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The effect of 1% chlorhexidine gel and 0.12% dentifrice gel on plaque accumulation: a 3-day non-brushing model

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Abstract: *Aim:* The purpose of the study was to compare the effects of four treatments on 'de novo' plaque accumulation. Treatments included tray application of 1% chlorhexidine gel (CHX-Gel), 0.12% chlorhexidine dentifrice-gel (CHX-DFG), a regular dentifrice (RDF) tray application, or 0.2% chlorhexidine mouthwash (CHX-MW) in a 3-day non-brushing model. *Material and methods:* The study was designed as a single blind, randomized parallel clinical trial. After professional prophylaxis, subjects abstained from all other forms of oral hygiene during a 3-day non-brushing period. Subjects were randomly assigned to one of the four test groups (CHX-Gel, CHX-DFG, RDF applied in a fluoride gel tray or rinsing with a CHX-MW). After 3 days, the Quigley & Hein plaque index (PI) and Bleeding on Marginal Probing (BOMP) index was assessed. Subsequently, all subjects received a questionnaire to evaluate their attitude, appreciation and perception towards the products used employing a Visual Analogue Scale. *Results:* After 3 days, the full-mouth PI means were 0.88 for the CHX-gel regimen, 0.79 for CHX-MW, 1.16 for CHX-DFG and 1.31 for the RDF regimen. The two dentifrices (CHX-DFG and RDF) were significantly less effective than the CHX-Gel or the CHX-MW. *Conclusion:* Within the limitations of the present 3-day non-brushing study design, it can be concluded that the effect of a 1% CHX-Gel application tray is significantly greater than that of 0.12% CHX-DFG or RDF in inhibiting plaque accumulation. The 1% CHX-Gel applied via a tray and 0.2% CHX-MW rinse were comparably effective.

Key words: chlorhexidine; dentifrice; gel; plaque; toothpaste

Introduction

Dental plaque is a multispecies biofilm of microorganisms that grows as an ecosystem on hard tissues in the oral cavity. Epidemiological studies revealed a high correlation between supragingival plaque levels and chronic gingivitis (1). Clinical research (2) showed that plaque was the primary etiologic factor in gingival inflammation. The formation of plaque on a tooth surface is a dynamic and ordered process commencing with the attachment of primary plaque-forming bacteria.

Efficient removal of dental plaque is essential for maintaining oral health. The mainstay and most reliable method currently used for supragingival plaque control is mechanical cleaning using a toothbrush (3). This can be manual or powered (4, 5). Mechanical tooth cleaning through

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toothbrushing with toothpaste is the most common and potentially most effective form of oral hygiene practiced in developed countries (6, 7).

However, for many individuals it is difficult to achieve a level of plaque control comparable with oral health by toothbrushing (with dentifrice) only. In general, individuals remove only around half of the plaque from their teeth even when brushing for 2 min (8). Patients' efforts are often compromised by the presence of hard-to-reach areas as well as inadequate skills, poor motivation, and lack of compliance. A significant proportion of all individuals appears to fail to practice a critical standard of plaque removal, and gingivitis is highly prevalent even at an early age (9, 10).

The adjunctive use of an antiseptic and/or chemical agent may therefore be justified. After three decades of use in dentistry, chlorhexidine digluconate (CHX) is still considered to be the leading antiseptic for combating biofilms in supragingival and oral musosal sites (11, 12). Despite the ideal nature of toothpaste as a vehicle, most chemical plaque-control agents have been evaluated and later formulated as mouthrinses. Mouthrinses vary in their constituents and are usually considerably less complex than toothpastes. Chlorhexidine is used in various vehicles and concentrations in commercially available products and may be purchased by consumers as mouthwash, spray, or gel.

In the Netherlands, two over-the-counter gels containing CHX are available: a 1% CHX-gel (Corsodyl®-gel; GlaxoSmithKline, Zeist, the Netherlands) and a dentifrice gel containing 0.12% CHX (Perio-aid®; Dentaid, Houten, the Netherlands). The 1% gel is meant for temporary use for a maximum of 15 days, whilst the 0.12% CHX dentifrice gel has been recommended for long-term use. A previous study showed that application of 0.12% CHX dentifrice gel does not significantly reduce plaque accumulation, compared to a regular dentifrice (RDF) (13). However, a head to head comparison between the 1% and 0.12% gel has not been reported.

