DE Slot
R Lindeboom
NAM Rosema
MF Timmerman
GA van der Weijden

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

Authors' affiliations:
DE Slot, School for Dental Hygiene, Inholland University of Professional Education, Amsterdam, The Netherlands, *R Lindeboom*, Department of Clinical Epidemiology and Biostatistics, University of Amsterdam, Academic Medical Center (AMC), Amsterdam, The Netherlands, *NAM Rosema*, *MF Timmerman*, *GA van der Weijden*, Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands

Correspondence to:
D.E. Slot MSc, RDH,
Inholland University of Professional Education
School of Health, School for Dental Hygiene
Louwesweg 1, Postvak 32
1066 EA Amsterdam
The Netherlands
Tel.: +31 20 5188643/313
Fax: +31 20 5188391
E-mail: dagmar.slot@inholland.acta.nl

Dates:
Accepted 16 November 2006

To cite this article:
Int J Dent Hygiene **5**, 2007; 45–52
Slot DE, Lindeboom R, Rosema NAM, Timmerman MF, van der Weijden GA. The effect of 0.12% chlorhexidine dentifrice gel on plaque accumulation: a 3-day non-brushing model

Abstract: *Background:* Maintaining an adequate low level of plaque through daily tooth brushing is often not feasible. Effective chemotherapeutic agents as an adjunct to mechanical plaque control would therefore be valuable. Chlorhexidine (CHX) mouthwash has proved to be an effective inhibitor of plaque accumulation. *Aim:* The purpose of the present study was to assess the effect of application of 0.12% CHX dentifrice gel on *de novo* plaque accumulation. *Material and methods:* The study was designed as a single blind, randomized three-arm parallel clinical trial. At the beginning of the test period all volunteers received a thorough professional oral prophylaxis. Subjects were randomly assigned to one of three regimens. During a 3-day non-brushing period, subjects abstained from all forms of mechanical oral hygiene. One regimen (test group) used 0.12% chlorhexidine dentifrice gel (CHX-DGel, Perio·Aid®) applied in a fluoride gel tray, the benchmark control group used a regular dentifrice applied in a fluoride gel tray (RegD, Everclean® HEMA). The positive control group rinsed with a 0.12% chlorhexidine mouthwash (CHX-MW, Perio·Aid®). The Quigley and Hein plaque index (PI) from all subjects was assessed after 3 days of *de novo* plaque accumulation. Subsequently, all subjects received a questionnaire to evaluate their attitude, appreciation and perception towards the products used employing a Visual Analogue Scale scores. After the experimental period, habitual oral hygiene procedures were resumed. *Results:* Ninety-six systemically healthy subjects completed the study. After 3 days, the full-mouth PI for the CHX-DGel regimen was 1.87 compared with 1.93 for the RegD regimen and 1.55 for the CHX-MW regimen. The two dentifrices (CHX-DGel and RegD) were significantly less effective as the CHX-MW ($P = 0.0006$). No

significant difference between scores of the dentifrices was found. *Conclusion:* Within the limitations of the present 3-day non-brushing study design, it can be concluded that application of 0.12% CHX dentifrice gel is not significantly different from application of regular dentifrice on plaque accumulation. Use of a 0.12% CHX mouthwash is significantly more effective. CHX-DGel appears a poor alternative for a dentifrice. It is not an effective inhibitor of plaque growth and does not possess fluoride.

Key words: chorhexidine; clinical trial; dentifrice; dentifrice gel; mouthwash, oral hygiene; plaque

Introduction

The most common method to prevent caries and periodontal diseases is mechanical supragingival plaque control by toothbrush, and interdental aids, such as dental floss, toothpicks and interdental brushes. For most people, however, total plaque removal seems not a realistic goal. Most people remove less than half of the plaque with brushing once a day, leaving approximately 60% after brushing responsible for rapid regrowth (1). Therefore, an adjunct to mechanical plaque control would be valuable. Several products for chemical plaque inhibition are available on the market. The bisbiguanide compounds, which include chlorhexidine (CHX) gluconate and alexidine, are the most effective agents currently in use (2). CHX is a cationic chlorophenyl biguanide with outstanding bacteriostatic properties. The drug was synthesized and first reported by ICI in 1954 following extensive investigations of its biological properties of polydiguanide compounds (3). CHX was initially used in dentistry for presurgical oral disinfection and endodontics (4). The application of CHX as an anti-plaque and calculus agent was suggested by Schroeder and Hirzel (5). CHX has been proved as an effective plaque inhibitor when used as an adjunct to mechanical cleaning procedures as well as when used alone (6).

