Clinical evidence in the treatment of white spot lesions following fixed orthodontic therapy: a meta-analysis

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Objective: This systematic review aims to determine the most effective method of treatment to remineralise post-orthodontic white spot lesions (WSLs).
Method: Six databases were accessed and searched for articles. Screening and selection were conducted according to the PRISMA guidelines using predetermined inclusion and exclusion criteria. Two reviewers independently assessed and extracted identified studies and relevance disagreement was resolved through consensus. Experimental studies were included that involved (i) patients of any age who had WSLs after the removal of fixed appliances, (ii) any treatment to remineralise the WSLs compared with no treatment or a placebo, and (iii) measurement of the changes in enamel mineralisation status after treatment. Eligible articles were assessed for internal bias and underwent narrative synthesis. A meta-analysis using random-effects modelling was performed to calculate a pooled estimate and assess between-study variability using Cochran's Q and I^2.
Results: The nine articles included in this review were found to have a medium or high risk of bias. The qualitative assessment provided contrasting results between studies. The meta-analysis showed both CPP-ACP – pooled *d* of -0.28 (N = 5 studies; 95% CI = -0.48- -0.07) – and fluoride – pooled *d* of -0.25 (N = 4 studies; 95% CI = -0.48- -0.02) – to generate improvement in the enamel mineralisation status, with CPP-ACP producing more consistent results compared with fluoride.
Conclusions: The meta-analysis found that CPP-ACP and fluoride were effective in reducing post-orthodontic WSLs. Due to the heterogeneity of the included studies with regard to the fluoride concentrations and mode of delivery, the current meta-analysis could not accurately establish which remineralising agent, CPP-APP or fluoride, is more effective. Further high quality studies of long-term duration are required to determine best clinical practice.
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Introduction

White spot lesions (WSLs) are localised porosities of subsurface enamel caused by demineralisation.[1] Light striking a hypocalcified enamel surface scatters, resulting in an opaque white appearance[2] that visually diagnoses WSLs in clinical practice. For research purposes, various indices have been used to quantify the severity of WSLs.[3] Quantitative light-induced fluorescence (QLF) is a commonly used and highly sensitive diagnostic tool that utilises the relationship between the mineral content of a tooth and the amount of fluorescence emitted from enamel after being exposed to high-intensity blue light. To illustrate, a low QLF reading would be produced from a demineralised tooth structure since it emits less fluorescence and a greater lesion depth is reflected by a higher loss of fluorescence (ΔF). QLF also allows researchers to precisely measure the area of the WSLs (A). Similarly, laser fluorescence (Diagnodent) can be used to determine the depth of a lesion by measuring the loss of dental fluorescence.[4]

WSLs are considered the most common iatrogenic complication of orthodontic treatment as the introduction of fixed appliances can unfavourably alter the oral environment.[5] Prevalence studies carried out using QLF reported that WSLs were found in 97% of post-orthodontic cases.[6] Orthodontic brackets, ligatures and bands physically obstruct effective cleaning in the area around these appliances and reduce the natural self-cleansing effect, giving rise to prolonged plaque accumulation and retention.[7,8] Consequently, the imbalance between the processes of demineralisation and remineralisation of enamel results in the creation of opaque hypocalcified lesions.[2] While demineralisation usually decreases after the removal of fixed orthodontic appliances, some lesions persist and result in compromised aesthetics.[1] In severe cases, WSLs may progress to cavitated carious lesions and require invasive restorative intervention.[9] Therefore, effective treatment following fixed orthodontic therapy is necessary to reverse and remineralise these potentially disastrous and unsightly lesions.

Many options have been attempted and described to treat WSLs. For example, fluoride supplements in the form of varnishes, dentifrices and mouthwashes have been routinely used.[9] While the strengthening capacity of fluoride on enamel and its effectiveness in preventing WSLs is widely accepted, the current literature has limited evidence to support the efficacy of fluoride in remineralising post-orthodontic WSLs.[10] In recent years, attention has been directed to calcium phosphate-based remineralisation agents. The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing agent functions by concentrating calcium and phosphate substrates in saliva, which enhances its buffering capacity.[11] As a result, the remineralisation process is enhanced whereas the demineralisation process is suppressed.[12] Previous in vitro studies and clinical trials that investigated the effectiveness of CPP-ACP in reducing demineralisation around orthodontic brackets have shown favourable results.[13,14] However, there is a lack of clinical consensus regarding the effectiveness of remineralising agents in treating post-orthodontic WSLs. A systematic review published in 2012 comparing the effectiveness of CPP-ACP against fluoride in reducing post-orthodontic WSLs produced inconclusive findings and a lack of robust quantitative results. The authors also reported that most of the available studies had methodological problems.[15] Hence, the present review aims to (1) examine the effectiveness of remineralising agents in reducing post-orthodontic WSLs and (2) produce a meta-analysis of the remineralising agents.

Methods

Search strategy

Six databases (PubMed, EMBASE CINAHL, Cochrane, Scopus and Web of Science) were searched using a combination of key search terms relating to 'orthodontic', 'white spot lesions', 'CPP-ACP', and 'fluoride' (see Appendix I for the detailed searched strategy). Articles were limited to those published between 2005 to 2016 and written in English.

A systematic screening of the searched articles was conducted using the PRISMA guidelines.[16] Following the initial search, duplicate articles were removed and the title and abstract of the resulting articles were assessed for relevance by both reviewers (TL and EY). The full texts of the remaining publications were analysed and articles that did not meet the inclusion criteria were excluded. The reference lists of all the full text papers were scanned to identify related studies.

The criteria for article inclusion in the present review were patients of any age who had WSLs after the removal of fixed appliance, those receiving treatment to remineralise the WSLs, and studies that compared no treatment with a placebo or alternative treatment. Moreover, the outcome of interest was the change in the status of enamel remineralisation, assessed through visual examination, photographs, quantitative light induced fluorescence or Diagnodent. Studies were also required to be an in vivo randomised controlled trial investigating treatment of white spot lesions following completed fixed orthodontics. Studies with artificial white spot lesions or those not comparing an intervention were excluded.

Appendix I. Search terms.