The purpose of the present study, therefore, was to evaluate whether 1% chlorhexidine gel (CHX-gel) is effective in preventing 'de novo' plaque accumulation when compared to a RDF or 0.12% CHX gel-toothpaste using a 3-day non-brushing model. A 0.2% CHX mouthwash was used as a positive control.

Materials and methods

Ethics approval

The study followed instructions based on the Helsinki principles. The protocol was approved by the Medical Ethics Committee of the Academic Medical Centre (AMC) of Amsterdam under registration number MEC 07/152 # 07.17.1074. The study has also been registered at the Dutch Trial Register (NTR1429). Subject participation in this study was voluntary. Before enrolment, all subjects were given oral and written instructions, information about the products, and a description of the purpose, aim, reason, duration, possible benefits and possible harms of study participation. All subjects willing to take part signed an informed consent form prior to the study procedures.

Subjects

A total of 115 non-dentally related subjects were recruited by e-mail and a flyer advertising the study. Inclusion criteria required that the subjects were ≥18 years of age, systemically healthy and possessed a dentition with at least 20 teeth (minimum of five evaluable teeth per quadrant). Exclusion criteria were open caries, pockets ≥5 mm, orthodontic appliances or removable (partial) dentures, a history of allergic reaction to erythrosine and/or CHX, use of antibiotics in the preceding 3 months, and pregnancy or medication that might interfere with the conduct of the study or possibly influence normal gingival health.

Design and (clinical) procedures

The study was designed as a prospective single-blind, randomized four-arm parallel clinical trial. At baseline, the teeth of all subjects were stained for plaque with an erythrosine disclosing solution applied with a cotton swab. Subjects subsequently received professional oral prophylaxis for a maximum of 30 min, performed by experienced dental hygienists. Teeth were scaled and polished so that they were plaque-, stain-, and calculus-free. An ultrasonic scaler (Sonosoft® KaVo, the Netherlands BV, Vianen, the Netherlands and EMS Electro Medical Systems SA, Nyon, Switzerland) and hand instruments (H6/7,SD204, 1/2, 12/13 11/14 American Eagle® American Eagle Instruments Inc., Missoula, MT, USA, and/or Hu-Friedy® Hu-Friedy Inc., Leimen, Germany) were used, followed by rotating polishing cups, points and brushes (Hawe-Prophy® #1802, #1805 and #0220), Hawe-Neos Dental Dr H.v.Weissenfluh AG, Bioggio, Switzerland) with polishing paste (Cleanpolish® #360, Hawe-Neos Dental Dr H.v.Weissenfluh AG, Bioggio, Switzerland).

After debridement, teeth were stained for plaque for a second time in order to make sure that all visible and stainable plaque had been removed. Subsequently, unwaxed floss (Johnson & Johnson, distributor, GABA B.V., Almere, the Netherlands) was used for a professional interdental cleaning. Distal to the last molars, bandage tape (Cotton Tamponing Bandage 1 cm 5 m sterile Hartmann®, Heidenheim, Germany) was used to make sure that all remnants of plaque were removed. Next, every subject received a unique trial number and was randomly assigned to one of the four regimens (Table 1) consisting of 1% CHX gel, 0.12% CHX dentifrice gel, RDF and 0.2% CHX mouthwash. Allocation concealment to treatment assignment was performed by keeping the registration form in an opaque sealed envelope which was stored by the study coordinator. Case record forms only include subject numbers and made no refer whatsoever to any treatment assignment.

