Não foram observadas diferenças, em relação à composição bioquímica, entre os grupos durante os diferentes períodos experimentais. Foi observado, após 1 semana, um aumento significativo nas concentrações de P_i , retornando aos valores iniciais após 4 semanas. Todos os grupos apresentaram uma redução significativa na concentração de PI por mais de 8 semanas após os tratamentos.

Conclusão: A aplicação do verniz resultou em uma modificação do biofilme dentário tornando-o menos cariogênico.

Palavras-chave: Clorexidina; biofilme dental; fosfato inorgânico; polissacarídeos insolúveis

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Received: May 10, 2010

Accepted: November 22, 2010

Conflict of Interest Statement: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

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Introduction

One strategy for controlling dental caries in patients with high levels of cariogenic microorganisms is their suppression by chemotherapeutic agents (1-3). Chlorhexidine (CHX) is an antimicrobial agent used extensively in dentistry. The importance of mutans streptococci (MS) in the etiology of dental caries has been clearly established (4,5) and among dental biofilm microorganisms, MS are one of the most sensitive to CHX (6).

Although most studies have focused on the microbiological composition of the dental biofilm, the concentration of calcium, inorganic phosphate, fluoride, and extracellular insoluble polysaccharides is also related to its cariogenicity. The dental biofilm formed in the presence of frequent sucrose exposition (8 times per day) showed 3 times more extracellular insoluble polysaccharide and lower inorganic concentration of calcium, phosphate, and fluoride than the control group (no sucrose exposure) (7). Mattos-Graner et al. (8) showed that caries incidence in children is more dependent on the capacity of MS to synthesize extracellular insoluble polysaccharide than on their number in the dental biofilm.

There are several vehicles used for CHX delivery, such as dentifrices, solutions, gels and varnishes (9-12). The latter were specially designed as a slow-release CHX agent, with the purpose of maintaining therapeutic concentrations over a period of several days (13). The varnish containing 1% CHX has been investigated the most. However, there is no agreement with respect to the total number of 1% CHX varnish applications (ranging from 1 to 3) and the interval between them (ranging from 1-7 days or monthly) (14-16).

No consensus has been reached in the literature about the ideal regimen of 1% CHX varnish applications and there is no published data about its effect on the biochemical composition of the dental biofilm. The aim of the present investigation was to use a randomized controlled study to evaluate the effect of different regimens of topical application of a varnish containing 1% CHX on the biochemical composition (inorganic phosphate and insoluble polysaccharide) of the dental biofilm.

Methods

One hundred and fifty-eight schoolchildren were screened for MS salivary levels. Those patients with $MS \geq 10^5$ CFU/mL saliva (colony forming units per milliliter of saliva) in 2 saliva samples were invited to participate in the study. Sixty subjects, 11-16 years old (mean \pm SD, 12.9 \pm 1.39 years old), were stratified according to the MS levels and randomly allocated in four groups according to varnish treatment. Five of the children left school. Therefore, the final sample comprised 55 subjects. The product used in this study was a varnish containing 1% chlorhexidine and 1% thymol (Cervitec[®], Schaan, Liechtenstein). The participants were divided in groups with the following treatments: group A

(n=14): single application of 1% CHX varnish; group B (n=14): 1% CHX varnish was applied once daily on three consecutive days; group C (n=15): 1% CHX varnish was applied 3 times with an interval of four days between each application; and group D (n=12): placebo varnish (without CHX and thymol) was applied once daily on three consecutive days. All participants were selected from a public school and had access to fluoridated public water supply (0.7 ppm F) and fluoride dentifrice (1500 ppm F). The study was approved by the Ethics Committee of the Faculty of Dentistry of the Federal University of Rio Grande do Sul (protocol number 08/04). All participants' parents signed an informed consent.

Prior to varnish application, the teeth were professionally cleaned, the interdental areas were flossed, each quadrant was isolated with cotton rolls and dried. The varnish was then applied on all teeth surfaces using a small brush supplied by the manufacturer. The patients were instructed to avoid eating for 3 h and brushing their teeth for 24 h, in accordance with the manufacturer's instructions.

Dental biofilm samples were collected at baseline and 1, 4, and 8 weeks after the final varnish application. Subjects were asked to refrain from oral hygiene of the posterior teeth 48 h before the sampling. Dental biofilm samples were collected with a sterile curette along the supragingival margins of posterior tooth surfaces (labial, lingual, and interdental sites). All analyses were blindly performed by the same researcher.

