

# Comparison of mouthrinses containing chlorhexidine and other active agents with chlorhexidine mouthrinse-gel: effects on de novo plaque formation

## Comparação de enxaguatórios de clorexidina e outros agentes ativos com enxaguatório-gel de clorexidina sobre a formação de biofilme

### Abstract

**Purpose:** Antiseptic mouthrinses containing chlorhexidine (CHX) seem to be the most effective chemical agents for plaque control. The CHX concentration is usually 0.12% or 0.20%, but formulations with lower concentrations of CHX (0.05%) in combination with other active agents such as sodium fluoride (0.05%) or cetyl pyridinium chloride (0.05%) (Cpc) are available. The aim of this study was to compare plaque formation at 24 hours after the use of 0.1% CHX mouthrinse-gel and mouthrinses containing 0.12% and 0.05% CHX plus other active agents.

**Methods:** A controlled, randomized, double-blind, crossover clinical trial was designed. Thirty subjects underwent four consecutive experimental phases with four treatments: CHX 0.1% + hydroxymethylcellulose 2.5% (HMC), CHX 0.12% + alcohol, CHX 0.12% + 0.05% sodium fluoride, and CHX 0.05% + 0.05% Cpc. On the day of study, the subjects discontinued all other oral hygiene habits and were randomly assigned for treatment with the experimental mouthwash. Each experimental phase was preceded by a 28-day washout period. Plaque formation was recorded after one undisturbed day.

**Results:** Formulations of CHX 0.12% with alcohol and sodium fluoride and CHX 0.1% + HMC 2.5% reduced de novo plaque formation to a greater extent than the mouthwash with CHX 0.05% + Cpc ( $P < 0.05$ ).

**Conclusion:** The 0.1% CHX gel presents an anti-plaque efficacy similar to that of mouthwashes containing 0.12% CHX and other active agents, and was more effective at inhibiting plaque formation than the mouthwash containing 0.05% CHX with Cpc.

**Key words:** Chlorhexidine; mouthrinse-gel; plaque index; mouthrinses; plaque inhibition

### Resumo

**Objetivo:** Enxaguatórios contendo clorexidina (CHX) parecem ser os mais eficazes agentes químicos para controle de placa. A concentração de CHX geralmente é de 0.12% ou 0.20%, mas formulações de clorexidina em concentrações mais baixas (0.05%) com outros agentes ativos, tais como fluoreto de sódio (0.05%), cloreto de cetilpiridínio (0.05%) estão disponíveis atualmente. Este estudo teve por objetivo comparar a eficácia de gel de clorexidina a 0.1% com enxaguatórios contendo 0.12% e 0.05% CHX e outros agentes ativos, em relação ao crescimento de placa bacteriana em 24 horas.

**Metodologia:** Um ensaio clínico com delineamento cruzado, randomizado, controlado e duplo-cego foi concebido. Trinta sujeitos foram submetidos a quatro fases experimentais consecutivas com quatro tratamentos (bochechos): CHX 0.1% + HMC 2.5%, 0.12% CHX + álcool, CHX 0.12% + 0.05% de fluoreto de sódio e CHX 0.05% + 0.05% cloreto de cetilpiridínio. No dia de estudo, os indivíduos interromperam todas as medidas de higiene oral e foram tratados os bochechos experimentais aleatoriamente. Cada fase experimental foi precedida por um período de 28 dias de *washout*. A formação de placa foi registrada depois de um dia sem distúrbio de formação.

**Resultados:** As formulações de CHX 0.12% com álcool e fluoreto de sódio e gel foram eficientes em retardar a formação de placa e foram superiores ( $P < 0,05$ ) para a CHX 0.05% com cloreto de cetilpiridínio.

**Conclusão:** O gel de clorexidina 0,1% para bochechos apresenta uma eficácia antiplaca semelhante aos bochechos de 0.12% CHX com outros agentes ativos, sendo superior aos bochechos de clorexidina a 0.05% com cloreto de cetilpiridínio.

