

Chlorhexidine mouthrinse in combination with an SLScontaining dentifrice and a dentifrice slurry

Van Strydonck DAC, Timmerman MF, Van der Velden U and Van der Weijden GA. Chlorhexidine mouthrinse in combination with an SLS-containing dentifrice and a dentifrice slurry. J Clin Periodontol 2006; 33: 340–344. doi: 10.1111/j/1600-051X.2006.00910.x

Abstract

Objectives: The aim of the present study was to compare the plaque-inhibitory effect of a 0.2% chlorhexidine digluconate (CHX) rinse when preceded by ordinary toothbrushing with a 1.5% sodium lauryl sulphate (SLS)-containing dentifrice to the effect of the same rinse when used alone, or when preceded by rinsing with an SLS-containing slurry.

Methods: The study was an examiner blinded, randomized three-arm, parallel design. It used a 4-day plaque accumulation model to compare three different oral hygiene regimens, which were performed under supervision. One hundred and twenty healthy volunteers were enrolled in the study and were randomly assigned to one of each group. At the beginning of each test period, they received a thorough dental prophylaxis. The experiment was performed in one randomly assigned (upper or lower) jaw, called the *study* jaw. The opposite jaw, referred to as the *dentifrice* jaw, served only to introduce the influence of toothbrushing with a dentifrice on the antiplaque efficacy of the CHX in the *study* jaw of the same mouth. At the end of the 4-day test period, plaque and gingival bleeding were scored in the study jaw. In all the regimens, the oral hygiene procedure was finalized by rinsing with a CHX 0.2% solution for 1 min. The study jaw was not brushed during the experiment. Regimen A (positive control) consisted of rinsing with CHX alone. In regimen B, rinsing with CHX was preceded by rinsing with an SLS-containing slurry, while in regimen C rinsing with CHX was preceded by toothbrushing with an SLS-containing dentifrice in the *dentifrice* jaw. No other oral hygiene measures were allowed. After 4 days of undisturbed plaque accumulation, the amount of plaque and level of gingival health were evaluated.

Results: The overall plaque index for regimens A, B and C was 1.17, 1.62, and 1.14, respectively. There was no significant difference in plaque accumulation between the CHX alone regimen (A) and the SLS–dentifrice–CHX regimen (C). Regimen B differed significantly from regimens A and C. The overall bleeding index for regimens A, B and C was 0.24, 0.18, and 0.20, respectively. There was no significant difference between the three regimens.

Conclusions: The present study shows that the anti-plaque efficacy of a 0.2% CHX rinse was not reduced when preceded by everyday toothbrushing with a SLS-containing dentifrice. However, when preceded by rinsing with an SLS-containing slurry, the anti-plaque efficacy of a 0.2% CHX rinse was reduced.

D. A. C. Van Strydonck, M. F. Timmerman, U. Van der Velden and G. A. Van der Weijden

Department of Periodontology, Academic Centre for Dentistry Amsterdam, ACTA, Louwesweg, EA Amsterdam, The Netherlands

Key words: chlorhexidine; dentifrice; plaque; sodium lauryl sulphate

Accepted for publication 21 January 2006

Effective plaque control is crucial for the maintenance of periodontal health. For most individuals, the most efficient, safe and economical method of removing plaque is toothbrushing with a dentifrice. However, for many, a plaque-free dentition obtained by toothbrushing with dentifrice only is a difficult goal to achieve. The adjunctive use of an antiseptic agent may therefore be justified. After three decades of use in oral medicine, chlorhexidine digluconate (CHX) is still considered as the leading antiseptic to combat biofilms in supragingival and oral musocal sites (Addy 1986, Addy & Moran 1997).