Dental biofilm was dehydrated in a vacuum desiccator over phosphorus pentoxide (P_2O_5) and the dry weight was obtained (± 0.01 mg, Sartorius BP 210D, Sartorius, Goettingen, Germany). For inorganic phosphate (P_i) and insoluble polysaccharide (IP) analysis, 0.5 M hydrochloric acid (HCl) was added to the microtube (0.1 mL HCl/mg dry weight dental biofilm). After 3 h at room temperature under constant agitation, the same volume of TISAB II pH 5.0 (containing 20 g NaOH/L) was added as a buffer. The samples were centrifuged for 10 min at 14000 rpm (Eppendorf 5410, Eppendorf AG, Hamburg, Germany) and the supernatant retained for determination of P_i . To the precipitate, 1.0 M NaOH (0.2 mL/mg dry weight plaque) was added. After 3 h at room temperature under constant agitation, the samples were centrifuged and the concentration of IP was determined in the supernatant (17). P_i was determined colorimetrically (18) and IP was determined using the phenol-sulfuric method (19).

The P_i and IP concentrations were evaluated using ANOVA for repeated measures and a Tukey test using SAS version 9.1 (SAS, Cary, North Carolina, USA). The level of significance was set at 5%.

Results

The results were expressed as micrograms of inorganic phosphate per milligram of dental biofilm (μ g P_i /mg dental biofilm) and micrograms of insoluble polysaccharide per milligram of dental biofilm (μ g IP/mg dental biofilm).

Table 1. Mean \pm standard deviation of inorganic phosphate concentration in dental biofilm at baseline (BL) and 1, 4, and 8 weeks after different 1% CHX varnish regimens and placebo varnish application ($\mu\text{g P}_i/\text{mg}$ dental biofilm).

Group*	BL	1 wk	4 wk	8 wk
A	6.63 \pm 4.73 ^{Ba}	10.19 \pm 6.28 ^{Aa}	7.59 \pm 4.83 ^{Ba}	6.33 \pm 2.58 ^{Ba}
B	7.57 \pm 3.62 ^{Ba}	10.40 \pm 8.09 ^{Aa}	7.06 \pm 3.42 ^{Ba}	7.78 \pm 4.82 ^{Ba}
C	5.56 \pm 2.41 ^{Ba}	8.05 \pm 5.57 ^{Aa}	7.78 \pm 4.15 ^{Ba}	6.58 \pm 2.55 ^{Ba}
D	7.63 \pm 5.98 ^{Ba}	10.16 \pm 6.29 ^{Aa}	7.65 \pm 7.74 ^{Ba}	7.50 \pm 4.45 ^{Ba}

* Group A: 1 varnish application; group B: once daily, 3 consecutive days; group C: 3 times, at 4-day intervals; group D: placebo varnish. Means followed by different capital letters (line) and small letters (column) differ significantly, ANOVA for repeated measures and Tukey test ($P < 0.05$).

Table 2. Mean \pm standard deviation of insoluble polysaccharide concentration in dental biofilm at baseline (BL) and 1, 4, and 8 weeks after different 1% CHX varnish regimens and placebo varnish application ($\mu\text{g IP}/\text{mg}$ dental biofilm).

Group*	BL	1 wk	4 wk	8 wk
A	44.66 \pm 21.69 ^{Aa}	38.79 \pm 20.73 ^{Ba}	32.08 \pm 9.71 ^{Ca}	37.90 \pm 9.74 ^{BCa}
B	47.42 \pm 9.77 ^{Aa}	44.18 \pm 11.16 ^{Ba}	30.12 \pm 12.76 ^{Ca}	36.08 \pm 9.04 ^{BCa}
C	50.63 \pm 17.15 ^{Aa}	39.81 \pm 17.34 ^{Ba}	32.37 \pm 11.29 ^{Ca}	33.49 \pm 10.83 ^{BCa}
D	50.86 \pm 12.00 ^{Aa}	35.75 \pm 9.47 ^{Ba}	27.67 \pm 9.40 ^{Ca}	28.05 \pm 6.79 ^{BCa}

* Group A: 1 varnish application; group B: once daily, 3 consecutive days; group C: 3 times, at 4-day intervals; group D: placebo varnish. Means followed by different capital letters (line) and small letters (column) differ significantly, ANOVA for repeated measures and Tukey test ($P < 0.05$).

At baseline, there was no statistically significant difference of P_i and IP between the groups in the dental biofilm. After 1 week, a significant increase in P_i concentration was observed in all groups (A, B, C, and D), returning to baseline values after 4 weeks. No difference was observed between all groups during the different experimental periods (Table 1).

All groups showed a significant decrease in IP concentrations for more than 8 weeks after treatment. No difference was observed between all groups during the different experimental periods (Table 2).