Chlorhexidine can be applied in a number of ways: as a mouthwash (7–11), as a gel (12–18) and as a spray (13, 14, 19–24). Its efficacy has been extensively investigated. CHX is most commonly used in a mouthwash form.

In the Netherlands, CHX gel has traditionally been available in a 1% concentration (Corsodyl®-gel, Glaxo Smith Kline, Zeist, the Netherlands). More recently, a dentifrice gel containing a 0.12% concentration CHX was brought on the market (Perio-aid®, Dent-Aid, Houten, the Netherlands). The 1% CHX gel was meant for temporary use with a maximum of 15 days, while the 0.12% concentration dentifrice gel has been advocated for long-term twice daily brushing use. So far, no efficacy data on the latter product are available.

The purpose of the present study was to evaluate, when compared with a regular dentifrice, whether 0.12% CHX dentifrice gel is effective in preventing *de novo* plaque formation in a 3-day non-brushing model. As a positive control, the effect of rinsing with 0.12% CHX mouthwash was assessed. In addition, the individual attitude towards the used products was evaluated.

Materials and methods

Ethical aspects/approval

This study protocol was approved by the Medical Ethics Committee of the Academic Medical Centre (AMC) of Amsterdam under registration number 05/189. The study has also been registered by the Dutch Trial Register, international standard randomized controlled trial ISRCT 57974544. Participation as a subject in this study was voluntary.

Subjects

A total of 127 subjects were recruited from a database of the Department of Periodontology Academic Centre for Dentistry Amsterdam (ACTA) and from students of Inholland University responding to an email advertisement. Before enrolment, all subjects were given oral and written instructions and information about the products and purpose, aim, reason, duration

demand of benefits and possible harm of study participation. All subjects willing to take part signed an informed consent prior to the study procedures.

Inclusion criteria were ≥18 years of age, systemically healthy and 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, history of allergic reaction to erythrosine and/or CHX, use of antibiotics in the last 3 months or medication that might interfere with the conduct of the study or possibly influencing normal gingival health.

Design and (clinical) procedures

The study was designed as a single-blind, randomized three-arm parallel clinical trial. At baseline, teeth of all subjects were stained for plaque with an erythrosine disclosing solution applied with a cotton swab subsequently received a professional oral prophylaxis for a maximum of 30 min performed by experienced dental hygienists. Teeth were scaled and polished with the purpose of making them 100% free of plaque, stain and calculus. An ultrasonic scaler (Sonosoft® KaVo Nederland BV Vianen, the Netherlands) 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) 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) were used.

After debridement, teeth were stained for a second time. Performed to make sure all plaque had been removed. Subsequently, unwaxed floss (Johnson & Johnson, distributor, GABA B.V., Almere, the Netherlands) was used for a professional interdental cleaning. Distal of the last molars bandage tape (Cotton Tamponning Bandage 1 cm × 5 m sterile Hartmann®, Heidenheim, Germany) was used to make sure that all remnants were removed.

Every subject received a unique trial number and was randomly assigned to one of the three regimens (Table 1) consisting of 0.12% CHX dentifrice gel, regular dentifrice and 0.12% CHX mouthwash. No brushing was allowed in any of the three regimens.

Randomization was performed using true random numbers obtained via http://www.random.org. The primary investigator and study coordinator (DES) was responsible for the allocation concealment, subjects were instructed not to reveal their group assignment in any way to the clinical examiner (NAMR).

Table 1. **Following regimens groups who were designed**

Regimen CHX-DGel	0.12% Chlorhexidine dentifrice gel* twice a day application in fluoride application tray† for 2 min No brushing was allowed
Regimen RegD	Regular dentifrice‡ twice a day application in fluoride application tray† for 2 min No brushing was allowed
Regimen CHX-MW	0.12% Chlorhexidine mouthwash* twice a day mouthwash rinsing with 15 ml for 1 min No brushing was allowed

*Perio·Aid®, Dentaid, Houten, the Netherlands
†Fluoride application tray large 10EL630 Elmex®, Johnson & Johnson distributor, GABA BV, Almere, the Netherlands.
‡Everclean®, HEMA, Amsterdam, the Netherlands.