One of the most widely used detergents in dentifrice is sodium lauryl sulphate (SLS). Unfortunately, in vitro, SLS and CHX may act as antagonists (Rölla et al. 1970, Kirkegaard et al. 1974, Rölla & Melsen 1975, Bonesvoll 1977, Barkvoll et al. 1988). In vivo, the interactions between CHX and SLS have been studied by Barkvoll et al. (1989) and Owens et al. (1997). They both concluded that CHX and SLS are not compatible in the oral cavity, even when they are introduced separately. Ever since, it has been recommended that the time between a CHX rinsing and toothbrushing with an SLS-containing dentifrice should be at least 30 min., probably close to 2h, in order to avoid reduction in the anti-microbial effect of CHX. To optimize the efficacy of a CHX rinse, toothbrushing with a dentifrice should be suspended or toothbrushing should be performed with dentifrice formulations without antagonistic ingredients (Owens et al. 1997) or without a dentifrice.

In these initial studies, SLS was used as an aqueous solution (Barkvoll et al. 1989) or as a water dentifrice slurry (Owens et al. 1997). The proposed inhibiting effect of SLS has not been tested if one uses this product as one would for daily oral hygiene.

Recently, these practical guidelines have been questioned by Van Strydonck et al. (2004a). In a 4-day plaque accumulation model, the plaque-inhibition of a 0.2% CHX rinse in one jaw was investigated under the influence of toothbrushing with a 1.5% SLS-containing dentifrice in the opposite jaw. On the basis of their clinical results, it appeared that the antiplaque efficacy of the 0.2% CHX mouthrinse was not reduced.

A second study (Van Strydonck et al. 2004b) with a similar design confirmed

- Products would only be used under supervision of two dental assistants twice daily at set times.
- Appointments (days and hours) were strict and could not be changed.
- It was not allowed to perform another form of oral hygiene other than the one assigned.
- It was not allowed to eat, drink or rinse with water within 30 min. after the rinsing procedure with the test rinse.
- Any change in medical status or medicine intake was to be reported.

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice.

Procedure

The study had a single-blind, randomized, three-arm parallel design. It used a 4-day plaque accumulation model to compare three groups of healthy volunteers with three different, supervised, oral hygiene regimens (A, B and C). The three groups (n = 40 each) were matched for sex and age.

The experiment was performed in one randomly assigned (upper or lower) jaw, called the *study* jaw. The opposite jaw, referred to as the *dentifrice* jaw, served only to introduce the influence of toothbrushing with a dentifrice on the antiplaque efficacy of the CHX in the study jaw of the same mouth. At the end of the 4-day test period, plaque and gingival bleeding were scored in the study jaw. In all the regimens, the oral hygiene procedure was finalized by rinsing with a CHX 0.2% solution for 1 min. The study jaw was not brushed during the experiment. Regimen A (positive control) consisted of rinsing with 0.2% CHX alone. In regimen B, rinsing with CHX was preceded by rinsing (60 s)with a 1.5% SLS-containing slurry, while in regimen C rinsing with 0.2% CHX was preceded by toothbrushing (60 s) with a 1.5% SLS-containing dentifrice in the dentifrice jaw (Table 1). After brushing, the dentifrice foam was thoroughly expectorated and the mouth was briefly (3s) rinsed with water. No other oral hygiene measures were allowed.

At baseline (day 1), all subjects received thorough professional prophylaxis by two subsequent operators. After staining the teeth with an aqueous

Subjects (n = 120)

Material and Methods

effects.

One hundred and twenty subjects, 54 males and 66 females, aged between 16 and 70 (mean age 43), were found to be suitable for the study. Subjects were in good general health without a medical history or medication that might interfere with the outcome of the study. All subjects were dentate with at least 24 scorable teeth, excluding third molars or crowned teeth with porcelain or golden restorations.

the findings of the first study. This time,

the study was performed under super-

vision, the order of rinsing-brushing

was reversed and different brands of

dentifrice, with and without SLS, were

compared. Again, the results showed

that the anti-plaque efficacy of CHX

was not reduced by everyday tooth-

brushing with a dentifrice. Irrespective

of whether the dentifrice contained SLS,

or was used before or after the rinse.

these two dentifrice studies by Van

Strydonck et al. (2004a, b) did not sup-

port the conclusions of the earlier work

(Barkvoll et al. 1989, Owens et al.

the conflicting results of Van Strydonck

et al. (2004a, b) compared with the

earlier CHX-SLS interaction studies

(Barkvoll et al. 1989, Owens et al.