Discussion

The present study was performed to investigate the effect of different regimens of a varnish containing 1% CHX on the biochemical composition of dental biofilm. The P_i and IP concentrations were evaluated. Three regimens were tested: (i) single application; (ii) once daily application, on three consecutive days; (iii) 3 applications with an interval of four days between each. No difference in the biochemical composition of the dental biofilm could be observed between the different groups. Treatment with placebo and CHX varnish demonstrated the same effect on P_i and IP concentrations. All treatments showed a transitory increase in the P_i biofilm concentration and a decrease in the IP biofilm concentration.

The presence of MS in dental biofilm has been used as an indicator of cariogenic biofilm (4,5). Studies that evaluated CHX have used the levels of MS to investigate its cariogenic effect (9,11,12,16). In relation to 1% CHX varnish, considering the microbiological aspects, there is no consensus in the literature on the ideal regimen. It has been applied in intensive modes with 2-3 applications within a 2-week period (9,14,20) or once a month (15,20). Intensive

short-term 1% CHX gel application (3-4 daily applications on two consecutive days) (11) showed MS reduction similar to less intensive treatment for a prolonged period of time (10-14 days) (21,22). Frequent CHX administration has been justified by the speculation that a repeated consecutive treatment strategy could increase the suppressive effect on MS levels by inhibiting re-growth. As a consequence, it would favor tooth surface colonization by other microorganisms, promoting modifications in the cariogenicity of the dental biofilm.

Few studies have been conducted to compare 1% CHX varnish regimens (12,23). Although Twetman and Petersson (12) have suggested that intensive treatment is more effective in reducing MS, Ribeiro et al. (23) have observed that repeated applications of 1% CHX varnish do not increase its effects and 1% CHX varnish caused a slight, short-term reduction in MS levels.

Most studies have evaluated the effect of CHX on the microbiological composition of the dental biofilm (24). However, it has been suggested that the structure of the biofilm formed can be more relevant than the number of cariogenic microorganisms with respect to caries development (8). The inorganic composition of the dental biofilm (calcium, inorganic phosphate, fluoride) is important in the equilibrium between demineralization and remineralization of enamel. The presence of IP in the dental biofilm increases its porosity and favors the adherence and accumulation of microorganisms on the tooth surface. The cariogenicity of the dental biofilm has been correlated with the IP (25). Cury et al. (7) demonstrated that the dental biofilm formed in the presence of sucrose showed higher IP and lower inorganic concentration, suggesting that these are the characteristics of a cariogenic dental biofilm. The present study demonstrated a significant alteration in biochemical composition of the

dental biofilm with an increase in the P_i concentration 1 week after the treatments. The source of this additional amount of P_i found in the dental biofilm is from oral environment, because the saliva is supersaturated with respect to enamel mineral (calcium and inorganic phosphate). These ions are important to maintain the environment supersaturated when biofilm pH falls. These ions can diffuse from saliva to biofilm forming a mineral reservoir, making this biofilm less cariogenic (7,25).

The literature shows that there is a close relation between concentrations of Ca and P_i . When low concentration of Ca is observed, simultaneously a low concentration of P_i is also verified (7,25). Therefore the analysis of the only one ion (P_i), performed in this study, was adequate to indicate a low inorganic composition of the dental biofilm.

High concentration of insoluble polysaccharides in dental biofilm may increase its porosity and cariogenicity (26,27). In the present study, a significant decrease in IP concentrations for more than 8 weeks after treatments was verified in the dental biofilm. These results suggest a change in the biofilm extracellular matrix, which contains polysaccharides, reducing its cariogenicity.

However, these modifications were also observed in the group treated with the placebo varnish, without the two active components (thymol and CHX). These results suggest that another component present in the varnish could be influencing the alterations of the biochemical composition of the dental biofilm and this effect could not be attributed

to the active components CHX and thymol. The data of the three experimental groups show an increase in P_i levels and decrease in IP levels after varnish treatment. One could conclude from these results that the CHX varnish decreased the cariogenicity of the dental biofilm. However, analyzing these data together with the control group, no difference was observed between the groups

Conclusion

The present study demonstrated that varnish application resulted in modification of the dental biofilm toward a less cariogenic one.

Acknowledgements

This study was supported by a grant from the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES-Brazil) and *Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS/0412881).

The authors thank the schoolchildren and their parents for their valuable participation. Thanks to Ivoclar-Vivadent (Schaan, Lichtenstein) for the supply of Cervitec® and placebo varnish.

This manuscript is derived from a thesis submitted by the first author to the Faculty of Dentistry, UFRGS (Porto Alegre, RS, Brasil) as a partial fulfillment of the requirements for the Master's Degree in Clinical Dentistry.

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