WSL, white spot lesion*, demineralization, enamel demineralization, demineralized enamel, teeth white spot*, opaque lesion*, hypocalcified enamel, hypocalcification, enamel porosities, hypocalcified white lesion*, tooth mousse, tooth mousse plus, casein phosphopeptide-amorphous calcium phosphate, calcium phosphate, CPP-ACP, remineralization, tooth remineralization, reduced demineralization.*

Quality assessment

To assess potential bias in the methodology of the selected studies, the Cochrane Collaboration's tool for bias appraisal was applied.[17] The two reviewers independently assessed each study for low, unclear or high risk of bias in terms of the randomisation method and blinding of randomisation, blinding of participants and examiners, blinding of outcome, attrition of data, bias in the reporting of results and other possible sources of bias. The studies were classified as low risk of bias (low risk of bias for all key domains), medium risk of bias (low or unclear risk of bias for all key domains) or high risk of bias (high risk of bias for one or more key domains) to summarise the assessment results. In cases of differing opinions between the reviewers, the concerns were discussed with an additional reviewer (HH) to reach a consensus.

Data extraction

Data from the eligible studies were extracted independently by the reviewers (TL and EY) and disagreement was resolved through consensus and after discussion with a third reviewer (HH). Information obtained included the study characteristics (year of publication, type of study) and sample characteristics (sample size, inclusion criteria, follow-up duration), as well as intervention and study results (including the method of assessment). Effect estimates such as regression coefficients were obtained from each study. In the absence of effect estimates, standardised mean difference was derived using the sample size, mean and standard deviation through Practical Meta-analysis Effect Size Calculator.[18]

Meta-analysis

Only studies that compared the remineralising treatment to a placebo, normal oral hygiene regimen or no treatment were considered in the meta-analysis. The meta-analysis was compiled using a quality-effects model instead of random-effects model as the former considers the quality score as it pooled the effect estimates of each study.[19] Between-study heterogeneity was assessed using Cochran's Q and I^2 statistical method. Separate meta-analyses were completed according to the type of intervention (CPP-ACP and fluoridation). Subgroup analysis per type of outcome (fluorescence score, lesion area and clinical index score) and sensitivity analyses were conducted to explore the changes in pooled estimates and the level of heterogeneity.

Results

Study characteristics

A total of 708 articles were retrieved from the six databases, as shown in Figure 1. After the removal of duplicates, 435 titles and abstracts were examined for relevance and 24 articles were selected. After evaluating the full texts, nine studies were finally considered eligible based on the selection criteria and subsequently reviewed. The description of each study is provided in Appendix II. In total, 15 articles[20-34] were excluded, six[20-25] because the experimental design used artificial lesions in vitro, two[26,27] because there was no comparison of a remineralising intervention against a control. The participants of one study[28] had not undergone orthodontic treatment, one study[29] did not include statistical data in the results section and was therefore excluded since a meta-analysis or comparison of results could not be performed. One article was written in Chinese with an English version unable to be located[30] and four articles[31-34] were reviews.

Of the nine eligible studies,[35-43] four experimental trials[36,38-40] focused on evaluating the effect of CPP-ACP. Two of the CPP-ACP studies[38,39] had control groups using a placebo with no active ingredients, one[36] had normal fluoridated toothpaste as the control and one[36] used fluoridated toothpaste and fluoridated mouthrinse as a control. Three of the included studies[37,41,42] examined the effect of sodium fluoride in different forms, namely: (1) a 0.5% fluoride chewing stick, (2) 5% fluoride varnish, and (3) 5% acidulated fluoride film. The remaining two studies[35,43] tested the effects of both CPP-ACP and fluoride (5% fluoride varnish and 0.025% fluoride mouthrinse, respectively) against a control (inactive placebo and fluoridated toothpaste, respectively). Various tools were used to evaluate the outcome of the experiment, including: (1) visually, (2) intraoral photographs, (3) ICDAS II clinical index, (4) QLF and (5) Diagnodent. The maximum length of the follow-up period in each eligible study ranged from four weeks to six months.[35-43]

Appendix II. Data extraction table.

Author and date	Type of study	Sample	Intervention and control	Assessment tool and data collection	Follow-up duration	Statistical analysis	Key findings
Akin et al. 2012[35]	RCT	80 participants who had undergone treatment at the orthodontic department. Records grouped according to date of debonding. Having a mean age of 14.5. Had developed multiple decalcified enamel lesions after fixed orthodontic therapy. Inclusion criteria: 1. Undergone fixed appliance therapy 12 months before study and post-orthodontic demineralised non-cavitated lesions involving maxillary or mandibular teeth 2. Non-smoker 3. No previous history of bleaching treatment 4. Absence of plaque accumulation and gingival inflammation 5. Absence of dental caries, prosthetic restorations, intrinsic and extrinsic discolourations and morphologic/anatomical deviations in the teeth 6. No health problems 7. Treatment of patients started after patients had undergone debonding	Intervention 1: mouth rinse with neutral 0.025% sodium fluoride (Colgate Plax) 20 mL for 30 seconds twice daily. Rinse immediately after brushing with toothpaste containing fluoride. Intervention 2: CPP-ACP treatment (GC Tooth Mousse) twice a day after brushing their teeth. Intervention 3: microabrasion 18% hydrochloric acid mixed with fine pumice powder to form a slurry, teeth cleaned with rubber cup in slow handpiece. Patients underwent four or five sessions of microabriasion at an in interval of two weeks, if necessary. Control: just brush teeth.	Photographs taken pre-op, after debonding and after six months treatment. Image processing software used to quantify size (mm2) of the visible area of demineralised enamel. Demineralised area expressed as percentage of total tooth surface.	Six months	Normality test of Shapiro-Wilks and the Levene's variance homogeneity test applied to data. Data found to be normally distributed and homogeneity of variance evident. Paired-sample *t*-test used to determine differences in mean changes within each treatment group.	Mean degree of white spot lesion decreased during the follow-up period. Statistically significant ($p <$ 0.001) difference between pre- and post-treatment extent of WSLs found for all groups. Turkey HSD test showed success rate of microabrasion was highest, followed by CPP-ACP. No significant differences between toothbrushing and mouth rinse. Fluoride mouth rinse only effective on small lesions Microabrasion success rate of 99% achieved for mild and moderate lesions and 94% for severe lesions.
Andersson et al. 2007[36]	Examiner blinded RCT	26 healthy adolescents (13 boys and 13 girls with mean age 14.5 years, 96% response rate) treated by orthodontic department in one calendar year. Inclusion criteria: 1. WSL on maxillary of mandibular incisors and canines immediately after debonding of fixed orthodontic appliance 2. Good oral health with no untreated carious lesions 3. Lived in community with low fluoride content in piped drinking water 4. Regular brushing habits with fluoride dentifrice twice/day Randomly assigned to groups with a dice.	Intervention: Brush teeth twice daily with CPP-ACP containing dental cream without fluoride (Topacal C5) for three months, followed by normal use of standard 1000 Fppm dentifrice for three months. Control: Conventional 0.05% sodium fluoride mouthrinse once daily together with a standard fluoride dentifrice for six months period.	Blinded examiner for clinical exam. Visual inspection at debonding, one, three, six and 12 months. Scored 0: no visible colour change 1: slight white colour change, only visible after air-drying 2. slight colour change, with certain marked white areas 3: white consistent colour change 4: distinct white colour change Laser fluorescence: Performed using DIAGNOdent after drying with air spray and reference value from intact buccal surface enamel obtained.	Twelve months	Pearson correlation coefficient used to calculate relationship between variables. Follow-up LF readings compared with baseline values using Student's paired 2-tailed test. Categorised scores compared using chi-squared test. Differences between groups assessed by the unpaired Wilcoxon test.	Clinical scored: a statistically significant improvement over time was evident in both groups but more sites became invisible in the test group. LF: No significant differences disclosed between the two treatment regimes either at baseline or at any of the follow-ups.