**Palavras-chave:** Clorexidina; gel de bochechos; índice de placa; enxaguatórios; inibição de placa

Ismael Yévenes<sup>a</sup>  
Sonia Rivera Alvarez<sup>b</sup>  
Miguel Neira Jara<sup>a</sup>  
Paula Maass Wolfenson<sup>a</sup>  
Ljubica Petrasic Smith<sup>b</sup>

<sup>a</sup> Department of Chemistry, Dental School, University of Chile, Santiago, Chile

<sup>b</sup> Department of Restorative Dentistry, Dental School, University of Chile, Santiago, Chile

### Correspondence:

Ismael Yévenes  
Department of Chemistry, Dental School  
University of Chile  
Sergio Livingstone 943 (ex-Olivos), Independencia  
Santiago, Chile  
E-mail: iyevenes@odontologia.uchile.cl

Received: March 9, 2009

Accepted: July 15, 2009

## Introduction

Several products for chemical inhibition of microbial plaque are available in the dental market. Compounds derived from bisbiguanide including chlorhexidine digluconate (CHX) and alexidina are the most effective agents currently used (1). CHX is considered the gold standard to which other antiplaque agents are compared. It is a cationic chlorophenyl bisbiguanide with bacteriostatic properties and low mammalian toxicity (2). The active ingredient was synthesized by ICI laboratory in 1954 and initially used in Dentistry as an antiseptic for pre-surgical and endodontic treatments (3); its use as an anticalculus and antiplaque agent dates from 1969 (4). The agent was approved by the American FDA (Food and Drug Administration) and is marketed in most countries worldwide (5). CHX has been demonstrated to have effective plaque inhibition effects when used alone or in combination with mechanical cleaning procedures (6), and can be delivered in different vehicles, such as mouthrinses (7), gel (8), and spray (9,10). Mouthrinses with CHX concentrations of 0.12% and 0.20% are most commonly used, but the prescribed rinse regimen and amount may vary.

Previous studies have confirmed the effectiveness of CHX in the prevention of plaque formation (5,10-12). Clinical trials showed that the anti-plaque effect can be observed with a minimum dose of 5-6 mg twice a day. At higher doses, the efficiency curve tends to flatten, resulting in small increases in anti-plaque efficiency (13). The reduced efficacy observed for the 0.05% concentration was solved by associating CHX with other active agents such as cetyl pyridinium chloride (Cpc). Cpc is known to have some antibacterial activity; however, the incorporation of Cpc in a CHX mouthrinse does not guarantee an additive effect since modifications of the formulation could also affect its action (14). Mouthrinses with 0.05% CHX have demonstrated *in vitro* and *in vivo* anti-plaque activity in several studies and in six-month clinical trials (15,16), but the effectiveness of CHX in combination with other active agents is still not clear.

The purpose of this clinical study was to compare and evaluate the anti-plaque activity of an aqueous 0.1% CHX gel with that of mouthrinses containing CHX at two concentrations (0.12% and 0.05%) as well as other active agents; the outcome measure was plaque formation using the one-day-without-brushing model.

## Methods

### Study Population

A total of 30 subjects from the Faculty of Dentistry of the University of Chile were invited to participate voluntarily in the project. All subjects received oral and written instructions and information about the products, objectives, reasons, duration, and possible risks of the study procedures, and signed an informed consent form. The inclusion criteria were adult patients, older than 18 years old, systemically healthy, and having at least 20 teeth. The exclusion criteria were patients with cavitated caries, periodontal pockets larger than 1.5 mm, orthodontic appliances or removable prostheses, allergies to erythrosine or CHX, use of antibiotics in the past 3 months and use of other drugs that might alter normal gingival health.

### Study Design and Clinical Procedures

The study was a double-blind, randomized, crossover clinical trial. At baseline, the volunteers brushed their teeth using toothpaste without any active ingredient for 2 min. Microbial plaque on the dental surfaces was stained with erythrosine solution, and oral prophylaxis using a rubber cup without polishing paste was performed to ensure that the teeth were free of plaque, stains and calculus.

The antimicrobial products tested are described in Table 1. Each subject received a single number and was randomly assigned to the experimental groups. During the four one-day trials, with a 4-week interval between trials, the volunteers rinsed their mouths according to the assigned random sequence of treatments: 0.12% CHX with alcohol, 0.12% CHX+0.05% NaF, 0.05% CHX+0.05% with Cpc (with double volume to match the total applied dose of CHX), and 0.1% CHX gel.