Each subject received a demonstration and verbal instruction from the study coordinator (DES) immediately following the professional dental prophylaxis. In addition, a written instruction form was provided explaining the use of the intervention products. The subjects were given a stopwatch with alarm to keep track of the assigned rinsing or application time. Drinking, eating or rinsing was not allowed for 30 min after the experimental procedures. During a 3-day experimental non-brushing period, subjects abstained from all other forms of mechanical oral hygiene. To check for compliance, subjects were asked to register the time of use of intervention products onto a calendar record chart.

At the second visit (3 days later), all plaque on the teeth was disclosed using cotton swabs with an 1% erythrosine disclosing solution from the same batch of disclosing solution for all subjects. All measurements were carried out under the same conditions and were preformed by the same experienced examiner (NAMR) who was blinded to the regimen. This examiner had been trained and calibrated in the plaque scoring system and had applied it in other studies (11, 25). Plaque was recorded at six sites per tooth on a five-point scale using the Quigley and Hein's (26) plaque index (PI) as modified by Turesky *et al.* (27) and further modified by Lobene *et al.* (28). Each subsequent full-mouth plaque assessment lasted approximately 10 min. After the experimental period, habitual oral hygiene procedures were resumed.

Finally, all subjects received a questionnaire to evaluate their attitude towards the used product. They were questioned about their opinion of appreciation of 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) at the right end (Visual Analogue Scale, VAS).

Statistical analyses

The Quigley and Hein index as assessed after 3 days of *de novo* plaque accumulation was used as the primary outcome variable. Full-mouth mean PI scores were calculated for each individual. Data considering the VAS scores from the questionnaire to evaluate the subjects' attitude, appreciation and perception towards the used products were secondary outcomes. All analyses comparing differences (PI, VAS scores) between the three regimens were performed using non-parametric tests (Kruskal–Wallis *H*-tests) with post-testing corrected for multiple comparisons. All data are presented as mean ± SD per regimen. For the difference in PI scores between regimens 95% confidence intervals were calculated. Values of $P \leq 0.05$ were considered as statistically significant.

Sample size

The American Dental Association (ADA) (29) states in its Acceptance Program Guidelines Toothbrushes (1998) that under unsupervised conditions, a 15% statistically significant reduction in plaque is needed to provide evidence of greater effectiveness in cleaning teeth. Sample size calculations with PS Power and Sample Size Program® showed that given a lower limit for superiority of 15%, a mean PI of 2.7, an SD of 0.3, a difference of 0.4. and an $\alpha = 0.05$ to obtain 80% power, 21 subjects would be sufficient for this study (seven subjects in each group). The ADA also requires that adequate evidence must be provided by entering at least 30 subjects for each into the study at baseline. At least 25 subjects for each product should be available for examination at the end of the study. Considering possible loss to follow-up for the present study 35 extra subjects were included per regimen.