Baeshen et al. 2011[37]	Double blind RCT	37 orthodontic patients (11 males, 26 females, mean age 17.2 years) recruited from three dental hospitals. Divided by randomisation list. Inclusion criteria: 1. Full fixed appliance therapy 2. At least two WSLs on both the left and right side of maxillary dentition, adjacent to site of orthodontic band or bracket After debanding, prophy with water and pumice and professional supragingival scaling if required. Randomly divided using randomisation list.	Intervention: 19 patients using fluoridated (soaked in 0.5% sodium fluoride) miswaks. Chewing sticks used on one side of the maxilla, five times a day, for six weeks. Individual plastic tray covering half the dentition placed immediately after brushing and while using miswak. All subjects told to use fluoridated toothpaste (1450 Fppm) twice a day and no other fluoride products. Control: 18 patients using non-fluoridated miswaks.	Visual examination and DIAGNOdent (laser fluorescence) used to measure WSL at debanding appt, two, four and six weeks Buccal surfaces of all test teeth photographed ICDAS II scoring 0. sound tooth surface 1. first visual change in enamel 2. distinct visual change in enamel when viewed wet 3. localised enamel breakdown due to varies with no visible dentin 4. underlying dark shadow from dentine with or without localised enamel breakdown 5. distinct cavity with visible dentin 6. extensive distinct cavity with visible dentin	Six weeks	Power analysis performed before study suggested sample size 17 patients. Mean and standard deviation calculated for both ICDAS II and DIAGNOdent measurements. Mean changes at baseline and at six weeks analysed using paired *t*-tests. For comparison between test and control groups, a non-paired *t*-test used. Correlation between ICDAS II index score and DIAGNOdent readings at baseline and at six weeks were analysed using Pearson's correlation coefficient. 20% of measurements for both ICDAS II scores and DIAGNOdent readings were recorded twice by same examiner, showing 91% readings the same.	Fluoridated miswaks impregnated with 0.5% sodium fluoride have a stronger remineralisation effect on WSLs compared with nonfluoridated miswaks. Slight decrease in WSL also seen in covered sites using fluoridated miswaks indicates that the mouth tray did not totally prevent fluoride from reaching buccal surface.
Bailey et al. 2009[38]	Double blind, parallel group, RCT	45 participants (mean age 15.5 years, 23 females and 22 males) invited to participate from nine private orthodontic practices in Melbourne. Computer generated random schedule used to assign products. Inclusion criteria: 1. Healthy 2. Exhibit at least two WSL 3. Scheduled for bracket removal Exclusion criteria: 1. Untreated dentinal decay in study teeth 2. Milk protein allergy 3. Chronic use of medication causing dry mouth 4. Pregnancy or any illness/condition that the investigator deemed could affect study outcome	Intervention: Cream containing 10% CPP-ACP (Tooth Mousse). After normal oral hygiene procedures using the supplied fluoridated dentifrice (1000 ppm F) and soft toothbrush, participant instructed to apply 1 g morning and night on buccal/labial surface for 12 consecutive weeks. At each assessment visit, participants received prophylaxis, oral hygiene reinforcement and supervised fluoride mouthrinse (900 ppm F). Control: Placebo cream.	Visual inspection of buccal/labial tooth surface wet and after sive seconds of drying Scored using ICDAS II criteria. Examinations conducted by three dentists with prior clinical trial experience. Measured at baseline, four, eight and 12 weeks. Product tubes weighted prior to dispensation and on return from the participant. Overall compliance calculated as total amount of product used.	Twelve weeks	For all WSL, transition scores analysed by proportional odds ordinal logistic regression model. Robust variance estimation to account for clustering of lesions within participants. No statistically significant difference in total product usage.	Difference in transition score distributions between two treatment groups was not statistically significant, even though there was a clear trend to greater regression of lesions by remineralising cream. When analysis restricted to lesions with severity codes 2 or 3, OR was statistically significant.