Each subject received oral and written instructions on the use of mouthrinses and was not allowed to eat or drink anything for 30 min following the application of the mouthrinses. A fact sheet was made available for recording of dental hypersensitivity, gingival irritation, or any other comments regarding the use of mouthrinses.

Oral hygiene was suspended for 24 hours, and accumulated plaque was revealed with erythrosine. All measurements were conducted under the same conditions by a qualified, experienced examiner who had participated in similar

**Table 1.** Technical description of the mouthrinses evaluated.

Mouthrinses	Composition	Delivery protocol
Perio-Aid® Laboratory DENTAID SA	0.12% CHX + alcohol	15 mL per 3 min for once
Cariax® Laboratory Chile Pasteur SA	0.12% CHX + 0.05% NaF	15 mL per 3 min for once
Perio maintenance-Aid® Laboratory DENTAID SA	0.05% CHX + 0.05% Cpc	30 mL per 3 min for once
Colutoriogel® Mouthrinse-gel Chemistry Laboratory, Faculty of Dentistry, Un. of Chile	0.1% CHX + 2.5% HMC (alcohol free)	15 mL per 3 min for once

studies using the plaque index (PI) of Quigley and Hein (17), modified by Turesky et al. (18) After each trial period, the usual oral hygiene habits were resumed.

### Statistical analyses

The plaque index data from the treatment groups were analyzed using the Wilcoxon test for non-parametric data. Inter-treatment data were analyzed using paired Student *t*-tests and analysis of variance at the 0.05 significance level and 95% confidence interval.

## Results

Table 2 shows the plaque index (PI) values for each type of CHX treatment. Comparison between the 0.12% CHX mouthrinses and 0.1% CHX mouthrinse-gel showed no significant differences (PI=0.66, 0.58, and 0.62;  $P>0.05$ ), but the performance of these treatments was different from that of the 0.05% CHX mouthrinse ( $P=0.02$  and  $P=0.015$ , respectively). The highest PI was recorded for 0.05% CHX mouthrinse with 0.05% Cpc (PI=1.06).

Table 3 shows the mean PI values for the different mouthrinses used. Significant differences ( $P<0.05$ ) were found between 0.05% CHX mouthrinse with Cpc (Perio maintenance-Aid®) and 0.12% CHX mouthrinse with alcohol (Perio-Aid®), 0.1% CHX gel alcohol free/2.5% HMC, or 0.12% mouthrinse/0.05% NaF (Cariax®). The formulations containing 0.12% CHX with and without alcohol and the 0.1% CHX gel with 2.5% HMC were equally effective in inhibiting plaque regrowth; the lowest efficacy was recorded for the formulation containing 0.05% CHX with Cpc.

No dental hypersensitivity, gingival irritation, or other comments regarding the use of mouthrinses and mouthrinse-gel were reported during the study.

## Discussion

This clinical study aimed to compare the inhibition of plaque formation by a CHX mouthrinse-gel with that by mouthrinses containing 0.12% or 0.05% CHX and other active agents. The experimental protocol consisting of no oral hygiene for one day has been used previously (19,20) because plaque formation can be measured in a short period without causing detectable harm to the study subjects. Additionally, this technique is a useful and fast method for screening potential plaque inhibitory agents and experimental formulations. However, long-term studies on the use of these mouthrinses should also be performed.

Previous studies compared the anti-plaque effects of CHX mouthrinses with and without alcohol and other active agents, and CHX was found to be the most powerful antiseptic agent against dental biofilm and gingivitis (11,12,14). Alcohol is commonly added to mouthrinses because it (1) dissolves other components in the formulation, (2) has antiseptic properties, (3) stabilizes certain active ingredients, and (4) improves the product shelf-life (14). However, some studies found that the presence of alcohol in oral hygiene products used for long periods may be related to an increased risk of developing oral cancer and mucositis (16,21). In the present study, no complaints were recorded after the use of CHX mouthrinses; however, this is most likely due to the short study period.