1997) seems to be the use of an SLS-

containing dentifrice by Van Strydonck

et al. (2004a, b) instead of an SLS rinse

therefore to compare the plaque-

inhibitory effect of a 0.2% CHX rinse

when preceded by everyday tooth-

brushing with a 1.5% SLS-containing

dentifrice in the opposite jaw with the

effects of the same rinse when pre-

ceded by rinsing with an SLS-contain-

ing dentifrice slurry and rinsing with

0.2% alone. The present parallel study

intended to eliminate any carry-over

The aim of the present study was

by the other authors.

The most plausible explanation for

1997).

They were excluded if they had fixed or removable orthodontic appliances or removable prosthesis, pockets >5 mm or attachment loss >2 mm. On approval, all the volunteers received a personal instruction schedule, signed an informed-consent paper and, in order to participate, agreed to the following:

Table 1. Regimens

| Regimen A | Rinsing only with 0.2% CHX rinse for 60 s.* No brushing was allowed |
|-----------|--|
| Regimen B | Rinsing with a 3 g/10 ml water SLS-containing dentifrice slurry for 60 s.^{\dagger} Expectoration of the slurry, not followed by rinsing with water. Rinsing with a 0.2% CHX rinse for 60 s.^{*} |
| Regimen C | No brushing was allowed Toothbrushing in the <i>dentifrice</i> jaw with 1 cm of a 1.5% SLS-containing dentifrice for $60 s^{\ddagger}$ Expectoration of remaining dentifrice, followed by rinsing with water for 3 s. Rinsing with 0.2% CHX rinse for $60 s.^*$ No brushing of the <i>study jaw</i> was allowed |

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.

*Corsodyl[®] (GlaxoSmithKline (gsk), Zeist, the Netherlands).

[†]Aquafresh Regular[®] slurry (gsk) contains 3 g dentifrice Aquafresh Regular[®] (GSK)/10 ml water solution.

[‡]Aquafresh Regular[®] (gsk) dentifrice contains 1.5% SLS, pyrophosphate, 0.24% NaF (0.3% NaF = 1500 p.p.m.).

erythrosine disclosing solution (0.9%), a first operator (Ph.D) removed all supragingival plaque, stain and calculus by a sonic airscaler and polished all the teeth. Subsequently, after a second disclosing, the second operator (L. V. D. S.) made sure that all visible remnants of plaque, stain and calculus were removed from the teeth.

Throughout the duration of the study, subjects were asked to refrain from all forms of oral hygiene other than assigned products (e.g. dentifrice, non-study toothbrushes and mouthrinses, floss, woodsticks, interdental brushes, electric toothbrushes, oral irrigators, etc.).

Subsequently, they were randomly assigned to one of the three regimens. Instructions for the allocated regimen were given to each subject by a dental assistant, who also supervised the further conduct of the study.

During the test week, the subjects performed their given test regimes, while two dental assistants supervised the brushing-rinsing twice daily at set times, during the study duration. After 4 days of undisturbed plaque accumulation, the amount of plaque was scored in the study jaw. After disclosing the teeth with an erythrosine solution, plaque was assessed at six sites around each tooth, according to the modifications of Turesky et al. (1970) and Lobene et al. (1982) of the Quigley & Hein (1962) plaque index. In addition, at day 4, bleeding was scored in the study jaw by the use of a WHOapproved ball-ended probe (Ash Probe EN15, Dentsply International, York, PA. USA) and assessed by bleeding on marginal probing (Van der Weijden et al. 1994, Lie et al. 2001). Briefly the marginal gingivae were probed at an angle of approximately 60° to the longitudinal axis of the tooth. The bleeding was assessed at six sites per tooth. The gingival units that bled upon probing were recorded (scores 0, 1 and 2) giving non-bleeding sites a (0), pin-prick bleeding sites a (1) and excess bleeding sites a (2). Bleeding was scored within 30 s after probing. After the test, subjects resumed their normal tooth cleaning habits. All clinical measurements were performed under the same conditions by one and the same, well-trained examiner (D. V. S), who was blinded to the treatment.