Beerens MW et. al. 2010[39]	Double blind RCT	65 participants (69% response rate) were recruited from the orthodontic department. Having a mean age of 15.5. Inclusion criteria: 1. Healthy adolescent between age 12 to 19 2. Two or more bracketed surfaces with buccal subsurface WSLs, seen without prolonged air drying and without sign of dentinal involvement 3. No systemic disease 4. No syndromatic abnormalities 5. No allergy to milk protein and benzoate All participants were randomly allocated to intervention and control group as determined by a computer randomisation scheme.	Intervention: Home use of CPP-ACP + sodium fluoride (0.2% w/w; 900 p.p.m.) (MI Paste Plus 35 ml, Recaldent; GC Benelux Europe, Leuven, Belgium) once a day. Control: Home use of fluoride-free control paste + calcium (Ultradent 100 ml; Kruidvat NL, Renswoude, the Netherlands). All participants were given the same oral hygiene instruction. No additional fluoride was administered for all participants.	QLF baseline images were captured immediately after debonding (T1). Follow-up QLF were captured at the 6th (T2) and 12th (T3) weeks after debonding. Caries regression, determined by assessment of lesion depth (DF), lesion area (A), and integrated fluorescence loss (IFL), at three different time-points (T1, T2, and T3) The follow-up QLF images were compared with those at baseline	Twelve weeks	Chi-square test and the Student's *t*-test to determine the statistical significance. Lesion progression or regression for the whole group of subjects was determined by repeated-measures ANOVA, followed by Bonferroni post-hoc testing.	The Bonferroni post-hoc test identified a significant decrease in fluorescence loss (DF) from baseline to T3 with for both groups ($p < 0.05$) and no difference between groups. The IFL did not change significantly over time and between the groups.
Brochner A et.al. 2010[40]	Examiner blinded RCT	60 participants (83% response rate) were recruited. Having a mean age of 15.2 years. Inclusion criteria: 1. Healthy individuals 2. At least one labial WSL visible during debonding appointment Exclusion criteria: 1. Ongoing medication for chronic disease 2. High caries risk behaviour The subjects were allocated to one of the two groups as determined with aid of computer randomisation.	Intervention: 1. 1 g of a CPP–ACP cream (Tooth Mousse, GC Europe N. V., Leuven, Belgium) to the teeth once daily (in the evening) for a period of four weeks 2. Standard fluoride toothpaste (Colgate, 1,100 ppm F) in the morning. . Control: 1. Fluoride toothpaste two times a day for a period of four weeks. All participants were asked to refrain from additional preventive measures based on fluoride during the experimental period.	QLF: QLF images were taken at the baseline (during bracket debonding) and at the end of the experiment. The change in fluorescence (ΔF) and area of lesion (A) were recorded. Clinical registration: The slides were scored independently by two examiners. The labial surfaces of the upper incisors, cuspids and the first premolar were scored as: 1 = no white spot formation, 2 = slight white spot formation (thin rim), 3 = excessive white spot formation (thicker bands) and 4 = white spot formation with cavity formation. In case of disagreement, a consensus was reached.	Four weeks	The follow-up mineral content was compared with baseline within each group with the aid of ANOVA for repeated measurements. The comparisons between the groups were computed by Student's *t*-test or a chi-square test. All data were checked for normality distribution.	The baseline ΔF values were similar in the intervention and control groups at Baseline. Statistically significant ($p < 0.05$) reductions of approximately 30–35% were disclosed in both study groups. The lesion area (A,mm2) was somewhat larger in the control group than in the intervention group at baseline, but the difference was not statistically significant. After the study period, the average lesion area was decreased by 58% in the CPP–ACP group and by 26% in the control group, which was significantly different to baseline in the intervention group ($p < 0.05$). The difference between two groups were not statistically significant ($p = 0.06$). Regarding the clinical score, there were no statistical significant difference between the two groups.

Du M et. al. 2012[41]	Examiner blinded RCT	110 (87% response rate) were recruited from the orthodontic department of Wuhan University. Having a mean age of 16.6 years. Inclusion criteria: 1.Good general health, no systemic diseases 2. Received conventional periodontal therapy after fixed orthodontic treatment 3. At least two teeth with WSLs Exclusion: 1. Enamel hypoplasia 2. Dental fluorosis 3. Tetracycline pigmentation 4. Periodontal pocket (>= 3mm) 5. Carious cavity Using a random number table, participants were assigned to intervention group or control group.	Intervention: Fluoride varnish was applied to the dry, clean, tooth surfaces with WSLs. Control: Saline was applied to the dry, clean, tooth surfaces with WSLs. Both groups were advised not to brush their teeth or chew food for at least four hours after treatment. No supplemental measure was taken to remove plaque from tooth surfaces, such as using dental floss and mouth rinse. Only standard tooth cleaning and oral hygiene instruction (tooth brushing twice a day with fluoride toothpaste) was provided.	Assessment tool: Diagnodent Diagnodent reading was taken at baseline, at three months and six months. The peak reading displayed on the panel of Diagnodent was recorded.	Six months	The recorded diagnodent reading were subjected to ANOVA test, comparing between groups at different time point and between groups at each time point. A Student's *t*-test was used to detect intergroup difference.	The mean baseline DD readings in the two groups were similar (*t*-test, $p > 0.05$). There were statistically significant differences between the mean DD readings of the two groups at the three-month ($p < 0.05$) and at the six-month follow-up visits ($p < 0.01$). There was a significant decrease in the mean DD reading in group 1 during the first three months ($p < 0.001$) and a further decrease after six months. The decrease in mean DD reading between the third and the six months were not statistically significant ($p > 0.05$). Similar findings were obtained in group 2.
He T et. al. 2016[42]	Examiner blinded RCT	240 who recently finished orthodontic treatment (87% response rate) were recruited from the orthodontic depart, having a mean age of 16.9. Inclusion criteria: 1. Age 12 to 15 2. Good general health 3. One maxillary anterior tooth with a WSL on the buccal surface Exclusion criteria: 1. Presence of oral ulcer 2. Presence of ulcerous gingivitis 3. Acute bronchial asthma 4. Pregnant and nursing women 5. Allergic reaction after using fluoride varnish, fluoride film and other dental products Randomisation was done with Excel.	Intervention 1: Duraphat contained 5% NaF. Intervention 2: 5% acidulated NaF. Control: Fluoride-free deliquescent toothpastes.	QLF image were taken at baseline and after three and six months. Change in fluorescene ΔF (%), area of demineralised enamel and ΔQ(ΔF x area) To minimise contamination, the unit of randomisation and analysis was the whole patient unit, instead of the tooth unit. The unit was achieved by obtaining average QLF measurements for each tooth (eg, if one patient had three teeth with WSLs, we averaged the values for the three teeth).	Six months	A mixed-effects linear model was used to analyse the QLF parameters (ΔQ, area, and DF), and restricted maximum likelihood was adopted for parameter estimation. The mixed model is a two-level model for repeated measurable data. The second level is patient, and the first level is the time at which the data for each patient were measured.	The baseline parameters (ΔQ, area, and DF) and numbers of teeth with a WSLs did not differ significantly among the three groups. The follow-up duration for patients lost to follow-up was similar in each group. ΔQ values were statistically associated with time. Significant decrease in ΔQ was observed in all groups after six months. ΔQ values of the varnish and film groups decreased by a significantly greater amount than that of the control Group ($p < 0.0001$). The Varnish group decreased by a significantly greater amount than that of the film group. The mean area of WSLs and the mean F showed trends similar to those for ΔQ.

