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The effect of an oxygenating agent on chlorhexidine-induced extrinsic tooth staining: a systematic review

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Abstract: *Background:* Although chlorhexidine digluconate (CHX) is currently the most effective mouthwash for reducing plaque and gingivitis, one of its side effects is extrinsic tooth staining. Interestingly, oxygenating agents may reduce this staining. *Objective:* The aim of this review was to systematically search the literature for data concerning the inhibiting effect of an oxygenating agent (OA) on CHX-induced tooth staining. *Methods:* MEDLINE-PubMed, Cochrane-CENTRAL, EMBASE and other relevant electronic databases were searched for articles that were published up to November 2011. Articles were included if they were randomized controlled trials or controlled clinical trials conducted with healthy subjects ≥ 16 years of age that compared the effects of CHX mouthrinse combined with an OA with the effects of CHX alone. *Results:* An independent screening of 1183 titles and abstracts resulted in 4 publications that met the inclusion criteria. The extracted data allowed meta-analyses of intermediate length studies and showed that combining an OA with CHX mouthrinses led to a significant reduction in tooth staining (mean difference: 0.27; $P = 0.02$) and plaque scores (mean difference: 0.10; $P = 0.003$) when compared with CHX alone. One of the included studies reported a side effect for one participant. The present review was limited by the availability of data, and the included studies were methodologically and clinically heterogeneous, which affected the quality and interpretation of the evidence. *Conclusion:* There is moderate evidence that a combination of CHX and an OA reduces tooth staining without interfering with plaque growth inhibition.

Key words: bleeding; chlorhexidine; gingivitis; hydrogen peroxide; mouthwashes plaque; systematic review; tooth staining

Introduction

Dental health practitioners often prescribe mouthrinses as an aid for preventing the formation of plaque. Currently, the most effective and widely investigated mouthwash for reducing plaque and gingivitis is chlorhexidine digluconate (CHX), which is the gold standard of antiplaque agents (1, 2). Interestingly, a number of local side effects have been reported for CHX mouthrinses. The most common side effect is extrinsic tooth staining and a brown discoloration of the tongue. The staining becomes worse when other products that are known to cause staining, such as tea, coffee, wine and cigarettes, are consumed at the same time. Importantly, the staining effects of CHX may have a negative effect on rinsing compliance (3–5).

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In vitro and *in vivo* studies have shown that hydrogen peroxide (H_2O_2) has a good stain-removing/preventive capability (6, 7). The potential of an oxygenating agent (OA) to inhibit CHX-induced stain formation was suggested by Ellingen *et al.* (8), proved by Eriksen *et al.* (9) and confirmed by Addy *et al.* (7). The mechanism by which H_2O_2 reduces extrinsic staining is not clear, which is not surprising given that the mechanism by which CHX causes staining is still under debate (10, 11). Clinical studies have demonstrated individual discoloration tendencies (12, 13) and shown that CHX binds food chromogens and dyes to surfaces (10). Warner *et al.* (14) indicated that chlorhexidine treatments alter the incorporation of natural sulphur-containing organic components, which are found in saliva or bacteria, into plaques. The natural sulphur-containing component appears to readily interact with transition metals, particularly iron, to produce stained materials. Indeed, both stannic and ferric sulphides are strongly coloured, and these colours correlate nicely with clinically observed extrinsic discolorations. In a more oxidized state, sulphide compounds transform into sulphates, which generally become greyish/white and soluble. These findings may explain why rinsing with an oxidizing solution inhibits the staining of teeth (10). Clinical studies that have investigated the potential synergistic effect of OA and CHX mouthwashes suggested that OA and CHX can be used to control dental plaque (15–17). Positive results regarding the inhibition of plaque growth have been observed when CHX was combined with an OA (16, 18).

The effects of OA mouthwashes on CHX-induced tooth staining have not been systematically evaluated. Because healthcare providers are often overwhelmed with the amount of available information (19), there is a need for systematic reviews to efficiently integrate existing information and provide data for clinical decision making. The objective of this systematic review was to assess the effects of CHX mouthwashes on tooth staining, plaque and gingivitis when used in combination with an OA or alone.

Materials and methods

This systematic review was conducted in accordance with the guidelines of Transparent Reporting of Systematic Reviews and Meta-analyses (PRISMA statement) (20).

Focused question

What is the effect of a CHX mouthrinse on tooth staining (primary outcome) and plaque and gingivitis (secondary outcomes) when used in combination with an OA (i.e. hydrogen peroxide/perborate) or alone?