Randomization was performed using true random numbers obtained via http://www.random.org. Each subject received a

Table 1. **Regimens**

Regimen	Product	Use of intervention
CHX-DFG	0.12% Chlorhexidine dentifrice gel Dentaid®	Twice daily application in fluoride application tray for 2 min No brushing was allowed
CHX-Gel	1% Chlorhexidine gel Corsodyl®	Twice daily application in fluoride application tray for 2 min No brushing was allowed
RDF	Regular dentifrice HEMA	Twice daily application in fluoride application tray for 2 min No brushing was allowed
CHX-MW	0.2% Chlorhexidine mouthwash Corsodyl®	Twice daily mouthwash rinsing with 10 ml for 1 min No brushing was allowed

demonstration and verbal instructions immediately following the professional dental prophylaxis. In addition, a written instruction form was provided to explain the use of the intervention products.

The dentifrice/gel groups received a large 10EL630 Elmex® fluoride application tray (Johnson & Johnson distributor, GABA BV, Almere, the Netherlands) for the twice daily application. All subjects were given a stopwatch with an alarm to keep track of the assigned rinsing or application time (Table 1). Drinking, eating and rinsing were not allowed for 30 min after the experimental procedures. During a 3-day experimental non-brushing period, subjects abstained from all other forms of oral hygiene. To check for compliance, subjects were asked to register the time of use of intervention products on a calendar record chart.

At the second visit (3 days later), all plaque on the teeth was detected using cotton swabs with an 1% erythrosine disclosing solution; the same batch was used for all subjects. All measurements were carried out under the same conditions and were performed by the same experienced examiner (NAMR). Plaque was assessed at six sites per tooth on a six-point scale using the Quigley & Hein's (14) plaque index (PI) as modified by Turesky *et al.* (15) and further modified by Lobene *et al.* (16), in which the absence or presence of plaque was recorded on a 0–5 scale (0 = no plaque, 5 = plaque covering more than two-thirds of the tooth surface). The level of gingival inflammation was then assessed by another examiner (DES) using the Bleeding on Marginal Probing (BOMP) score (17–19). Bleeding was elicited with a WHO-approved ball-ended probe (Ash Probe EN15, Dentsply International, York, PA, USA).

The gingival margin was briefly probed at an angle of approximately 60° to the longitudinal axis of the tooth. The absence or presence of bleeding was scored within 30 s of probing on a scale of 0–2 (0 = non-bleeding, 1 = pinprick bleeding, 2 = excess bleeding).

Both examiners (NAMR, DES) were calibrated and blinded to the regimens. Subjects were instructed not to reveal their group assignment in any way to the clinical examiners.

Finally, all subjects received a questionnaire to evaluate their attitude towards the product they had used. They gave their opinions of the product taste, alteration of taste, comfort of use, duration of taste, and perception of plaque control. Subjects marked a point on a 10-cm-long uncalibrated line with the negative extreme response (0) on the left and the positive extreme (10) on the right end [Visual Analogue Scale (VAS)]. After the experimental period, the subjects resumed their normal oral hygiene procedures.

Sample size

The American Dental Association (ADA) (20) Toothbrush Acceptance Program Guidelines state that adequate evidence from at least one clinical investigation of at least 25 subjects per group at baseline must show that the product can provide a 15% statistically significant reduction in plaque versus baseline when employed under unsupervised conditions by the average layman. Therefore, 15% is generally accepted as a clinically relevant difference in PI.

Sample size calculations were performed with PS Power and Sample Size Program®. These analyses provided a lower limit for 15% superiority, with a mean PI of 1.87 based on an earlier study (13). With a group standard deviation (σ) of 0.4, a difference (δ) of 0.28 and $\alpha = 0.05$ to obtain 80% power, 88 subjects would be sufficient for this study (22 subjects in each group). A sufficient number of additional subjects were included to compensate for possible loss to follow-up.

Statistical analyses

Subject demographics (gender, mean age) are presented by regimen; the statistical differences amongst groups were calculated. The Quigley & Hein PI (14) as assessed after 3 days of 'de novo' plaque accumulation was the primary outcome variable. Full-mouth mean PI scores were calculated for each individual. Secondary outcome variables were BOMP after 3 days as well as the VAS scores from the questionnaire. All analyses comparing differences (PI, BOMP, VAS scores) amongst the four regimens were performed using a one-way ANOVA test. All data are presented as mean and SD per regimen and analysed by 'Intention to Treat'. Normality was tested by Kolmogorov–Smirnov (with Lilliefors Significance Correction) and by Shapiro–Wilk analyses. For post-testing between the regimens the *T*-test was used to test for differences between regimens. The 95% confidence intervals were calculated for differences in plaque and BOMP scores between groups. *P*-values ≤ 0.05 were considered statistically significant. The statistical analyses were performed before breaking the allocation code.