Huang GJ et. al. 2013[43]	Single blinded RCT	135 patients (85% response rate) were recruited. Having age ranging from 12 to 20. Inclusion criteria: 1. Completed fixed appliance orthodontic therapy within the past two months 2. Had at least one WSL on the facial surface of a maxillary central incisor or lateral incisor that was not present before orthodontic therapy Exclusion criteria: 1. Unwillingness to be randomly assigned to one of the three treatment group 2. Any abnormal oral, medical, or mental condition 3. Any therapy for WSLs after orthodontic treatment 4. Any WSLs present on qualifying teeth with frank cavitation 5. Non-English speaking or non-English reading patients The three groups were well matched in all characteristics at enrollment. The randomisation sequence was created by using statistical software (Axio Research) and was stratified by each office by using random block sizes of three and six.	Intervention 1: Patient received an eight weeks supply of MI Paste Plus at the start of the study and instructions to apply a pea-sized amount to each arch twice daily. Intervention 2: Patient received 0.4 ml of 5% NaF as a single application at the start of the study. Both the intervention group patients received the usual home-care oral hygiene instructions and a packet with non-prescription fluoride toothpaste (1100 ppm of fluoride), a manual toothbrush, and dental floss. Control: These patients received the usual home-care oral hygiene instructions and a packet with non-prescription fluoride toothpaste (1100 ppm of fluoride), a manual toothbrush, and dental floss.	Photographs were taken digitally as intraoral frontal views with the patient's head tilted up approximately 5 to 10 to limit reflection from the flash to the incisal third of the maxillary incisors. Several digital photographs were taken at each time point to allow selection of the optimal image. The photographs (Fig 1)were cropped to include only the four maxillary incisors, adjusted for brightness, colour matched between sets, and saved in a 3000 3 1200 pixel 24-bit bitmap format with Photoshop Two blinded panels (expert and lay) rated the improvement in WSLs over the eight-week period using the visual analog scale. Median value of each panel was used for analysis. Objective assessment was performed by subtracting the % surface area of pretreatment WSLs to the one of post-treatment.	Eight weeks	The analyses for the aims were performed by using a linear regression model, accounting for possible effects associated with age, sex, and initial WSLs severity.	The mean improvement by Expert Panel: 1. Intervention 1 - 21% 2. Intervention 2 - 29% 3. Control - 27% The mean improvement by Lay Panel: 1. 29% 2. Intervention 2 - 31% 3. Control - 25% Objective improvement score: 1. Intervention 1 - 16% 2. Intervention 2 - 25% 3. Control - 17% No statistically significant differences were observed when comparing either active group with the control group.

The sample size of the included studies ranged from 24 to 240. All of the participants of the randomised control trials were recruited from the orthodontic department of the respective universities and underwent fixed orthodontic treatment immediately before the trials. Common inclusion criteria between the selected studies included the presence of white spot lesions on the labial or buccal surfaces of the teeth identified during debonding of the brackets, and the absence of systemic disease. Most of the respondents in the included studies were in adolescence, having a mean age ranging from 14.5 to 17.2.[35-43]

Fluoride versus inactive control

In a study that compared the efficacy of 5% fluoride varnish against 5% acidulated fluoride film and a control paste, the authors reported that the improvement in ΔQ ($\Delta F \times A$) was highest in the fluoride varnish group, followed by the fluoride film group and the control group, respectively. The difference between the three groups was statistically significant ($p < 0.05$).[42] Similarly, Du et al. examined the effect of 5% fluoride varnish against a control paste by Diagnodent assessment over six months and reported a greater decrease in the Diagnodent reading ($p < 0.05$) in the intervention group.[41] In addition, Baeshen et al. compared a 0.5% fluoride chewing stick against a placebo, the results of which were measured using the ICDAS II index and Diagnodent. At the end of six weeks, both the Diagodent reading and ICDAS II index showed greater improvement in the intervention group. The results were statistically significant ($p < 0.01$).[37]

CPP-ACP versus inactive control

Bailey et al. compared the effect of CPP-ACP against an inactive placebo cream for 12 weeks using ICDAS II. The authors reported that although CPP-ACP resulted in a higher percentage of regression of WSLs compared with the placebo cream, the outcome was not statistically significant (OR:1.67; 95% CI: 0.81, 8.45). Interestingly, when the analysis was restricted to more severe lesions (visual scores of 2 and 3), CPP-ACP showed greater regression and had a statistically significant result (OR: 2.33; 95% CI: 1.06, 5.14).[38] Moreover, Beerens et al. compared CPP-ACP paste against an inactive control paste for three months, measured by using QLF. A statistically significant

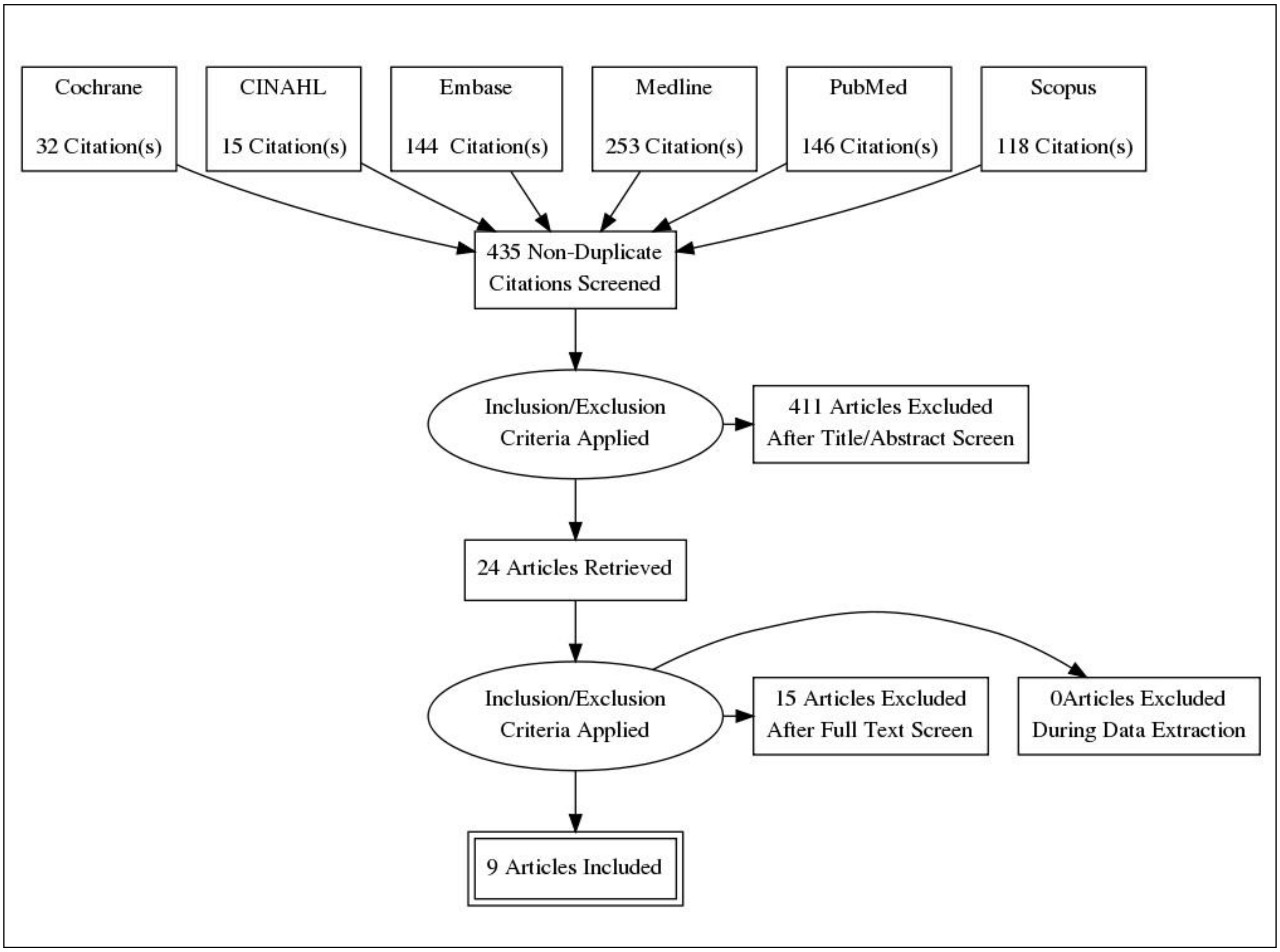

Figure 1. Study search.