Search strategy

The following three electronic databases were searched for relevant trials: The National Library of Medicine, Washington DC (MEDLINE-PubMed), The Cochrane Central Register of Controlled Trials (CENTRAL) (the Cochrane Library current issue) and EMBASE (Excerpta Medical Database by Elsevier). The search strategy was developed in MEDLINE and revised appropriately for each database searched. The databases were searched up to November 2011 using the search terms that are presented in Box 1. The reference lists of the included studies were handsearched to identify potential relevant studies. In addition, national (http://www.trialregister.nl) and international trial registries (http://www.controlled-trials.com, ClinicalTrials.gov) were searched for relevant unpublished or ongoing studies. Furthermore, the following database sources were searched for possible relevant studies that had not reached full publication: OpenGrey (http://www.opengrey.eu/), British Library Inside (http://www.bl.uk/inside), the European Federation of Periodontology (http://www.epf.net), the International Association for Dental Research (http://www.iadr.org), Web of Science® and BIOSIS Previews®, (http://www.ovid.com).

Box 1

The following terms were used in the PubMed-MEDLINE search strategy: The search strategy was customized according to the database been searched. *The asterisk (*) was used as a truncation symbol*

[<*Agent* OR *brandname*> AND *vehicle* AND *outcome*]

[<**Agent**

(MeSH terms) chlorhexidine OR (text words) chlorhexidine OR chlorhexidine di-gluconate OR chlorhexidine gluconate OR zinc-chlorheidine OR chlorhexidine gluconate lidocaine hydrochloride OR CHX OR CHX formulations OR chlorhexidine phosphanilate

Brandname

peridex OR eludril OR corsodyl OR hibitane OR periogard OR perioaid maintenance OR hibidex

OR

Agent

(MeSH terms) hydrogen peroxide OR peroxides OR(text words) oxygenating agents OR oxidizing agents OR sodium perborate OR buffered sodium peroxyborate OR peroxyborate OR peroxycarbonates OR OA

OR

Brandname

bocasan OR amosan OR peroxyl OR ascoxal >

AND

Vehicle

(MeSH Terms) mouthwashes OR (Text Words) mouthwash OR mouthwash* OR mouthrinses OR mouthrinse)

AND

Outcome

(MeSH terms)gingivitis OR gingival haemorrhage OR gingival pocket OR periodontal diseases OR periodontal pockets OR tooth staining OR(text words) gingivitis OR gingival inflammation OR gingival diseas* OR gingivit* OR gingival index OR gingival haemorrhage OR bleeding on probing OR papillary bleeding OR papillary bleeding index OR gingival bleeding OR bleeding index OR sulcus bleeding index OR periodontitis OR pocket depth OR gingival pocket OR periodontal pocket OR periodontal diseas* OR pockets OR probing depth OR probing-depth OR probing pocket depth OR pocket-depth OR periodontal attachment loss OR plaque index OR dental plaque OR plaque OR interdental plaque OR interproximal plaque OR plaque index OR dental deposit* OR stain OR staining OR stain index OR calculus OR tartar]

Screening and selection

All of the titles and abstracts that were obtained in the searches were independently screened by two reviewers (NMS, GAW) to select studies that potentially met the inclusion criteria. Randomized controlled trials (RCTs) and controlled clinical trials (CCTs) were included, conducted in healthy humans (≥16 years) and comparing the tooth staining effects of CHX by itself or in combination with an OA. No language restrictions were imposed. Based on the title and/or the abstract, the full-text versions of potentially relevant articles were acquired. The articles were categorized (NMS, GAW) as definitely eligible, definitely not eligible or questionable. Disagreements concerning eligibility were resolved by consensus or, if a disagreement persisted, by arbitration through a third reviewer (DES).

Assessment of heterogeneity

The factors that were used to evaluate the heterogeneity of the results of the studies chosen for this review included the study design and the evaluation period, the characteristics of the study participants, the comparison and regimen and industry funding.

Data extraction

Two reviewers (NMS, DES) used a specially designed data extraction form to independently extract data from all of the studies regarding characteristics of the populations, intervention methods, comparisons and outcomes. These forms were piloted on a few studies to ensure their suitability for data extraction. Disagreement between the reviewers was resolved through discussion and consensus. If a disagreement persisted, the judgment of a third reviewer (GAW) was decisive.

Quality assessment

The methodological quality of the included studies was independently scored by two reviewers (NMS, DES) as previously described by Higgings *et al.* (21). The quality assessment was performed by combining the proposal criteria of the RCT checklist of the Dutch Cochrane Centre (22), Montenegro *et al.* (23) and Needleman *et al.* (24). An aspect of the score list was given a '+' for an informative description of the item at issue for a study design that met the quality standard, a '–' for an informative description and a study design that did not met the quality standard and a '?' for missing or insufficient information. A study was considered to have a 'low risk' of bias when 'random allocation, defined inclusion/exclusion, blinding to patient and examiner, balanced experimental groups, an identical treatment between groups except for intervention and report of follow-up criteria were adequately addressed'. Studies that scored five of these six criteria were considered to have a potential 'moderate risk' of bias, and the absence of two or more of these six criteria was considered to represent a potential 'high risk' of bias as proposed by Van der Weijden *et al.* (25). In addition, the 'Levels of Evidence' of the Oxford Centre for Evidence-Based Medicine (OCEBM) were used to rate the hierarchy of evidence (26).