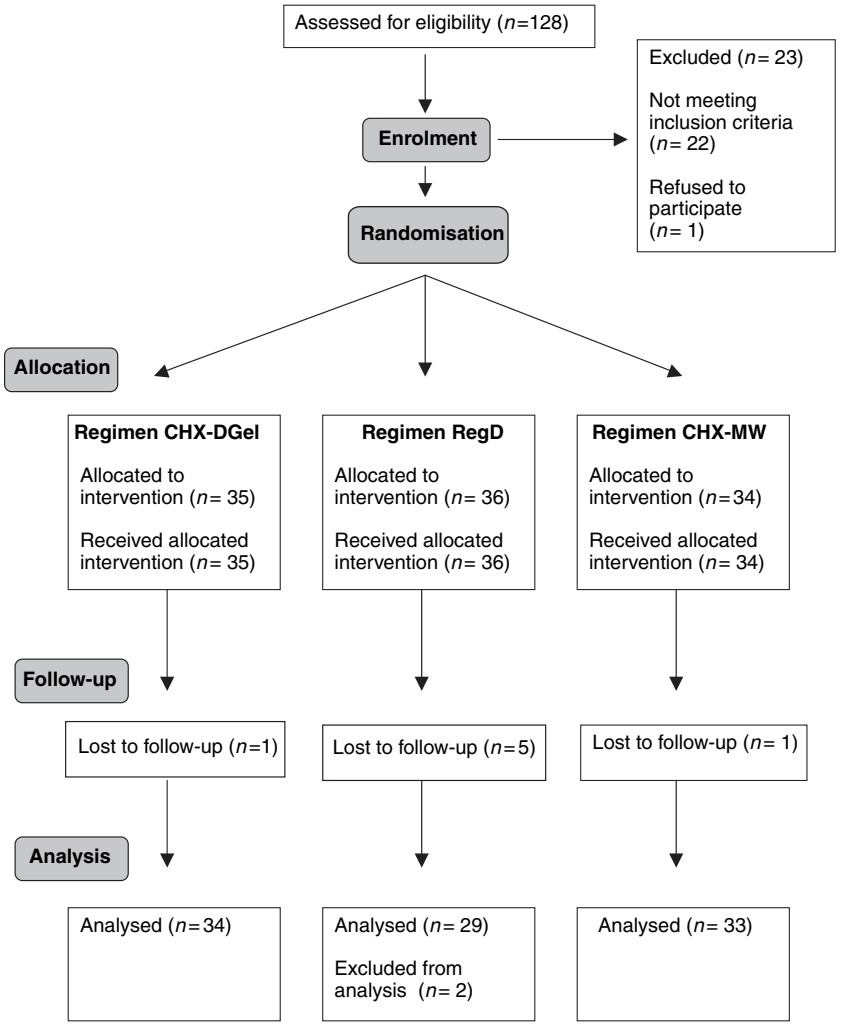

Fig 1. Flow chart subject enrolment.

Results

Figure 1 shows a flow chart of the participants that were enrolled for this study. A total of 127 systemically healthy recruited subjects (≥18 years of age) were screened; 22 were excluded for open caries or pockets ≥5 mm. Of the 105 subjects, who were enrolled into the study, 98 completed the protocol. Seven subjects (one in the CHX-Dgel group, five in the RegD group and one in the CHX-MW group) were lost to follow-up because they did not attend the second appointment. Being absent was unrelated to the study products. In the end, 96 completed the study protocol without any protocol violation. Two male volunteers, both from the RegD group were excluded from the analysis because each had one protocol violation. One had brushed once and the other had forgotten to use his product once. Subjects' demographics of those included in the analysis are presented in Table 2. Groups were comparable in age. However, due to chance, the randomization procedure resulted in an unequal distribution of the sexes over the groups. There were significantly fewer women in the RegD group.

Table 3 provides the results for the primary endpoints, the mean PI scores for each regimen after 3 days of plaque accumulation. Mean whole mouth PI for the CHX-DGel was 1.87 compared with 1.93 for the RegD regimen and 1.55 for the CHX-MW regimen. A statistically significant difference between the three regimens was found ($P = 0.0006$). Post-testing between the regimens revealed that PI scores when using dentifrices (CHX-DGel and RegD) were significantly higher when compared with using CHX-MW ($P \leq 0.05$). No statistically significant difference between PI scores of the two dentifrices (CHX-DGel and RegD) was found (Table 4).

Table 2. **Subjects' demographics presented by regimen**

	CHX-DGel	RegD	CHX-MW	*P*-value
n	34	29	33	
♀ (female)	25	16	28	0.033†
♂ (male)	9	13	5	0.033†
Mean age in years (SD, range)	21.9 (4.50, 18–39 year)	23.5 (4.15, 18–39 year)	21.5 (3.20, 18–32 year)	0.440†

†Chi^2 test.

Table 3. **Mean overall plaque index (PI) scores (standard deviation in parentheses) for each regimen after 3 days of plaque accumulated and minimum and maximum scores**

	CHX-DGel	RegD	CHX-MW	*P*-value
Mean overall PI	1.87 (0.37)	1.93 (0.46)	1.55 (0.37)	0.0006*
Minimum	1.07	0.77	0.74	
Maximum	2.86	2.73	2.24	

*Kruskal–Wallis *H*-test with post-testing corrected for multiple comparison.

Table 4. **Results from the statistical analysis Kruskal–Wallis *H*-test with post-testing corrected for multiple comparisons and 95% confidence intervals for differences for mean plaque scores between the regimens**

	Kruskal–Wallis *H*-test	Confidence interval
CHX-DGel – RegD	NS	−0.27 to 0.15
CHX-DGel – CHX-MW	≤0.05	0.13 to 0.50
RegD – CHX-MW	≤0.05	−0.59 to −0.17

Table 5 shows the complete question and the two extremes of the answering possibilities. Table 6 shows the results of the questionnaire. A statistically significant difference between the three groups was found with respect to perception of taste, alteration of taste, comfort of use and duration of taste. No statistically significant differences were found by the application/rinsing time and subjects' perception of plaque control.