**Table 2.** Plaque index (mean and standard deviation) after 24 hours according to the mouthrinses tested.

Mouthrinses	Composition	Mean Plaque Index	Standard deviation
Perio-Aid® Laboratory DENTAID SA	0.12% CHX + alcohol	0.66	0.16
Cariax® Laboratory Chile Pasteur SA	0.12% CHX + 0.05% NaF	0.58	0.14
Perio maintenance-Aid® Laboratory DENTAID SA	0.05% CHX + 0.05% Cpc	1.06	0.26
Colutoriogel® Mouthrinse-gel Chemistry Laboratory, Faculty of Dentistry, Un. of Chile	0.1% CHX + 2.5% HMC (alcohol free)	0.62	0.15

**Table 3.** Application of paired-samples T-test to Index Plaque.

	0.12% CHX + alcohol*	0.12% CHX + 0.05%†	0.05% CHX + Cpc‡	0.1% CHX + 2.5% HMC§
0.12% CHX + alcohol*	–	$P=0.77$	$P=0.015^{  }$	$P=0.91$
0.12% CHX + 0.05% NaF†	$P=0.77$	–	$P=0.018^{  }$	$P=0.87$
0.05% CHX + Cpc‡	$P=0.015^{  }$	$P=0.018^{  }$	–	$P=0.038^{  }$
0.1% CHX + 2.5% HMC§	$P=0.91$	$P=0.87$	$P=0.038^{  }$	–

\* 0.12% CHX mouthrinse/alcohol (Perio-Aid®)

† 0.12% CHX mouthrinse/NaF (Cariax®)

‡ 0.05% CHX mouthrinse/Cpc (Perio maintenance-Aid®)

§ 0.1% CHX mouthrinse-gel/alcohol-free/2.5% HMC.

|| Significant differences

Some studies reported similar effects for CHX mouthrinses with or without alcohol (22,23), suggesting that the addition of different active chemical compounds to CHX mouthrinses without alcohol might help to enhance their anti-plaque and anti-gingivitis effects. The combination of 0.12% CHX with sodium fluoride was shown to have a reduced ability to delay new plaque formation (24), but in the present study, this was not observed, probably because we evaluated plaque the accumulated over a 24-hour period.

Other studies have suggested the reduction of CHX concentration to avoid common side effects such as stained teeth and tongue as well as burning and irritation of soft tissues (16,25), given that efficacy of the mouthrinses is likely derived from the overall qualities of the formulation and not solely on the presence of CHX. However, our results showed that 0.05% CHX combined with Cpc had the lowest effect on plaque growth, even when used with

twice the volume to match the CHX dose applied in the other conditions. However, CHX doses seem to be more important for the efficacy of mouthrinse formulations than the CHX concentration (14).

The advantages of the mouthrinse-gel have been described previously (19,26). The present study confirmed, within the limitations of this experimental model, that the anti-plaque activity of CHX mouthrinse-gel was similar to that obtained with 0.12% CHX mouthrinse alone or with other active agents, and was higher than that of 0.05% CHX.

## Conclusions

The 0.1% CHX mouthrinse-gel showed antiplaque efficacy similar to that of mouthrinses containing 0.12% CHX and other active agents, and greater efficacy than the 0.05% CHX mouthrinse supplemented with cetyl pyridinium chloride.