Data analysis

The Cochrane Collaboration's statistical guidelines were followed to determine the choice of summary statistics and estimates of the overall effect (21). A fixed-effect model meta-analysis was used (21, 27, 28). Continuous data and pooled outcomes were expressed as difference in means (MD) with their associated 95% confidence interval. Analyses were performed in Review Manager Software in accordance with the PRISMA guidelines (20, 29). The outcome of the meta-analysis was evaluated by identifying five factors that might decrease the quality and body of evidence using criteria proposed by GRADE (30).

Results

Search selection results

The search and selection process are summarized in Fig. 1. A search of PubMed-MEDLINE, Cochrane-CENTRAL and the EMBASE search resulted in 1182 unique titles. Records identified through other sources resulted in one additional publication (31).

In total 17 articles were excluded after reviewing the full text because they failed to fulfil the inclusion criteria of this review (7, 16, 32–46). An overview of these studies, including the reasons for exclusion is given in the online Appendix S1. A total of four studies met the inclusion criteria (9, 31, 47, 48), and these studies consisted of a total of 227 subjects (with a range of 28–119 subjects) who completed the follow-up.

Assessment of study heterogeneity

Table 1 reveals the characteristics of the included studies and shows that there was considerable heterogeneity in the study design, the evaluation period and the characteristics of the participants. There was also heterogeneity regarding the order of rinsing, the rinsing time and the amount of mouthwash that was used. In two of the four studies, rinsing with water immediately after the use of mouthrinse was not allowed (47, 48). In the study by Winer *et al.* (48), participants were asked to brush their teeth immediately prior to the use of the mouthrinse and to expectorate thoroughly afterwards. They were required to repeat this protocol twice a day (after breakfast and before bedtime). A professional prophylaxis was provided immediately prior to entering the experiment in three of the studies (9, 47, 48). Rahmani *et al.* (31) performed scaling and root planing on their subjects two weeks prior to the start of the trial. Winer *et al.* (48) and Rahmani *et al.* (31) only included non-smoking participants, whereas Gründemann *et al.* (47) did not provide any data concerning their subjects' smoking habits.