Results

Figure 1 is a flow chart of the participants who were enrolled in this study. A total of 115 systemically healthy recruited subjects (±22 years of age) were screened. Three were excluded for not meeting the inclusion criteria and 112 subjects were enrolled in the study. Groups were comparable in age and sex ratio (Table 2). All but one subject (in the CHX-DFG group) completed the protocol without any protocol violation; she was lost to follow-up because she did not attend the second appointment. Her absence was determined to be unrelated to the study products.

Table 3 provides the results for the primary response variable, i.e., the mean PI scores for each regimen after 3 days of plaque accumulation. Mean whole-mouth PI was 1.16 (0.46) for the chlorhexidine dentifrice-gel (CHX-DFG) group and 0.88 (0.39) for the CHX-Gel group, compared to 1.31 (0.40) for the RDF group and 0.79 (0.36) for the chlorhexidine mouthwash (CHX-MW) group. A statistically significant difference was found amongst the four regimens (P = 0.000).

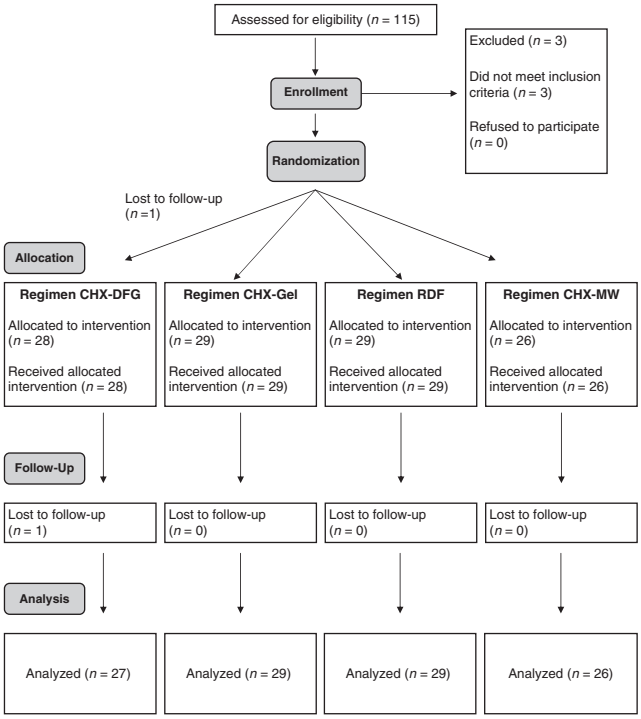

Fig. 1. Flowchart of subject's enrolment.

Post-testing between the regimens revealed that the PI scores of both CHX-MW and CHX-Gel groups were significantly lower than those of the CHX-DFG and RDF groups. No statistically significant differences were found between the PI scores of CHX-DFG and RDF or between CHX-MW and CHX-Gel (Table 4). The mean bleeding index (BOMP) for each regimen is presented in Table 5. No significant differences in the BOMP score were found amongst the four different regimens.

Table 6 shows the complete questionnaire and the two extremes of the response options. Table 7 shows the results of the questionnaire. A statistically significant difference amongst the four groups was found with respect to perception of taste, comfort of use and subjects' perception of plaque control. No statistically significant differences were found for alteration of taste, duration of taste, or the application/rinsing time. Both data from plaque and bleeding scores were normal distributed. With respect to perception of taste, CHX-MW and CHX-gel were not as well appreciated as the CHX-DFG and RDF. The comfort of use of the mouthwash was perceived as significantly higher than that of the application tray. Subjects using RDF considered plaque control to be less effective when compared to CHX-GEL and CHX-MW.