($p < 0.05$) improvement in fluorescence loss in both the intervention and control groups was reported, but no difference between the two groups. There was also no clinical or statistical significance in lesion area (A) over time.[39]

CPP-ACP versus fluoride

Andersson et al. compared the effect of CPP-ACP against a combination of fluoridated toothpaste and 0.05% fluoridated mouthrinse for 12 months. Diagnodent and visual scoring were used to evaluate the outcomes. At the end of the treatment, the Diagnodent readings showed statistically significant improvements in the test and control groups, although the difference between the two groups was not statistically significant. However, visual scoring showed a higher percentage of lesion regression in the test group compared with the control group (64% vs 23%), the difference noted as statistically significant ($p < 0.01$).[36] Bröchner et al. compared the effect of CPP-ACP against normal fluoridated toothpaste over four weeks, no clinical or statistical differences were found using QLF and clinical scoring. While there was a 58% decrease in the average lesion area (A) in the CPP-ACP group and 26% in the control group, the difference was not statistically significant ($p = 0.06$). An evaluation using clinical scoring also reported no statistically significant differences between the reduction of WSLs in the two groups.[40]

CPP-ACP versus fluoride versus non-active control

Huang et al. conducted a study involving two intervention groups, CPP-ACP and 5% fluoride varnish, which were compared with a control group in which the respondents were instructed to brush with 1100 ppm fluoridated toothpaste. A linear regression model showed no statistically significant difference

Table I. Bias risk assessment of included studies.

Domain	Akin et al.	Andersson et al.	Baeshen et al.	Bailey et al.	Beerens et al.	Brochner et al.	Du et al.	He et al.	Huang et al.
Selection bias									
Random sequence generation	0	1	1	1	1	1	1	1	1
Allocation concealment	-1	1	-1	1	1	1	-1	1	1
Performance bias									
Blinding of participants and personnel. Assessments should be made for each main outcome (or class of outcomes).	-1	1	1	1	1	1	1	1	1
Detection bias									
Blinding of outcome assessment. Assessments should be made for each main outcome (or class of outcomes).	1	1	1	1	1	1	1	1	1
Attrition bias									
Incomplete outcome data. Assessments should be made for each main outcome (or class of outcomes).	1	1	1	1	-1	0	1	1	1
Reporting bias									
Selective reporting	1	1	1	-1	1	1	1	1	1
Other bias									
Other sources of bias	0	0	0	-1	0	0	0	0	0
Risk of bias	High	Medium	High	High	High	Medium	High	Medium	Medium

Key
1: low risk of bias
0: unknow risk of bias
-1: high risk of bias

between the improvement in the three groups.[43] In contrast, Akin et al. reported that the CPP-ACP group showed a higher success rate in the treatment of WSLs compared with the 0.025% fluoride rinse group and the inactive control group ($p < 0.01$).[35]

Quality assessment

The outcome of quality assessment of the studies is shown in Table 1. Overall, five studies[35,37-39,41] were evaluated to have a high risk of bias and four[36,40,42,43] assessed to be of medium risk. Baeshen et al.[37] and Du et al.[41] were graded as a high risk of bias in allocation concealment as a randomisation list/table was used, while Akin et al.[35] grouped participants according to the date of debonding, which may have led to selection bias. Beerens et al.[39] was graded as high risk and Bröchner et al.[40] was graded unclear in attrition bias due to the high and moderately high loss of follow-up data, respectively. Bailey et al.[38] did not report the pre-specified QLF result and also had a high risk of bias due to funding of the study provided by the manufacturer of the product.

Only eight studies[35-42] were included for meta-analysis as one article[43] failed to provide information related to mean difference. The CPP-ACP group had a pooled d of -0.28 (N = 5 studies; 95% CI = -0.48- -0.07). The odds ratios of each subgroup showed similar results (see Figure 2) with a negligible level of heterogeneity between the studies ($I^2 = 0$; Q = 2.22, $p = 0.898$).

Surprisingly, CPP-ACP estimates improved in statistical significance across all types of WSL