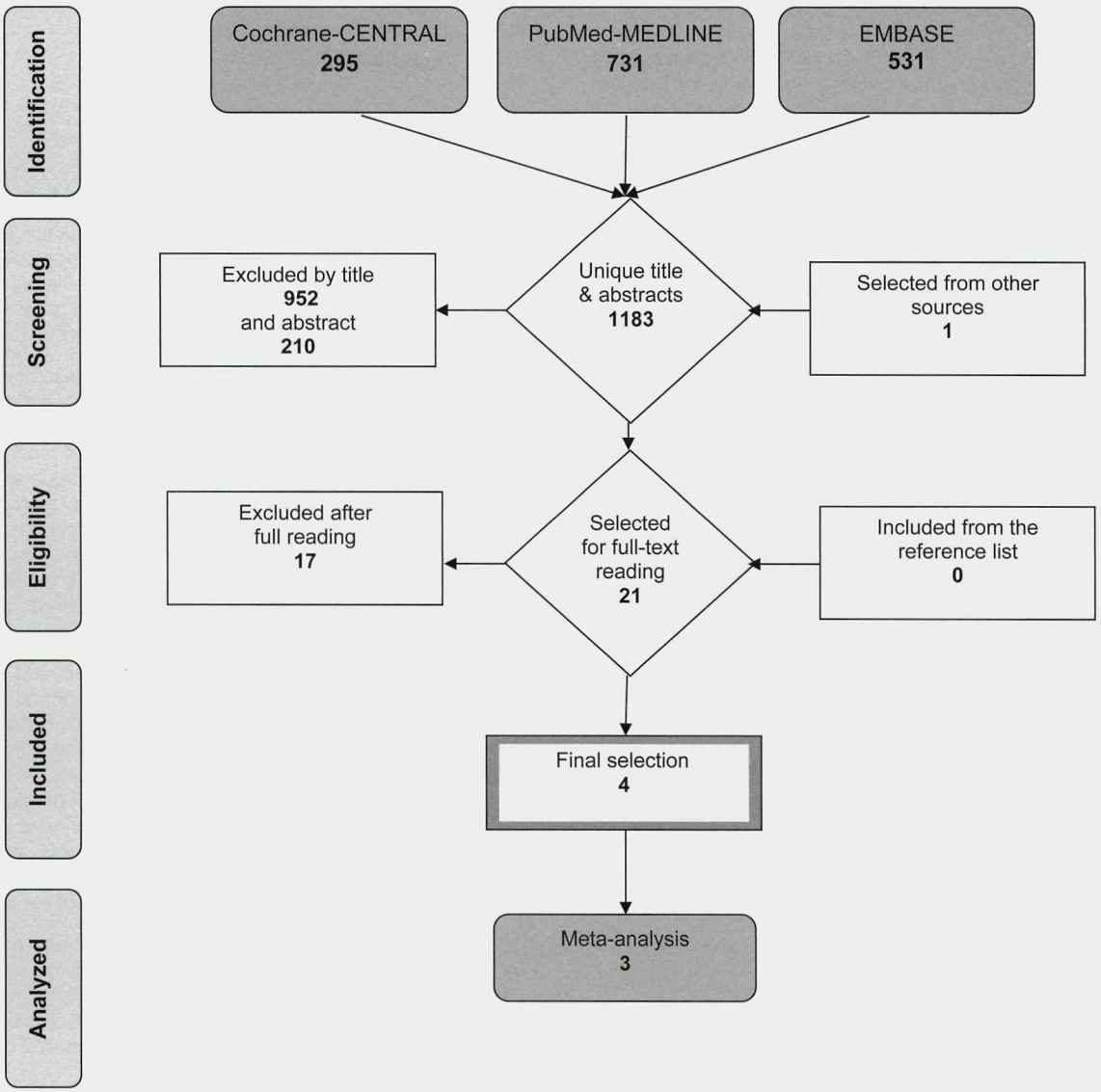

Fig. 1. Search, selection and analysis process.

Erikson *et al.* (9) performed a subanalysis based on tea consumption and smoking status and found that tea consumption and smoking were more frequent in the participants that developed extrinsic tooth staining.

Side effects

Two studies discussed side effects. Winer *et al.* (48) did not find any intraoral soft tissue side effects that were attributed to product use, whereas Gründemann *et al.* (47) reported that one participant complained of a burning sensation of his/her tongue, loss of taste perception, nausea and a dry mouth.

Industry funding

The study by Winer *et al.* (48) was supported by a grant from Colgate-Hoyt, Canton, MA, and the Erikson *et al.* study (9) was supported by the Norwegian Research Council for Science and the Humanities.

Assessment of study quality

A summary of the quality assessment of the various studies is presented in Table 1. Winer *et al.* (48) had a low potential risk of bias, whereas the other three studies revealed a moderate potential risk of bias (9, 31, 47). The level of evidence (OCEBM) for the four included studies was 2 (26), which represents randomized trials with dramatic effects. For more detail, please see Appendix S2.

Study outcomes

Base and end-of-trial scores for parameters of interest are shown in Tables 2 and 3.

Table 1. **Overview of the studies that were processed for data extraction**

Ref #	Author (year) Title	Design and evaluation period (washout period) Risk of bias	Participants *n* base (end) Gender Age	Regimen % Brand	Authors conclusion
31	Rahmani *et al.* (2006) Effects of combined use of Hydrogen peroxide and chlorhexidine mouthrinses on gingivitis, plaque and tooth staining	RCT Double-blind Parallel 14 days Non-brushing Moderate*	Mild to moderate gingivitis 30 ♀: 4 ♂: 26 Mean: 24 Range: 16-32	OA +CHX CHX OA: 15 ml, 30 s, twice daily CHX: 10 ml, 30 s, twice daily CHX: 0.2%? OA: 1.5%?	The use of hydrogen peroxide prior to CHX may cause significant reduction in tooth staining. Hydrogen peroxide does not have negative effects on plaque and gingivitis reduction ability of CHX.
47	Gründemann *et al.* (2000) Stain, plaque and gingivitis reduction by combining chlorhexidine and peroxyborate	RCT Single-blind Parallel 14 days Non-brushing Moderate*	Minimal gingivitis (non dental students) 30 (30) ♀: 20 ♂: 10 Mean: ? Range: 22–27	OA + CHX CHX OA: 30 ml, 60 s, twice daily CHX: 15 ml, 60 s, twice daily CHX: 0.12% Oral-B® OA: 1.35% Bocasan®	The adjunctive use of an oxidizing agent peroxyborate to CHX proved to be superior to CHX alone with regard to the inhibition of plaque and development of gingivitis. In addition, the proportion of stained surfaces was significantly less when adding the oxidizing mouthrinse to CHX.
48	Winer *et al.* (1991) Effect of Peroxyl® mouthrinse on chlorhexidine staining of teeth	RCT Single-blind Parallel 90 days Brushing Low*	Non advantage periodontitis 142 (119) ♀: 68 ♂: 51 Mean: ? Range: ?	OA + CHX placebo + CHX OA: 10 ml, 60 s, four times a day CHX: 15 ml, 30 s, twice daily Placebo: 10 ml, 60 s, four times a day CHX: 0.12% Peridex® OA: 1.5% Peroxyl®	Use of OA mouthrinse significantly reduced extrinsic tooth stain produced by CHX rinsing. The mean stain scores for all teeth were significantly lower for the OA + CHX. group compared with the placebo + CHX group. No intraoral soft tissue side effects, attributed to product use, were observed or reported throughout the study
9	Eriksen *et al.* (1983) Chemical plaque control and prevention of extrinsic toot staining *in vivo*	RCT Double-blind Crossover 14 days (7 days) Non-brushing Moderate*	Military recruits 50 (50) ♀: ? ♂: ? Mean: ? Range: 19–24	CHX + OA CHX + placebo CHX: ? ml, 60 s, twice daily OA: ? ml, 60 s, twice daily Placebo: ? Ml, 60 s, twice daily CHX: 0.2% Hibitane® OA: 1% Caroat®	The plaque-preventive capacity of CHX was maintained, and a marked reduction in extrinsic tooth staining could be observed when a CHX + OA were used.