Discussion

Model

Short-term plaque regrowth studies are perhaps the most commonly used clinical experiments for screening chemical oral hygiene products. They have the advantage of assessing the chemical action of the formulation separate from the indeterminate variable of toothbrushing. Typically, plaque regrowth from a zero baseline is recorded to determine the influence of the test agent. This method was originally used for mouthrinses and has been modified for toothpaste by delivering the formulation in a tray applied to the teeth (21). Study periods range from 24 h to several days. A negative (benchmark) control and a positive control such as chlorhexidine may be used. These help to determine the activity of the test formulations in relation to known formulations. The present study evaluated the plaque-inhibiting effect of CHX products in a 3 day non-brushing model during which plaque was allowed to accumulate freely. This design has been used previously to assess the effect of 0.12% DFG (13). The results of the present study confirm the observations of a previous study, which showed no significant difference between CHX-DFG and RDF. In

Table 2. **Subject demographics, presented by regimen**

	CHX-DFG	CHX-Gel	RDF	CHX-MW	*P*-value
n	27	29	29	26	
♀ Female	21	23	22	20	0.991*
♂ Male	6	6	7	6	
Mean (SD) age in years	22.1 (2.55)	22.3 (3.05)	23.5 (3.64)	22.2 (2.23)	0.838*
Age range	19–29	19–31	19–32	18–26	

*Chi-square comparison amongst the four groups.

Table 3. **Mean (standard deviation), minimum, and maximum overall plaque (PI) scores for each regimen after 3 days of plaque accumulation**

	CHX-DFG	CHX-Gel	RDF	CHX-MW	*P*-value
Mean overall PI	1.16 (0.46)	0.88 (0.39)	1.31 (0.40)	0.79 (0.36)	<0.001*
Minimum	0.38	0.27	0.51	0.27	
Maximum	2.21	1.99	2.21	1.68	

*One-way ANOVA test.

Table 4. ***Post-hoc* statistical analysis: *t*-tests and 95% confidence intervals for differences in mean plaque scores between the regimens**

Regimens	*t*-test	Confidence interval	Significant
CHX-Gel : RDF	<0.001	[−0.63; −0.21]	Yes
CHX-Gel : CHX-MW	0.343	[−0.11; 0.30]	No
CHX-Gel : CHX- DFG	0.018	[−0.50; −0.05]	Yes
RDF : CHX-MW	<0.001	[0.31; 0.73]	Yes
RDF : CHX-DFG	0.210	[−0.08; 0.37]	No
CHX-MW : CHX-DFG	0.002	[−0.60; −0.15]	Yes

addition the present study showed that the inhibition of plaque formation with a 1% CHX gel was not significantly different from a 0.2% CHX mouthwash.

1% CHX-gel

The 1% CHX-Gel product is commercially available over the counter and can be delivered via a toothbrush or in trays. The distribution of a gel throughout the mouth over the tooth surfaces by toothbrush appears to be poor, and preparations must be delivered to all surfaces to be effective (22). CHX-Gel delivered via a tray was found to be particularly effective against plaque and gingivitis in handicapped individuals (23). However, the acceptability of the tray delivery system to the recipients and the care-takers was found to be poor (24). The 1% CHX gel has also been used in subgingival applications after scaling and root planing. This results in a statistically lower gingival index than scaling and root planing alone (25). Bleeding on probing was also significantly reduced compared to a placebo gel (26). Other studies have shown a reduction in the frequency and detection of several peridontopathic microorganisms (25, 26).

Dose response

The anti-plaque effect of the 0.12% CHX dentifrice gel may be similar to that of a RDF due to the amount of CHX digluconate per application. The CHX-MW, CHX-Gel and the CHX-DFG all contained various percentages of CHX. Given a specific gravity of 1.080 g ml^{-1} for CHX digluconate, each CHX-DFG application with a fluoride tray of approximately 10 g contained 12 mg of available CHX digluconate. For the 1% CHX gel, the application of approximately 10 g provided around 100 mg of CHX digiclonate. Although no direct comparison can be made between a gel and a mouthwash, it is clear that the 12 mg provided by CHX-DFG is insufficient to exceed the effect of RDF. The reason for this may be 2-fold.