## References

- Baker PJ, Coburn RA, Genco RJ, Evans RT. Structural determinants of activity of chlorhexidine and alkyl bisbiguanides against the human oral flora. *J Dent Res* 1987;66:1099-106.
- Jones CG. Chlorhexidine: is it still the gold standard? *Periodontol* 2000 1997;15:55-62.
- Clarke JR, Blacklock Z. The use of chlorhexidine and "savlon" for decontamination of "medihaler" mouthpieces. *Med J Aust* 1965;13:827-8.
- Schroeder HE, Hirzel HC. A method of studying dental plaque morphology. *Helv Odontol Acta* 1969;13:22-7.
- Löe H. Oral hygiene in the prevention of caries and periodontal disease. *Int Dent J* 2000;50:129-39.
- Slot DE, Lindeboom R, Rosema NA, Timmerman MF, van der Weijden GA. The effect of 0.12% chlorhexidine dentifrice gel on plaque accumulation: a 3-day non-brushing model. *Int J Dent Hyg* 2007;5:45-52.
- Yévenes IL, Reyes JY, Campos NP, Saragoni VF. The effects of a chlorhexidine rinses on the development of plaque and antibacterial. *Av Periodon Implantol* 2003;15:19-24.
- Porras R, Anderson GB, Caffesse R, Narendran S, Trejo PM. Clinical response to 2 different therapeutic regimens to treat peri-implant mucositis. *J Periodontol* 2002;73:1118-25.
- Clavero J, Baca P, Junco P, González MP. Effects of 0.2% chlorhexidine spray applied once or twice daily on plaque accumulation and gingival inflammation in a geriatric population. *J Clin Periodontol* 2003;30:773-7.
- Pizzo G, Guiglia R, Imburgia M, Pizzo I, D'Angelo M, Giuliana G. The effects of antimicrobial sprays and mouthrinses on supragingival plaque regrowth: a comparative study. *J Periodontol* 2006;77:248-56.
- Paraskevas S, Rosema NA, Versteeg P, Van der Velden U, Van der Weijden GA. Chlorine dioxide and chlorhexidine mouthrinses compared in a 3-day plaque accumulation model. *J Periodontol* 2008;79:1395-400.
- Takenaka S, Trivedi HM, Corbin A, Pitts B, Stewart PS. Direct visualization of spatial and temporal patterns of antimicrobial action within model oral biofilms. *Appl Environ Microbiol* 2008;74:1869-75.
- Jenkins S, Addy M, Newcombe RG. A comparison of cetylpyridinium, triclosan and chlorhexidine mouthrinses formulations for effects on plaque regrowth. *J Clin Periodontol* 1994;21:441-4.
- Van Strydonck DAC, Timmerman MF, van der Velden U, van der Weijden GA. Plaque inhibition of two commercially available chlorhexidine mouthrinses. *J Clin Periodontol* 2005;32:305-9.
- Calsina-Gomis G, Serrano-Granger J. Are there any differences between different concentrations of chlorhexidine? Comparison of mouthrinses. *RCOE* 2005;10:457-64.
- Santos S, Herrera D, López E, O'Connor A, González I, Sanz M. A randomized clinical trial on the short-term clinical and microbiological effects of the adjunctive use of a 0.05% chlorhexidine mouth rinse for patients in supportive periodontal care. *J Clin Periodontol* 2004;31:45-51.
- Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc* 1962;65:26-9.
- Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamin C. *J Periodontol* 1970;41:41-3.
- Rivera S, Yévenes I, Reyes J, Norero H. Comparative effect again mouthrinses-gel of chlorhexidine with commercial mouthrinses in the growth of plaque in 24 hours. *Av Periodoncia* 2006;18:163-9.
- Claydon N, Addy M. The use of plaque area and plaque index to measure the effect of fluoride and chlorhexidine toothpastes on 24-h plaque regrowth. *J Clin Periodontol* 1995;22:540-2.
- Weaver A, Fleming SM, Smith DB, Park A. Mouthwash and oral cancer: carcinogen or coincidence? *J Oral Surgery* 1979;37:250-3.
- Martínez IL, Joan T, Muñoz V, Calatayud M, Ramón RM, Cuenca E. Study of the effectiveness of two chlorhexidine-based mouthrinse without alcohol to 0.2% and 0.12: control of supragingival plaque. *Arch Odontol Estomatol* 2003;19:100-4.
- Bascones A, Morante S, Mateos L, Mata M, Poblet J. Influence of additional active ingredients on the effectiveness of non-alcoholic chlorhexidine mouthwashes: a randomized controlled trial. *J Periodontol* 2005;76:1469-75.
- Quirynen M, Avontroodt P, Peeters W, Pauwels M, Coucke W, Van Steenberghe D. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. *J Clin Periodontol* 2001;28:1127-36.
- Herrera D, Roldán S, Santacruz I, Santos S, Masdevall M, Sanz M. Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: an *in vitro* contact test and salivary bacterial counts study. *J Clin Periodontol* 2003;30:307-14.
- Yévenes I, Reyes J, Monardes V, Rivera S. Evaluation of physico-chemical and microbiological properties of Colutoriogel® of chlorhexidine to 0.1%. *Rev Fac Odontol Univ Chile* 2003;21:16-24.