CHX, chlorhexidine; OA, hydrogen peroxide/perborate; ?, unknown.
*The author's estimated potential risk of bias (for details, please see Appendix S2).

A descriptive summary of the data concerning significant differences between the combination of an OA and CHX and CHX alone is presented in Table 4.

Primary outcome: tooth staining

Rahmani *et al.* (31) showed that the reduction in staining scores in the body and gingival region of the tooth surface was significantly higher in the OA and CHX group compared with the CHX alone group. There was a significant difference between the groups regarding the stain intensity score in the body region ($P = 0.004$), but not in the gingival region (31). Gründemann *et al.* (47) reported a significant difference between the proportions of stained surfaces in the OA and CHX group compared with the CHX alone group. The whole-mouth assessment showed a trend towards less staining for the OA and CHX group compared with the CHX-alone group. For the gingival sites, the scores in the CHX and OA group were approximately half of the scores in the CHX-alone group (47). Winer *et al.* (48) observed significantly less staining ($P < 0.005$) after 90 days in the OA and CHX group compared with the CHX-alone group. In addition, Eriksen *et al.* (9) found a significant difference ($P < 0.01$) between the extrinsic staining in the OA and CHX group compared with CHX alone.

Secondary outcome: plaque and gingivitis

Compared with CHX alone, rinsing with OA and CHX resulted in a synergistic effect on the plaque scores ($P = 0.03$) in one of four studies (47). The mean plaque

Table 2. **The outcomes of the selected studies with respect to tooth staining. The baseline scores were set at zero because all of the studies provided a professional prophylaxis prior to treatment**

Items/scores	Ref #	Authors	Index	Group	Mean (SD) End
Tooth staining	31	Rahmani *et al.* (2006)	Gingival region of the tooth surface Lobene (1968) (49) Intensity	OA + CHX CHX	1.17 (0.36) 1.34 (0.40)
			Gingival region of the tooth surface Lobene (1968) (49) Area	OA + CHX CHX	2.11 (0.56) 2.68 (0.51)
			Body region of the tooth surface Lobene (1968) (49) Intensity	OA + CHX CHX	0.84 (0.38) 1.38 (0.50)
			Body region of the tooth surface Lobene (1968) (49) Area	OA + CHX CHX	1.18 (0.61) 2.10 (0.45)
	47	Gründemann *et al.* (2000)	Modification of the Stain Index (Lobene 1968) (49) Intensity	OA + CHX CHX	0.36 (0.25) 0.77 (0.59)
			Gingival region of the tooth surface Modification of the Stain Index (Lobene 1968) (49) Intensity	OA + CHX CHX	0.41 (0.32) 0.89 (0.68)
	48	Winer *et al.* (1991)	Stain scoring based on the adapted method of Quigley & Hein Index (1962) (61)	OA + CHX Placebo + CHX	0.69 (0.60) 0.87 (0.56)
	9a 9b	Eriksen *et al.* (1983)	Staining Index (Eriksen *et al.* 1979) (62) Intensity	CHX + OA CHX + placebo CHX + OA CHX + placebo	0.36 (0.70)* 1.37 (1.01)* 0.33 (0.56)* 1.15 (0.99)*

For abbreviations, please see Table 1.
*Calculated by the authors of this systemic review based on the presented data in the selected paper.