Post-testing showed a significant difference between the perception of taste, alteration of taste and use of comfort for the CHX-MW when compared with the two dentifrices (CHX-DGel and RegD). For the duration of the taste of the study products, it appeared that taste of the CHX-DGel product remained shorter when compared with CHX-MW and RegD.

Discussion

This study aimed at evaluating whether CHX-DGel had a potential to inhibit *de novo* plaque formation. It used a 3-day non-brushing model which allows for plaque accumulation. This design has been used previously to assess the effect of various mouthwashes (30–36). Zee *et al.* (35) and Simonsson (36) also used this 3-day model to discern between 'rapid' and 'slow' plaque formers.

The application of the dentifrice in trays was based on a suggestion by Saxton and van der Ouderaa (37) to apply undiluted dentifrice directly to the test teeth. The method of applying undiluted dentifrice via a tooth shield was reported in an earlier 4-day plaque study by Saxton *et al.* (38), which was a modification of a full-mouth technique used by Gjermo and Rølla (39) and Strålfors (40). This technique eliminates the variability introduced by the mechanical action of tooth brushing, thus permitting the assessment of chemotherapeutic activity only. Putt *et al.* (41) confirmed that this was an effective short-term model to investigate the chemotherapeutic effects of CHX dentifrice on plaque.

Table 5. **Complete questions from VAS score (from 0 to 10)**

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

Table 6. **Visual Analogue Scale scores questionnaire response (0.0–10.0) of the mean response to the questionnaire (standard deviation in parentheses) presented by regimen**

Question	CHX-DGel	RegD	CHX-MW	*P*-value
Taste perception	6.68 (1.86)*	6.95 (1.17)*	5.18 (2.21)	0.0008
Alteration of taste	4.79 (0.99)*	4.73 (0.91)*	3.74 (1.52)	0.0052
Use comfort	5.67 (2.27)*	5.38 (2.69)*	7.62 (2.13)	0.0003
Duration of taste	4.41 (2.21)	6.01 (1.95)**	6.38 (2.02)**	0.0014
Plaque control	4.69 (2.37)	4.67 (2.77)	5.77 (2.55)	NS

*Significant differences when compared with CHX-MW
**Significant differences when compared with CHX-DGel.

The CHX-DGel was positioned against a RegD as benchmark control. This was a commercially available fluoride dentifrice not claiming anti-plaque efficacy. As positive control, a CHX-MW was used which at present is considered the standard and most effective anti-plaque agent (42). A positive control compares and positions the efficacy of CHX-DGel and RegD and is frequently used in early no oral hygiene study protocols (43).

The results from the present study show that 0.12% CHX-DGel is not significantly different from using RegD. Both dentifrices were less effective than the CHX-MW with respect to the plaque inhibition. Considering the small differences between 0.12% CHX-DGel and RegD, one could suggest that the present study suffers from inadequate power. If the observed difference between the 0.12% CHX-DGel and RegD regimen would have been powered with at least 80% and an $\alpha = 0.05$, a sample size of approximately 275 subjects per regimen would have been necessary. Clearly, one could then discuss the clinical relevance of this study design. In this perspective, using the model as chosen with inclusion of both a benchmark RegD as well as positive control (CHX-MW) was an elegant and powerful (power >99%) way to position the CHX-DGel regimen with only approximately 10% of such a large sample size.

The fact that application of a dentifrice use does not contribute to plaque growth inhibition does not necessarily mean an abolishment of its use. Dentifrices are also most effective fluoride carriers and their contribution to caries prevention is well established (44). The CHXDGel, however, neither has an effect on plaque growth nor does contain fluoride.

The CHX-DGel has a manufacturer's instruction for use, that states brushing twice daily allows long-term usage in analogy to a regular dentifrice. This might explain the absence of an anti-plaque effect because CHX can be inactivated by flavour and detergent in dentifrice formulations (45–47). One of the most widely used synthetic detergents in dentifrice is sodium lauryl sulphate (SLS). Unfortunately, CHX and SLS may counteract. Previous studies (46, 48) have shown that CHX and SLS are not compatible even when they are introduced separately in the oral cavity. Earlier Barkvoll *et al.* (49) showed that CHX and sodium monofluorophosphate are also not compatible in clinically relevant concentrations.