Data analyses

Full-mouth mean plaque and bleeding scores were calculated. Plaque scores were considered as the primary outcome variable. Kruskall-Wallis tests were used to test for differences between the three regimens. Mann-Whitney tests were used for post-testing. The *p*-values were adjusted for multiple testing using Bonferoni's corrections (a factor three was used as in total, three tests were performed). Furthermore, 95% confidence intervals were calculated for differences between the groups. p-values < 0.05 were considered as statistically significant. Power calculations showed that the study design was able to discern a difference of 0.35 with an n of 40 and a pooled standard deviation of 0.56 at a power of 80%.

Results

One hundred and nineteen of the selected subjects (n = 120) completed the study without protocol violation.

One subject was withdrawn from the trial because of non-compliance with the study protocol and was not included in the analysis. No adverse effects were noted. Table 2 shows the mean plaque scores of the study jaws for the three regimens. The mean plaque index for the "CHX rinsing only" regimen (A) was 1.17, 1.62 for the "Slurry–CHX" regimen (B) and 1.14 for the "brushing-CHX'' regimen (C). Analysis showed a significant difference between the three regimens (p = 0.006). Explorative testing revealed a higher plaque index for the "Slurry-CHX" as compared with each of the other two regimens (Table 3). The mean bleeding index in the study jaw for regimen A was 0.24, 0.18 for B and 0.20 for C (Table 2). There was no significant difference in bleeding on marginal probing score between the three different regimens (Table 4).

Discussion

Dentifrice ingredients such as SLS have been shown to inhibit the activity of CHX (Barkvoll et al. 1989, Owens et al. 1997). Barkvoll et al. (1989) allowed their subjects to rinse with an aqueous solution of 0.2% SLS. In the study of Owens et al. (1997), the dentifrice, being a sodium fluoride and sodium monofluorophosphate SLS-containing product, was made into a 3 g/10 ml water slurry. In both these studies on CHX-SLS interaction, the oral cavity was not cleared from SLS before rinsing with CHX. In previous dentifrice studies by Van Strydonck et al. (2004a, b), and also in the present study, the SLS detergent was tested in an "everyday oral hygiene'' situation, i.e. toothbrushing with an SLS-containing dentifrice. As in daily life, the panelists expectorated the remnants of the dentifrice and rinsed with water immediately after brushing with the dentifrice. This cleared the oral cavity of the residual SLS dentifrice. A lower intra-oral SLS concentration and a shorter contact time of SLS with CHX are considered to be responsible for the observed absence of reduction in plaqueinhibition when using a CHX rinse in combination with a dentifrice (Van Strydonck et al. 2004a, b). This supposition has been confirmed in the present study. Compared with the CHX-alone group. the dentifrice group showed no reduced plaque-inhibition, while the slurry group, which is comparable with earlier studies (Barkvoll et al. 1989, Owens

et al. 1997), showed a significantly higher level of plaque accumulation.

Analogous to the studies of Barkvoll et al. (1989) and Owens et al. (1997). both studies by Van Strydonck et al. (2004a, b) used a cross-over model, using each patient as their own control. This design was chosen to provide considerable power to detect differences with relatively small sample sizes. Cross-over experiments can yield great savings if the assumption of a no carryover effect is valid (Grizzle 1965, Louis et al. 1984). The 4-day plaque accumulation model includes a thorough dental prophylaxis before the commencement of each test regimen. The magnitude and duration of this were not established, and frequent prophylaxis may influence the level of gingival health. It has been shown that, with healthy gingival tissues, less plaque develops (Ramberg et al. 1994, 1995). This may introduce an unwanted carry-over effect, which could possibly have obscured the interaction that is the subject of the former CHX–SLS interaction studies. The present study was designed to eliminate any possible carry-over effect. For this reason, it has a parallel design.