Table 3. **The secondary outcomes of selected studies plaque and gingivitis scores. The baseline plaque scores were considered to be zero because the subjects received a professional prophylaxis prior to treatment*.**

Items/ scores	Ref #	Authors	Index	Group	Mean (SD) Baseline*	End	Difference
Plaque	31	Rahmani *et al.* (2006)	Sillness & Löe (1964) (50)	OA + CHX CHX		1.44 (0.57) 1.43 (0.49)	
	47	Gründemann *et al.* (2000)	Sillness & Löe (1964) (50)	OA + CHX CHX		0.08 (0.08) 0.18 (0.12)	
	9a 9b	Eriksen *et al.* (1983)	Sillness & Löe (1964) (50)	CHX + OA CHX + placebo CHX + OA CHX + placebo		0.4 (0.2) 0.5 (0.3) 0.5 (0.3) 0.5 (0.3)	
	48	Winer *et al.*1991)	Quigley & Hein (1962) (61)	OA + CHX placebo + CHX		1.25 (0.63) 1.16 (0.59)	
Gingivitis	31	Rahmani *et al.* (2006)	Löe & Sillness (1963)(63)	OA + CHX CHX	1.14 (0.45) 1.54 (0.32)	0.86 (0.32) 1.05 (0.03)	0.28 (0.30) 0.49 (0.32)
			Ainamo & Bay (1975)(64)	OA + CHX CHX	0.53 (0.30) 0.74 (0.30)	0.36 (0.31) 0.35 (0.20)	0.18 (0.27) 0.39 (0.28)
	47	Gründemann *et al.* (2000)	BOMP (Van der Weijden *et al.* (1994) (65)	OA + CHX CHX	0.42 (0.23) 0.48 (0.22)	0.21 (0.10) 0.38 (0.15)	0.21† 0.10†

For abbreviations, please see Table 1 and 2.
†Calculated by the authors of this systemic review based on the presented data in the selected paper.

scores for the treatment and control groups in the other studies did not differ (9, 31, 48). Gründemann *et al.* (47) reported a decrease in the bleeding tendency in the OA and CHX group, which was significant ($P < 0.001$) compared with CHX alone.

Meta-analyses

Due to the limited number of eligible studies, 'fixed-effect' meta-analyses were used to combine quantitative data (21). Because the subjects were enrolled following a thorough pro-

Table 4. **Descriptive summary of the statistical significance of the comparisons between the interventions OA/CHX, or CHX/OA, and control CHX**

Ref #	Authors	Intervention	Tooth staining Extend/Area	Intensity	Plaque	Gingivitis	Comparison
31	Rahmani *et al.* (2006)	OA + CHX	+	?	O	O	CHX
47	Gründeman *et al.* (2000)	OA + CHX	+	O	+	+	CHX
48	Winer *et al.* (1991)	OA + CHX	+	□	O	□	Placebo + CHX
9	Eriksen *et al.* (1983)	CHX + OA	□	+	O	□	CHX + placebo

+, significant difference in favour of intervention; 0, no significant difference; □, no data available; ?, inconclusive data that did not allow us to draw conclusions concerning statistical significance.
For abbreviations, please see Tables 2 and 3.

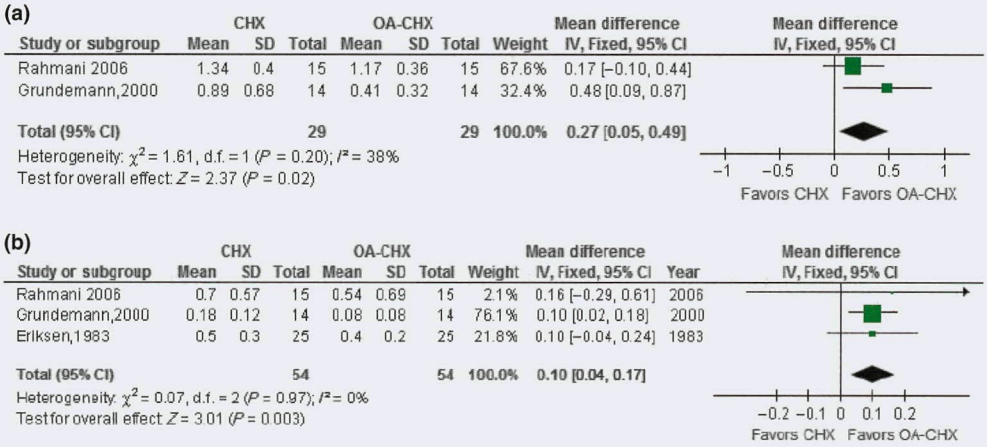

Fig. 2. (a) Meta-analysis of the stain intensity index according to Lobene (49) focusing on the gingival region comparing OA–CHX versus CHX. (b) Meta-analysis of the Plaque Index according to Sillness & Löe (50) comparing OA–CHX versus CHX.