An other explanation for the absence of an anti-plaque effect could be the amount of CHX digluconate per application. Both CHX-DGel and CHX-MW in this present study contained 0.12% CHX. Each CHX-MW application with 15 ml had delivered 18 mg of CHX digluconate. With a specific gravity of 1.080 g ml^{-1} for CHX digluconate, each CHX-DGel application with a fluoride tray of approximately 10 g had 12 mg of CHX digluconaat available. Based on studies by Cumming and Loë (50) and Lang and Ramseier-Grossmann (51), this amount of CHX should be sufficient to results in plaque growth inhibition. However, diffusion of CHX from the dentifrice formulation might have been prevented, by dentifrice components or may have been decreased (52).

In this respect, the manufacturers should be careful when reformulating the CHX-DGel. Children usually apply ± 0.25 g of dentifrice (53) on their brush, while adults 0.5 g for electric and 0.9 g for manual tooth brushing (54). So using CHX-DGel on a toothbrush would result in 0.6–1.1 mg of CHX digluco-

nate application. This is not enough to have a sufficient antiplaque effect (50, 51). To have sufficient amounts of CHX available, the concentration should be raised to a level of 2.0% to have an applicated dose comparable with the 0.12% CHX-MW.

A suggestion for further research would be to raise the CHX concentration in the CHX dentifrice gel to at least 1% level similar to a competitive product already available on the market. However, before another clinical research trail is started, with the involvement of a large group volunteers, it is obligatory to test the efficacy of the new formulation(s) in a laboratory setting first.

Summary and conclusion

Within the limitations of the present 3-day non-brushing study design, it can be concluded that the effect of application of 0.12% CHX dentifrice gel is not significantly different from that of regular dentifrice on plaque accumulation. Using 0.12% CHX mouthwash is significantly more effective. CHX-DGel appears a poor alternative for a dentifrice. It is not an effective inhibitor of plaque growth and does not possess fluoride.

Acknowledgements

The authors gratefully acknowledge Dentaid Benelux (Perioaid®) in Houten, the Netherlands, for providing study products and the School of Health Inholland University for their hospitality and the gift vouchers as compensation for the panellists. We also thank the colleague's dental hygienist for their help with the professional oral prophylaxis and Paula Versteeg for her support.