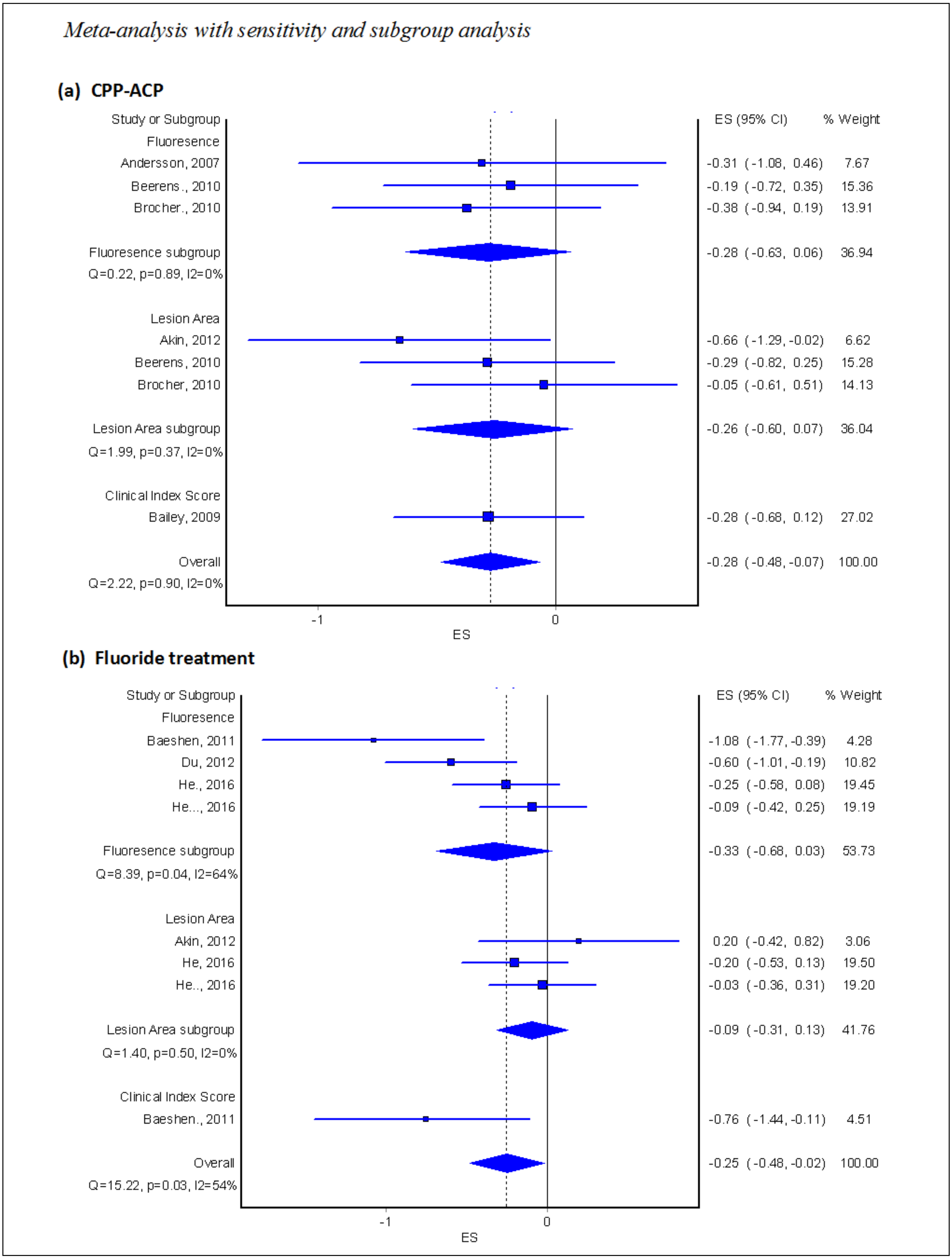

Figure 2. Pooled *d*: Effectiveness of CPP-ACP and fluoride treatment in reducing WSL outcomes using quality-effects model.

outcomes in this analysis despite the non-significant findings in each subgroup. Unlike CPP-ACP, fluoridation treatment showed a moderate level of heterogeneity ($I^2 = 54.02$; $Q = 15.22$, $p = 0.033$) with a lower standardised mean difference magnitude of -0.25 (N = 4 studies; 95% CI = -0.48- -0.02). Furthermore, it was observed that the fluorescence subgroup primarily contributed to the high between-study heterogeneity ($I^2 = 64\%$). A sensitivity analysis, removing the study conducted by Bailey et al.[38] which measured a clinical score index and the study by Beerens et al.[39] which had a high attrition rate, showed minimal changes in the effect estimates. The removal of the study conducted by Baeshen et al.[37] which also measured the clinical index score, as well as the study by Akin et al.[35] which had poor randomisation, improved the estimates for fluoride treatment and diminished the score for CPP-ACP (see Table II).

Discussion

The present systematic review incorporating a meta-analysis of the pooled data focused on determining the most effective method of remineralising post-orthodontic WSLs. Alternative treatment methods such as micro-abrasion and resin infiltration were not considered. Although there were in vitro studies[13,14,44] that reported positive and significant results, these were excluded as they did not adequately replicate the oral environment. Additionally, many confounding factors such as patient compliance, diet and salivary buffering capacity can also affect the clinical result of WSLs.[41,45,46] Hence, only randomised controlled trials were considered because the randomisation aimed to distribute any confounders equally between the intervention and control groups.

It is widely accepted that the introduction of fluoride as an anti-cariogenic agent was one of the most important events in the history of dentistry.[47] In addition to the enhancement of remineralisation and inhibition of demineralisation, fluoride has also been proven to have limited anti-microbial activity against bacteria in the oral biofilm.[48] While most of the included studies[35,37,41,42] in the review reported that additional fluoride has significant clinical benefit in reducing post-orthodontic WSLs, Huang et al.[43] reported that fluoride varnish was not more effective compared with normal oral hygiene instruction and

Table II. Sensitivity analysis of quality-effects meta-analysis of CPP-ACP and fluoride treatment in reducing WSL outcomes.

	Pooled estimate			Between-study heterogeneity				
Excluded study	*d*	LCI 95%	HCI 95%	Q	*p*-value	I^2	I^2 LCI 95%	I^2 HCI 95%
CPP-ACP								
Akin, 2012	-0.25	-0.47	-0.03	0.80	0.977	0.00	0.00	0.00
Andersson, 2007	-0.27	-0.49	-0.06	2.22	0.818	0.00	0.00	42.78
Bailey, 2009	-0.27	-0.52	-0.03	2.22	0.818	0.00	0.00	42.78
Beerens, 2010	-0.27	-0.50	-0.05	2.22	0.818	0.00	0.00	42.84
Beerens, 2010	-0.29	-0.52	-0.07	2.06	0.841	0.00	0.00	38.30
Brocher, 2010	-0.31	-0.54	-0.09	1.39	0.926	0.00	0.00	8.64
Brocher, 2010	-0.26	-0.48	-0.04	2.12	0.832	0.00	0.00	40.21
Fluoride treatment								
Akin, 2012	-0.26	-0.49	-0.03	13.12	0.041	54.28	0.00	80.44
Baeshen, 2011	-0.21	-0.40	-0.02	9.41	0.152	36.22	0.00	73.10
Baeshen, 2011	-0.22	-0.46	0.01	12.90	0.045	53.50	0.00	80.15
Du, 2012	-0.20	-0.44	0.03	12.04	0.061	50.16	0.00	78.87
He, 2016	-0.26	-0.54	0.02	15.14	0.019	60.36	9.10	82.71
He, 2016	-0.25	-0.53	0.03	15.22	0.019	60.59	9.70	82.80
He, 2016	-0.30	-0.56	-0.04	13.16	0.041	54.40	0.00	80.49
He, 2016	-0.29	-0.56	-0.02	14.14	0.028	57.56	1.74	81.67

fluoridated toothpaste. Huang et al. also argued that the use of high-concentration fluoride varnish may prevent the infiltration of calcium and phosphate into the deeper layer of the enamel, therefore inhibiting deeper remineralisation.[43] Akin et al. suggested that low-concentration fluoride rinse was more effective in small compared with large lesions due to better uptake of fluoride in shallow lesions.[35]