fessional prophylaxis, the meta-analysis was performed with the data from the end-of-trial assessment. Studies were included in this meta–analysis irrespective of the order in which the OA and CHX were used (17). Both Rahmani *et al.* (31) and Gründemann *et al.* (47) reported data for the gingival region of the tooth surface using the Lobene Stain Index (49). Pooled data from these two studies showed a significant reduction in tooth staining in the OA and CHX group compared with CHX alone, with a mean difference of 0.27 ($P = 0.02$) (Fig. 2a). Heterogeneity tests did not show any evidence of a different treatment effect ($P = 0.2$). Plaque was scored according to the Sillness & Löe Plaque Index (50) in three of the studies (9, 31, 47). There was a significant synergistic effect on plaque scores when a combination of OA and CHX was used, with a mean difference of 0.10 ($P = 0.003$). Heterogeneity tests did not show any evidence of a different treatment effect between the studies ($P = 0.97$) (Fig. 2b).

Grading the 'body of evidence'

Table 5 shows a summary of the various factors that were used to grade the evidence of the meta-analysis and rate its quality. Allocation concealment was unclear in all of the studies. In addition, one study did not blind the participants, and two studies did not report that the statistics were performed using an intention-to-treat approach. Subsequently, this affected the level of evidence which therefore was downgraded to a 'moderate' quality of evidence.

Discussion

This systematic review summarized the effects of combining OAs with CHX mouthwashes with respect to tooth staining, plaque and gingivitis scores. Four studies met the inclusion criteria, and three of the four studies observed significantly less CHX-induced tooth staining with the combination of CHX and an OA compared with CHX alone (31, 47, 48). Regarding the secondary outcomes, one study (47) found that combining an OA with CHX was more effective than CHX alone in preventing plaque formation and gingivitis; however, the other studies did not observe any significant difference.

This lack of added effects is surprising considering previously reported data. Indeed, plaque inhibition has been reported when CHX was combined with OAs, such as H_2O_2, peroxymonosulfate or Bocasan® (7, 9, 47, 48, 51). Dona *et al.* (16) compared the combination of CHX and Bocasan® with CHX alone in a 3-day plaque accumulation model and found significantly lower plaque scores for the

Table 5. **GRADE evidence profile of the meta-analysis (Figs 2a and b) for the combination of OA/CHX, or CHX/OA, compared with CHX on tooth staining and plaque scores**

# of studies (No. of participants)	Quality assessment					Summary of findings	
	Study limitations	Consistency	Directness	Precision	Publication bias	Mean Difference (95% CI)	Quality of the evidence (GRADE)
Staining							
2 (53)	Potential limitations	No serious inconsistency	No serious indirectness	No serious imprecision	Unlikely	0.27 (0.05,049)	+++ Moderate*,†
Plaque							
3 (108)	Potential limitations	No serious inconsistency	No serious indirectness	No serious imprecision	Unlikely	0.10 (0.04,0.17)	+++ Moderate*,†,‡

*Gründemann *et al.* (2000) (47): unclear allocation concealment, no intention-to-treat analysis, patients not blinded.
†Rahmani *et al.* (2006) (31): unclear allocation concealment, no intention-to-treat analysis.
‡Eriksen *et al.* (1983) (9): unclear allocation concealment.

combination of CHX and Bocasan®. Gründemann *et al.* (47) compared the combination of CHX and Bocasan® to CHX alone in a 14-day non-brushing protocol and found that the combination resulted in significant improvements in stain, plaque and bleeding scores. The results of the Gründemann *et al.* study correlate well with other studies that have investigated the synergistic effects of mouthwashes. Charbonneau *et al.* (15) tested a combination of CHX and monoperoxyphthalic acid in beagle dogs, and Steinberg *et al.* (17) assessed the synergistic effects of CHX and hydrogen peroxide in an *in vitro* study. Both studies indicated a superior effect of the combination of CHX and an OA on the inhibition of dental plaque formation. Recently, Rosema *et al.* (52) evaluated a preventive programme that consisted of an instructional lesson in oral hygiene and an oral prophylaxis treatment, which was followed by a 3-week rinsing regimen using a combination of CHX and sodium peroxyborate. The study showed a beneficial effect on oral/gingival health that lasted up to 9 months. Unfortunately, there was no control group in this study.

The comprehensiveness and applicability of the evidence obtained through this systematic review process are limited. The four studies that are included in this review were methodologically and clinically heterogeneous, which limited the ability to compare and synthesize the results. In addition, one cannot extrapolate the findings to the general population. Three of the included studies used a parallel design (31, 47, 48), and one study used a crossover design (9). Crossover designs offer a number of possible advantages over parallel group trials because each participant acts as his or her own control, which limits variations among the participants. Crossover trials are not appropriate in statistical analyses, however, when there is a strong likelihood of a carryover effect. There is concern that the CHX effect might be prolonged, and the 7-day washout period that was used by Eriksen *et al.* (9) may not have been sufficient; therefore, longer washout periods are preferable (53). However, the study by Eriksen *et al.* (9) did not report any carryover effects or effects between periods with this 7-day washout. Therefore, the concerns regarding the crossover design study are not applicable for the inclusion of Eriksen *et al.* (9) in this review.