Several studies have shown that the development of plaque may be dependent on a number of factors such as diet (Rateitschak-Pluss & Guggenheim 1982), surface roughness (Ouirynen et al. 1990), periodontal condition (Rowshani et al. 2004) and bacterial salivary load (Dahan et al. 2004). Hillam & Hull (1977) showed in an experimental gingivitis study that the amount of plaque that developed in 24 h in gingival health at baseline was considerably less as compared with the amount of plaque developed in 24 h at the end of the experimental gingivitis period. More extensive studies performed by

Table 2. Mean overall plaque and bleeding scores for each regimen after 4 days of plaque accumulation, standard deviation in parenthesis

| | CHX alone $(n = 40)$ | Slurry–CHX $(n = 40)$ | Dentifrice—CHX $(n = 39)$ | <i>p</i> -value* |
|----------------|----------------------|-----------------------|---------------------------|------------------|
| Plaque index | 1.17 (0.62) | 1.62 (0.55) | 1.14 (0.51) | 0.0006 |
| Bleeding index | 0.24 (0.17) | 0.18 (0.15) | 0.20 (0.17) | 0.2842 |

*Kruskall-Wallis test.

Table 3. p-values of post-testing^{*} and 95% confidence intervals for differences in plaque indices between regimens

| Regimens | Original p-values (Mann–Whitney test) | 95% confidence interval |
|----------|--|----------------------------|
| A–B | 0.0027* | 0.20-0.72 |
| A–C | 0.8947 | -0.23-0.28 |
| B–C | 0.0002* | 0.25-0.72 |

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.

Regimen A: CHX alone.

Regimen B: Slurry-CHX.

Regimen C: Dentifrice-CHX.

*Significant after Bonferroni's corrections (factor 3) for multiple comparisons.

Table 4. p-values of post-testing^{*} and 95% confidence intervals for differences in marginal bleeding indices between regimens

| Regimens | Original p-values (Mann–Whitney test) | 95% confidence interval |
|----------|--|----------------------------|
| A–B | 0.1018* | - 0.12-0.018 |
| A–C | 0.6948* | -0.04-0.11 |
| BC | 0.3339* | -0.09-0.05 |

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.

Regimen A: CHX alone.

Regimen B: Slurry--CHX.

Regimen C: Dentifrice-CHX.

*Significant after Bonferroni's corrections (factor 3) for multiple comparisons.

© 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Lang et al. (1973), Breckx et al. (1980), Goh et al. (1986), Quirynen et al. (1991), Ramberg et al. (1994, 1995), Daly & Highfield (1996) and Rudiger et al. (2002), all confirmed that the periodontal condition is of foremost importance in the rate of de novo plaque formation. Use of three separate groups, in the present parallel design, may introduce an unwanted effect as a result of varying levels of gingival health. Therefore, in this study, in addition to plaque levels, the level of gingival health was assessed to make sure that this was not an interfering factor with the outcome of the study. In terms of bleeding on marginal probing, no significant difference was found between the three regimens. Table 4 shows the 95% CI for the differences in bleeding scores. These intervals are narrow and "0" is not far from the middle of the interval. It can therefore be concluded that the level of gingival health was not the origin of a more elevated plaque index as observed in the slurry group.

The present study has shown that the anti-plaque efficacy of a 0.2% CHX rinse is reduced under the influence of an SLS-containing dentifrice solution, which is in agreement with the earlier findings of Barkvoll et al. (1989) and Owens et al. (1997). However, their conclusions about the influence of everyday toothbrushing with an SLScontaining dentifrice on the anti-plaque efficacy of a 0.2% CHX may have been premature. Both the results of the present study and the two previous studies on the efficacy of CHX, combined with the interaction of different dentifrices for toothbrushing by Van Strydonck et al. (2004a, b), clearly indicate that the anti-plaque effect of a CHX mouthrinse is not reduced under the influence of normal everyday toothbrushing with a dentifrice, irrespective of whether the dentifrice contains SLS, or was used before or after the rinse. This observation has a practical implication for periodontal treatment. After periodontal surgery in, for instance, one quadrant in the mouth, the use of a CHX mouthrinse is frequently subscribed to optimize adequate woundhealing of the operated area.