Currently, in vitro[49,50] and in vivo studies[51,52] have demonstrated and validated the anti-cariogenic effects of CPP-ACP. Shen et al.[53] reported that CPP-ACP had superior efficacy to 5000 ppm fluoride in reducing the depth of WSLs. However, there is insufficient evidence to establish a clinical benefit of CPP-ACP in managing post-orthodontic WSLs. In the present review, two RCTs compared the efficacy of CPP-ACP against an inactive control paste and reported a benefit of CPP-ACP that was clinically but not statistically significant.[38, 39] A variation in oral hygiene habits between the respondents may be considered as a confounding factor. Two of the included studies compared CPP-ACP against fluoridated toothpaste (± 0.025% fluoridated mouthrinse).[36,40] One[36] reported significant clinical improvement in the CPP-ACP group whereas the other[40] reported no significant result. Interestingly, two of the included studies compared CPP-ACP and fluoride against a normal oral hygiene routine but reported completely different results. Akin et al.[35] demonstrated that CPP-ACP was superior compared with fluoride and fluoride produced superior results to the control group in reducing post-orthodontic WSLs. In contrast, Huang et al.[43] reported no difference in effectiveness between the three groups.

Importantly, it was observed that ‘no treatment’ in most of the included studies seemed to result in lesion regression.[35,37-39,41-43] As the ‘no treatment’ groups were not restrained from tooth brushing with fluoridated toothpaste, it can be postulated that fluoridated toothpaste may be responsible for the improvement. This is supported by the study carried out by Argawal et al., which concluded that fluoridated toothpaste can cause a significant reduction of post-orthodontic WSLs.[29] This also implies that the therapeutic benefits of the remineralising agents studied may be masked by the therapeutic effects of the daily oral hygiene regimen.

In general, the studies analysed in the present review yielded different results regarding the effectiveness of the remineralising agents on WSL outcomes. However, the meta-analysis indicated that CPP-ACP and fluoride are both effective in reducing post-orthodontic WSLs. Studies concerning CPP-ACP demonstrated more consistent results (lower I^2 and Q). This finding could be related to the consistent dosage in CPP-ACP cream compared with the various concentrations of fluoride products available. The present analysis also inferred a possible clinical significance despite the statistical disadvantage of the results (e.g., low statistical power).[54] This was clearly observed in the improvement of the effect size of CPP-ACP after pooling individual study estimates. It was not possible to accurately establish which remineralising agent, CPP-ACP or fluoride, is more effective due to the heterogeneity in the included studies with regards to the concentration of fluoride applied and the mode of fluoride delivery.

Significantly, the inability to include the study by Huang et al.[43] may undermine the validity of the meta-analysis. However, it was methodologically impossible to include this study due to the lack of information regarding the mean difference. All of the other included studies[35-42] reported the pre- and post-treatment value of the enamel mineralisation status while Huang et al.[43] only reported the improvement score after treatment.

Significant heterogeneity was found in the instruments used to measure the improvement of WSLs, as each of the instruments had different sensitivity and specificity, which may have led to error when comparing the effectiveness of the treatment methods. Many studies have advocated that QLF is precise and consistent in quantifying and monitoring the mineral content and size of WSLs.[55-57] While the Diagnodent device can be useful in quantifying WSLs, it was suggested that its statistically significant difference may not coincide with a clinically significant difference.[58] Furthermore, stain and calculus can also affect the Diagnodent readings.[59] Visual assessment is also relevant when measuring the WSLs as this is the most common approach in clinical practice. Clinical indices provide a means to quantify WSLs but lack sensitivity in detecting small change. Photographic techniques, as described by Huang et al.[43] and Akin et al.,[35] allow quantification of the area of WSLs. However, bias may be introduced with inconsistent angles and lighting. According to Bröchner et al., a combination of QLF and visual assessment could be beneficial in future studies.[40] The disparity related to the instruments used was found in the subgroup analysis of fluoride treatment but not the CPP-ACP analysis. Studies with fluorescence as the outcome, measured using QLF and Diagnodent, showed a higher pooled estimate compared with the lesion area score measured by imaging software.

Strength and limitations

Having a robust statistical analysis that clarifies the findings of the systematic review is the most significant strength of the present review. However, there are several limitations as the included studies show clinical heterogeneity in regards to the type and dosage of treatment and outcome measurement tools. This prevents direct comparison of the effectiveness of different treatment methods. The exclusion of non-English papers also introduced a level of bias by preventing an assessment of results from other countries.[60] The small number of eligible studies limited the ability to examine the relationship between the effect of treatment and the severity of WSLs at this baseline. Limited data also prevented the investigation of the aggregate dosage-response relationship of each treatment method and the effect modification across different follow-up periods. The inability to include the study by Huang et al.[43] may have also undermined the validity of the meta-analysis.

Recommendation

From the present review, it is apparent that practising good oral hygiene plays an important role in reducing post-orthodontic WSLs. Therefore, it is the clinician's responsibility to advocate meticulous oral care for every patient who develops post-orthodontic WSLs. The meta-analysis for this review implied that both CPP-ACP and fluoride have additional benefits related to routine oral hygiene. The analysis of the current literature also revealed no side effects following the use of these products. However, the findings of this review are still inconclusive because of the inability to include all of the eligible studies in the meta-analysis, the small number of studies and the lack of representation from different settings.

Additional high-quality studies are required to provide more reliable evidence regarding the effectiveness of various remineralising agents and to establish the best clinical practice. Areas to be considered in future trials include standardising the oral hygiene regimen to prevent confounded results. The use of QLF in combination with image analysis would be preferred as both tools allow the measurement of the lesion area. Consequently, crosschecking the results would be made possible to provide more reliable evidence on this topic.[40] As fluoride may have different therapeutic effects for post-orthodontic WSLs at different dosages, including different fluoride dosages in a future study may provide valuable information.[61] Investigating the effect of using CPP-ACP in combination with various forms and dosages of fluoride may also be beneficial.[62] To study the longevity of treatment effects, a longitudinal analysis to measure the difference of treatment results in each follow-up period is recommended.

Conclusion

It may be concluded that simply removing fixed appliances and tooth-brushing with fluoridated toothpaste is effective in reducing post-orthodontic white spot lesions. Furthermore, the current meta-analysis revealed that CPP-ACP and fluoride both improved the enamel mineralisation status (statistically significant). However, the meta-analysis could not accurately establish which remineralising agent, CPP-ACP or fluoride, was more effective due to the heterogeneity in the included studies and with regard to the concentrations and the mode of fluoride delivery. Further high quality randomised controlled trials are required and imperative to establish good clinical practice.

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