Experimental non-brushing models (9, 31, 47) are useful for determining and comparing the efficacy of mouthrinses on tooth staining, plaque and gingivitis. Indeed, non-brushing models allow researchers to evaluate the effects of mouthrinses in the absence of any other oral hygiene aids. Non-brushing models, however, are not an accurate reflection of patients' daily use of mouthrinses. Another limitation of non-brushing models is that researchers cannot investigate the long-term effects of chemotherapeutics, which may more accurately reflect a patient's use of mouthrinses (54). The guidelines of the American Dental Association (ADA) recommend that studies that evaluate chemotherapeutic agents and their ability to control gingivitis and supragingival plaque be conducted for a minimum of 6 months with intermediate evaluation at 3 months to determine efficacy, safety and patient compliance (55). The data in this review were extracted from three experimental non-brushing studies of intermediate length with an evaluation period of 14 days (9, 31, 47) and from a 90-day brushing trial (48). Based on the ADA recommendations, long-term studies are required. Staining, however, is an adverse effect that can be visible after 1 week of therapy (56); therefore, studies with a short evaluation period are appropriate for the topic of this review.

The studies that were included in this review were heterogeneous in terms of CHX concentration, volume, frequency, duration and brand of the interventions used (Table 2). Berchier *et al.* (57) showed a small but statistically significant difference in favour of the 0.2% CHX concentration with respect to plaque growth inhibition; however, differences in concentration seem unlikely to substantially influence the clinical outcomes. Little is known about the relationship between differences in CHX concentration and volume and their potential effects on staining. For one study that was included in this review, the volume of CHX was unknown. The other three studies involved rinsing with either 15 ml of 0.12% CHX or 10 ml of 0.2% CHX (i.e. 18 mg CHX and 20 mg CHX, respectively, per rinse) (58).

Potential limitations in the review process

A rigorous search was conducted without language restrictions across a comprehensive list of electronic databases in an effort to locate all relevant trials. Despite all of efforts, one cannot rule out that relevant papers may have been missed. This comprehensive search eventually yielded four studies with moderate qualities of evidence. The formal testing for publication bias that was proposed by Egger *et al.* (59) could not be used owing to insufficient statistical power because less than 10 studies were included in the meta-analysis (21). Furthermore, a sensitivity analysis to examine the effects of random sequence generation, allocation concealment and blind outcome assessment on the overall estimates of the effects could not be conducted because of the limited number of studies.

The studies included in this review provided limited information on smoking and dietary habits. Through the interaction with dietary chromogens, CHX staining has been shown to be markedly increased in subjects who drink tea, coffee or red wine, which cause staining on their own. In addition, CHX staining has been shown to be increased in smokers (3, 4, 60). The potential impact of smoking and/or consuming beverages that have been shown to cause staining on their own on the overall outcomes of the included studies could not be estimated in this systematic review, but a subanalysis by Eriksen *et al.* (9) substantiated the need for this analysis. The outcomes of the meta-analyses have wide confidence intervals, which are attributed to the small number of studies and the small sample size within each study. Of the four studies, two (31, 47) were appropriate for a meta-analysis for the 'Lobene Stain Index' (49), and three were appropriate for the Sillnes &-Löe Plaque Index (9, 31, 47). Table 5 shows the overall conclusions with respect to tooth staining, which were supported by the results of the individual studies.

Conclusion

There is moderate evidence that the combination of CHX and an OA reduces tooth staining. The results of this study also show that the ability of CHX to inhibit supragingival plaque does not seem to be disturbed when CHX is used in combination with an OA.

Implications for practice

- Given the evidence in favour of the combined use of CHX and an OA with respect to tooth staining and plaque scores, the dental health practitioners should consider using the combination of CHX and an OA rather than CHX alone.

Implications for research

- More in-depth studies are necessary to support this conclusion with stronger evidence. Future studies should use more thoroughly controlled protocols, particularly regarding regimens and dietary chromogens, such as those found in tea, coffee and red wine.
- To ensure subject compliance, the development of a mouth wash that combines CHX and an OA in one solution needs attention.

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Conflict of interests

The authors declare that they have no conflict of interest. This study was self-funded by the authors and their institutions.

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Supporting information

Additional Supporting information may be found in the online version of this article:

Appendix S1. An overview of the excluded studies.

Appendix S2. Methodological quality scores of the included studies.

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