References

1 De la Rosa M, Zacarias Guerra J, Johnston DA, Radike AW. Plaque growth and removal with daily toothbrushing. *J Periodontol* 1979; **50:** 661–664.
2 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:** 1099–1106.
3 Davies GE, Francis J, Martin AR, Rose FL, Swain G. Laboratory investigation of a new antimicrobial agent of high potency. *Br J Pharmacol* 1954; **9:** 192–196.
4 Clarke JR, Blacklock Z. The use of chlorhexidine and ''savlon'' for decontamination of ''medihaler'' mouthpieces. *Med J Aust* 1965; **13:** 827–828.
5 Schroeder HE, Hirzel HC. A method of studying dental plaque morphology. *Helv Odontol Acta* 1969; **13:** 22–27.
6 Hull PS. Chemical inhibition of plaque. *J Clin Periodontol* 1980; **7:** 431–442.
7 Keijser JA, Verkade H, Timmerman MF, Van der Weijden GA. Comparison of 2 commercially available chlorhexidine mouthrinses. *J Periodontol* 2003; **74:** 214–218.
8 Löe H, Schiøtt CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J Periodontal Res* 1970; **5:** 79–83.
9 Löe H, Schiøtt CR, Karring G, Karring T. Two years oral use of chlorhexidine in man. I. General design and clinical effects. *J Periodontal Res* 1976; **11:** 135–144.
10 Van Strydonck DA, Timmerman MF, van der Velden U, van der Weijden GA. Plaque inhibition of two commercially available chlorhexidine mouthrinses. *J Clin Periodontol* 2005; **32:** 305–309.
11 Van der Weijden GA, Timmerman MF, Novotny AGA, Rosema NAM, Verkerk AAJ. Three different rinsing times and inhibition of plaque accumulation with chlorhexidine. *J Clin Periodontol* 2005; **32:** 89–92.
12 Cutress TW, Brown RH, Barker DS. Effects on plaque and gingivitis of a chlorhexidine dental gel in the mentally retarded. *Community Dent Oral Epidemiol* 1977; **5:** 78–83.
13 Francis JR, Hunter B, Addy M. A comparison of three delivery methods of chlorhexidine in handicapped children. I. Effects on plaque, gingivitis and toothbrushing. *J Periodontol* 1987; **58:** 451–455.
14 Francis JR, Addy M, Hunter B. A comparison of three delivery methods of chlorhexidine in handicapped children. II. Parent and house preferences. *J Periodontol* 1987; **58:** 456–459.
15 Kohler B, Andreen I. Influence of caries-preventive measures in mothers on cariogenic bacteria and caries experience in their children. *Arch Oral Biol* 1994; **39:** 907–911.
16 Kohler B, Bratthall D, Krasse B. Preventive measures in mothers influence the establishment of the bacterium *Streptococcus mutans* in their infants. *Arch Oral Biol* 1983; **28:** 225–231.
17 Pienihakkinen K, Soderling E, Ostela I, Leskela I, Tenovuo J. Comparison of the efficacy of 40% chlorhexidine varnish and 1% chlorhexidine-fluoride gel in decreasing the level of salivary mutans streptococci. *Caries Res* 1995; **29:** 62–67.
18 Porras R, Anderson GB, Caffesse R, Narendran S, Trejo PM. Clinical response to 2 different therapeutic regimens to treat periimplant mucositis. *J Periodontol* 2002; **73:** 1118–1125.
19 Burtner PA, Low DW, Mc Neal DR, Hassel RM, Smith RH. Effects of chlorhexidine spray on plaque and gingival health in institutionalized persons with mental retardation. *Spec Care Dentist* 1991; **11:** 97–100.
20 Clavero J, Baca P, Junco P, Gonzalez 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:** 773–777.
21 Dever GJ. Oral hygiene in mentally handicapped children. A clinical trial using a chlorhexidine spray on plaque and gingivitis in handicapped children. *Aust Dent J* 1979; **24:** 301–305.
22 Francetti L, Del Fabbro M, Testori T, Weinstein RL. Chlorhexidine spray versus chlorhexidine mouthwash in the control of dental plaque after periodontal surgery. *J Clin Periodontol* 2000; **27:** 425–430.
23 Kalaga A, Addy M, Hunter B. Comparison of chlorhexidine delivery by mouthwash and spray on plaque accumulation. *J Periodontol* 1989; **60:** 127–130.
24 Kalaga A, Addy M, Hunter B. The use of 0. 2% chlorhexidine spray as an adjunct to oral hygiene and gingival health in physically and mentally handicapped adults. *J Periodontol* 1989; **60:** 381–385.