One need not be concerned when toothbrushing is performed before or after the rinsing procedure.

In conclusion, when 0.2% CHX rinse was preceded by rinsing with an SLS-containing slurry, the anti-plaque effi-

cacy of CHX was reduced. However, when everyday toothbrushing with an SLS-containing dentifrice preceded a 0.2% CHX rinse, there was no significant difference from 0.2% CHX alone.

Acknowledgements

The support of Dr. Philippe Demoor, Dr. Luc Van der Sanden and the dental assistants of Paro Clinic Brasschaat is gratefully acknowledged. The authors would also like to express their gratitude to GlaxoSmithKline for financially supporting this project, and to Mr. John Pearman for his helpful comments in the preparation of this manuscript.

References

- Addy, M. (1986) Chlorhexidine compared with other locally delivered antimicrobials. A short review. *Journal of Clinical Periodontology* **13**, 957–964.
- Addy, M. & Moran, J. (1997) Clinical indications for the use of chemical adjuncts to plaque control: chlorhexidine formulations. *Periodontology 2000* 15, 52–54.
- Barkvoll, P., Rolla, G. & Bellagamba, S. (1988) Interaction between chlorhexidine digluconate and sodium monofluorophosphate in vitro. Scandinavian Journal of Dental Research 96, 30–33.
- Barkvoll, P., Rolla, G. & Svendsen, A. K. (1989) Interaction between chlorhexidine gluconate and sodium lauryl sulfate in vivo. *Journal of Clinical Periodontology* 16, 593–595.
- Bonesvoll, P. (1977) Influence of ionic strength, calcium, sodium dodecyl sulfate and urea on the retention of chlorhexidine in the human mouth after mouthrinses. *Archives of Oral Biology* 22, 273–279.
- Breckx, M., Theilade, J. & Attström, R. (1980) Influence of optimal and excluded oral hygiene on early formation of dental plaque on plastic films. A quantitative and descriptive light and electron microscopic study. *Journal of Clinical Periodontology* 7, 361–373.
- Dahan, M., Timmerman, M. F., Van Winkelhoff, A. J. & Van der Velden, U. (2004) The effect of periodontal treatment on the salivary bacterial load and early plaque formation. *Journal of Clinical Periodontology* 31, 772–777.
- Daly, C. G. & Highfield, J. E. (1996) Effect of localized experimental gingivitis on early supragingival plaque accumulation. *Journal* of Clinical Periodontology 23, 160–164.

- Goh, C. J., Waite, I. M., Groves, B. J. & Cornick, D. E. (1986) The influence of gingival inflammation and pocketing on the rate of plaque formation during non-surgical periodontal treatment. *British Dental Journal* 161, 165–169.
- Grizzle, J. E. (1965) The two- period changeover design an its use in clinical trials. *Biometrics* 21, 467–80.
- Hillam, D. G. & Hull, P. S. (1977) The influence of experimental gingivitis on plaque formation. *Journal of Clinical Periodontology* 4, 56–61.
- Kirkegaard, E., Fehr, F. R. & von der Rolla, G. (1974) Influence of chlorhexidine on in vitro uptake of fluoride in dental enamel. *Scandinavian Journal of Dental Research* 82, 566–569.
- Lang, N. P., Cumming, B. R. & Löe, H. (1973) Toothbrushing frequency as it relates to plaque development and gingival health. *Journal* of *Periodontology* 44, 396–405.
- Lie, M. A., Van de r Weijden, G. A., Timmerman, M. F., Loos, B. G., van Steenbergen, T. J. & Van der Velden, U. (2001) Occurrence of Prevotella intermedia and Prevotella nigrescens in relation to gingivitis and gingival health. *Journal of Clinical Periodontology* 28, 189–193.
- Lobene, R. R., Soparkar, P. M. & Newman, M. B. (1982) Use of dental floss. Effect on plaque and gingivitis. *Clinical Preventive Dentistry* 4, 5–8.
- Louis, T. A., Lavori, P. W., Bailar, J.C 3rd & Polansky, M. S. (1984) Crossover and selfcontrolled designs in clinical research. *The New England Journal of Medicine* **310**, 24–31.
- Owens, J., Addy, M., Faulkner, J., Lockwood, C. & Adair, R. (1997) A short-term clinical study design to investigate the chemical plaque inhibitory properties of mouthrinses when used as adjuncts to toothpastes: applied to chlorhexidine. *Journal of Clinical Periodontology* 24, 732–737.
- Quigley, G. & Hein, J. (1962) Comparative cleansing efficiency of manual and power brushing. *Journal of the American Dental Association* 65, 26–29.
- Quirynen, M., Dekeyser, C. & van Steenberghe, D. (1991) The influence of gingival inflammation, tooth type, and timing on the rate of plaque formation. *Journal of Periodontology* 62, 219–222.
- Quirynen, M., Marechal, M., Busscher, H. J., Weerkamp, A. H., Darius, P. L. & van Steenberghe, D. (1990) The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. *Journal of Clinical Periodontology* 17, 138–144.
- Ramberg, P., Axelsson, P. & Lindhe, J. (1995) Plaque formation at healthy and inflamed