Table 5. **Mean (standard deviation), minimum, and maximum overall Bleeding on Marginal Probing (BOMP) scores for each regimen after 3 days of plaque accumulation**

	CHX-DFG	CHX-Gel	RDF	CHX-MW	*P*-value
Mean overall BOMP	0.36 (0.19)	0.28 (0.16)	0.33 (0.13)	0.30 (0.17)	0.325*
Minimum	0.11	0.03	0.08	0.07	
Maximum	0.85	0.68	0.60	0.74	

*One-way ANOVA test.

Table 6. **Complete set of questions from Visual Analogue Scale questionnaire (scored from 0 to 10)**

		With extremes	
Paraphrase	Complete question	From	To
Taste perception	How was the taste of the product?	Very bad	Very good
Duration of taste	How long did the taste remain?	Very short	Very long
Alteration of taste	How was your taste of food and drinks affected?	Negative change	Positive change
Time of application	What is your opinion about the application time of the product?	Very short	Very long
Use of comfort	What is your opinion about the ease in use of the product?	Not easy	Very easy
Plaque control	What is your perception of plaque control during this 3 days?	Insufficient	Very efficient

Table 7. **Results of the questionnaire response on the Visual Analogue Scale Mean scores (standard deviation) are presented by regimen**

Question	CHX-DFG	CHX-Gel	RDF	CHX-MW	*P*-value*
Taste perception	6.26 (2.42)†,‡	2.47 (1.93)†	6.14 (1.99)†,‡	4.68 (2.30)	<0.001
Alteration of taste	4.54 (0.39)	4.24 (1.74)	4.34 (0.92)	3.93 (1.40)	0.351
Duration of taste	5.28 (2.06)	5.20 (2.72)	5.91 (2.09)	6.08 (2.53)	0.413
Time of application	5.49 (1.79)	4.57 (2.02)	5.18 (1.68)	4.27 (1.79)	0.061
Comfort of use	4.55 (2.17)†,‡	5.84 (2.47)†	5.83 (2.70)†	8.08 (1.55)	<0.001
Plaque control	4.65 (2.77)	6.02 (2.95)	3.66 (2.73)†,‡	5.72 (2.76)	0.007

*One-way ANOVA test.
†Post-tested with *t*-test, significant differences ≤0.05 compared with CHX-MW.
‡Post-tested with *t*-test, significant differences ≤0.05 compared with CHX-Gel.

CHX in CHX-DFG could be inactivated by dentifrice components (27–29), and diffusion of CHX from the dentifrice formulation might be inhibited or decreased by dentifrice components (30). Alternatively, for a gel dosing should be higher. The effect of the dosis has been shown to be the case with application of CHX via an oral irrigator; in this situation 80 mg was found to be the optimal dosage (31).

Bleeding scores

Several studies have shown that the development of plaque may be dependent on a number of factors such as diet (32), tooth surface roughness (33), periodontal condition (34) and bacterial salivary load (35). An experimental gingivitis study by Hillam & Hull (36) showed that the amount of plaque developing in 24 h in patients with good gingival health at baseline was considerably less than the amount of plaque developed in 24 h at the end of the experimental gingivitis period. More extensive studies were performed earlier (37–45) all confirmed that periodontal condition is of foremost importance in the rate of 'de novo' plaque formation. The use of four separate groups in the present parallel design could have introduced an unwanted effect as a result of varying levels of gingival health. Therefore, in this study, the level of gingival health was assessed in conjunction with the to plaque levels to evaluate whether this factor potentially could have impacted the outcome of the study. In other words whether differences in plaque scores after 3 days could be explained by differences in the level of gingival inflammation this appeared not to be the case (Table 5). In terms of BOMP, bleeding scores were found to be comparable amongst groups and therefore not considered to be a confounding factor for the plaque scores.

Conclusion

Within the limitations of the present 3-day non-brushing study design, it can be concluded that the tray application of 1% CHX gel is significantly more effective than 0.12% CHX dentifrice gel or RDF in the inhibition of plaque accumulation. When applied via a tray, the 1% CHX gel was not significantly different from rinsing with 0.2% CHX-MW.

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Conflict of interest and source of funding statement

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