25 Rosema NA, Timmerman MF, Piscaer M et al. An oscillating/pulsating electric toothbrush versus a high-frequency electric toothbrush in the treatment of gingivitis. *J Dent* 2005; **33 (Suppl. 1):** 29–36.
26 Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc* 1962; **65:** 26–29.
27 Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. *J Periodontol* 1970; **41:** 41–43.
28 Lobene RR, Soparker PM, Newman BS. Use of dental floss. Effect of plaque and gingivitis. *Clin Prev Dent* 1982; **4:** 5–8.
29 American Dental Association. Acceptance Program Guidelines Toothbrushes. Council on Scientific Affairs, 1998.
30 Addy M, Bates JF. The effect of partial dentures and chlorhexidine gluconate gel on plaque accumulation in the absence of oral hygiene. *J Clin Periodontol* 1977; **4:** 41–47.
31 Binney A, Addy M, Newcombe RG. The plaque removal effects of single rinsings and brushings. *J Periodontol*. 1993; **64:** 181–185.
32 Daly CG, Highfield JE. Effect of localized experimental gingivitis on early supragingival plaque accumulation. *J Clin Periodontol* 1996; **23:** 160–164.
33 Dills SS, Olshan AM, Goldner S, Brogdon C. Comparison of the antimicrobial capability of an abrasive paste and chemical-soak denture cleaners. *J Prosth Dent* 1988; **60:** 467–470.
34 Dona BL, Grundemann LJ, Steinfort J, Timmerman MF, van der Weijden GA. The inhibitory effect of combining chlorhexidine and hydrogen peroxide on 3-day plaque accumulation. *J Clin Periodontol* 1998; **25:** 879–883.
35 Zee KY, Samaranayake LP, Attstrom R. Scanning electron microscopy of microbial colonization of 'rapid' and 'slow' dental-plaque formers *in vivo*. *Arch Oral Biol* 1997; **42:** 735–742.
36 Simonsson T. Aspects of dental plaque formation with special reference to colloid-chemical phenomen. *Swed Dent J* 1989; **58:** 1–67.
37 Saxton CA, van der Ouderaa FJ. The effect of a dentifrice containing zinc citrate and Triclosan on developing gingivitis. *J Periodontal Res* 1989; **24:** 75–80.
38 Saxton CA, Svatun B, Lloyd AM. Antiplaque effects and mode of action of a combination of zinc citrate and a nonionic antimicrobial agent. *Scand J Dent Res* 1988; **96:** 212–217.
39 Gjermo P, Rølla G. Plaque inhibition by antibacterial dentifrices. *Scand J Dent Res* 1970; **78:** 464–470.
40 Strålfors A. Deninfection of dental plaques in man. In: Mühleman HR, Köning KG, eds. *Proceedings of Caries Symposium, Zürich. The Present Status of Caries Prevention by Fluorine-containing Dentifrices*. Berne, Switzerland, Hans Huber Publishers, 1961, 154–161.
41 Putt MS, Van der Weijden GA, Kleber CJ, Saxton CA. Validation of a 21-day, partial-mouth gingivitis model for evaluating chemotherapeutic dentifrices. *J Periodontal Res* 1993; **28:** 301–307.
42 Jones CG. Chlorhexidine: is it still the gold standard? *Periodontology 2000* 1997; **15:** 55–62.
43 Addy M. Use of antiseptics in periodontal therapy. In: Lindhe J, Karing T, Lang NP, eds. *Clinical Periodontology and Implant Dentistry*, 4th edn. Munskgaard, Oxford Blackwell, 2003, 464–493.
44 Davies RM, Elwood RP, Davies GM. The rational use of fluoride toothpaste. *Int J Dent Hyg* 2003; **1:** 3–8.
45 Addy M, Jenkins S, Newcombe R. Studies on the effect of toothpaste rinses on plaque regrowth. (I). Influence of surfactants on chlorhexidine efficacy. *J Clin Periodontol* 1989; **16:** 380–384.
46 Barkvoll P, Rolla G, Svendsen K. Interaction between chlorhexidine digluconate and sodium lauryl sulfate *in vivo*. *J Clin Periodontol* 1989; **16:** 593–595.
47 Jenkins S, Addy M, Newcombe R. The effects of 0.5% chlorhexidine and 0.2% triclosan containing toothpastes on salivary bacterial counts. *J Clin Periodontol* 1990; **17:** 85–89.
48 Owens J, Addy M, Faulkner J, Lockwood C, Adair R. A short-term clinical study design to investigate the chemical plaque inhibitory properties of mouthrinses when used as adjuncts to toothpastes: applied to chlorhexidine. *J Clin Periodontol* 1997; **24:** 732–737.
49 Barkvoll P, Rolla G, Bellagamba S. Interaction between chlorhexidine digluconate and sodium monofluorophosphate in vitro. *Scand J Dent Res* 1988; **96:** 30–33.
50 Cumming BR, Loë H. Optimal dosage and method of delivering chlorhexidine solutions for the inhibition of dental plaque. *J Periodontal Res* 1973; **8:** 57–62.
51 Lang NP, Ramseier-Grossmann K. Optimal dosage of chlorhexidine digluconate in chemical plaque control when applied by the oral irrigator. *J Clin Periodontol* 1981; **8:** 189–202.
52 Putt MS, Saxton CA, Milleman JL, Kleber CJ. Effect of dentifrice dilution on developing plaque and gingivitis. *J Dent Res* (Spec Issue) 1991; **70:** 327, Abstract #497.
53 Cochran JA, Ketley CE, Duckworth RM et al. Development of a standardized method for comparing fluoride ingested from toothpaste by 1.5–3.5-year-old children in seven European countries. Part1. Field work. *Community Dent Oral Epidemiol* 2004; **32 (Suppl. 1):** 39–46.
54 Buijs MJ, Loveren van C, Ung S, Ho Si T. *Fluoride retention after manual and electric tooth brushing.* Amsterdam, International Association for Dental Research, 2005, abstract book 2005 abstract #275.