gingival sites in young individuals. *Journal* of Clinical Periodontology **22**, 85–88.

- Ramberg, P., Lindhe, J., Dahlen, G. & Volpe, A. R. (1994) The influence of gingival inflammation on de novo plaque formation. *Journal* of Clinical Periodontology **21**, 51–56.
- Rateitschak-Pluss, E. M. & Guggenheim, B. (1982) Effects of a carbohydrate-free diet and sugar substitutes on dental plaque accumulation. *Journal of Clinical Periodontology* 9, 239–251.
- Rölla, G., Löe, H. & Schiött, C. R. (1970) The affinity of chlorhexidine for hydroxyapatite and salivary mucins. *Journal of Periodontal Research* 5, 79–83.
- Rölla, G. & Melsen, B. (1975) On the mechanism of the plaque inhibition by chlorhexidine. *Journal of Dental Research* 54, 1357–1362.
- Rowshani, B., Timmerman, M. F. & Van der Velden, U. (2004) Plaque development in relation to the periodontal condition and the bacterial load of the saliva. *Journal of Clinical Periodontology* **31**, 214–218.
- Rudiger, S. G., Carlen, A., Meurman, J. H., Kari, K. & Olsson, J. (2002) Dental biofilms at healthy and inflamed gingival margins. *Journal of Clinical Periodontology* 29, 524–530.
- Turesky, S., Gilmore, N. D. & Glickman, I. (1970) Reduced plaque formation by the chloromethyl analogue of victamine C. *Jour*nal of Periodontology 41, 41–43.
- Van Strydonck, D. A. C., Demoor, Ph., Timmerman, M. F., Van der Velden, U. & Van der Weijden, G. A. (2004b) The anti-plaque efficacy of a chlorhexidine mouthrinse used in combination with toothbrushing with dentifrice. *Journal of Clinical Periodontology* **31**, 691–695.
- Van Strydonck, D. A. C., Scalé, S., Timmerman, M. F., Van der Velden, U. & Van der Weijden, G. A. (2004a) Influence of a SLS containing dentifrice on the anti-plaque efficacy of a chlorhexidine mouthrinse. *Journal* of Clinical Periodontology **31**, 219–222.
- Van der Weijden, G. A., Timmerman, M. F., Danser, M., Nijboer, A., Saxton, C. A. & Van der Velden, U. (1994) Effect of pre-experimental maintenance care duration on the development of gingivitis in a partial mouth experimental gingivitis model. *Journal of Periodontal Research* 29, 168–173.

Address:

Danielle Van Strydonck Department of Periodontology Academic Center for Dentistry Amsterdam ACTA Louwesweg 1 1066 EA AMSTERDAM The Netherlands E-mail: d.v.strydonck@acta.nl This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.