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REVIEW ARTICLE

Remineralization potential and caries preventive efficacy of CPP-ACP/Xylitol/Ozone/Bioactive glass and topical fluoride combined therapy versus fluoride mono-therapy – a systematic review and meta-analysis

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ABSTRACT
Objective: To compare the remineralization potential and caries preventive efficacy of CPP-ACP/bioactive glass/xylitol/ozone and topical fluoride (TF) combined therapy versus TF mono-therapy.
Material and methods: Embase, PubMed, Scopus, Web of Science and Cochrane databases were searched. 4457 records were screened and 26 trials were included. Data from 16 trials was pooled using Review Manager 5.4. Level of significance was $p < .05$. The certainty of the evidence was evaluated using GRADE.
Results: Pooled analysis of two trials for white spot lesions (WSLs) regression (SMD −0.6, 95% CI: [−1.07 to −0.14], $p = .01$) and three trials for post-intervention DIAGNOdent values (SMD −1.24, 95% CI: [−1.96 to −0.52], $p = .0007$) significantly favoured CPP-ACP-TF combined therapy over TF mono-therapy. The sub-group analysis for caries increment (SMD −0.14, 95% CI: [−0.21 to −0.07], $p < .0001$) and the post intervention *S mutans* count (SMD −0.42, 95% CI: [−0.62 to −0.23], $p < .0001$) significantly favours 'xylitol-TF' and 'CPP-ACP-TF' combined therapy respectively. The high/unclear risk of bias, imprecision and indirectness of the included trials presented a low certainty of evidence.
Conclusion: CPP-ACP-TF exhibits superiority over TF monotherapy in remineralizing existing lesions and demonstrates better antibacterial effect, whereas it is not more effective for preventing caries incidence. However, Xylitol exerts an added benefit over fluoride alone in preventing caries increment. The low-certainty evidence highlights the need for more good quality trials.

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Dental caries; remineralization; prevention; fluoride; Mutans streptococci

Introduction

Dental caries is an easily preventable yet highly prevalent disease affecting individuals across all age groups [1]. The oral-cariogenic bacteria act on the dietary sugars to release acid that lowers the oral pH favouring demineralisation of the tooth surface. As soon as the salivary pH returns to normal, calcium and phosphate ions present in the oral environment maintain an overall mineral balance and promote remineralization of the tooth surface [2,3]. An imbalance in this demineralisation-remineralization process leads to development of new lesions or further progression of existing ones [4]. For many decades, the sole management of dental caries had been through a surgical approach involving restorations or endodontic therapy. Recently, there has been a paradigm shift to a more conservative and medical approach that focuses on preventive strategies at an early stage of demineralisation to avoid disease progression.

Fluoride use has been the mainstay of non-invasive regimens for treating initial tooth decay [4]. Though the main action of fluoride is remineralization and formation of acid-resistant fluorapatite, it also has an anti-cariogenic effect owing to its inhibitory activity on bacterial metabolism [5,6]. Literature has shown a significant decline in caries incidence in the post-fluoride-use era [4,7]. It is stated that fluoride remineralizes the porous surface layer of enamel blocking the enamel pores that limits the exchange of mineral ions, thereby preventing remineralization of the under surface [8]. However, it is also reported that fluoride ions alone are not adequate for the highly cariogenic/low pH oral environments and that fluoride has a limited effect in high-risk individuals [9]. In fact, it has been observed that in populations with appropriate fluoride exposure, the dental caries prevalence has reached a plateau level, and no further decline seems to be possible [10]. Therefore, even though fluorides in its various forms (toothpaste, varnish and gel) is conventionally used to prevent caries incidence and progression, it is concluded that fluoride alone is insufficient to prevent caries, especially in high risk populations [11]. Thus, new strategies that can enhance the remineralizing potential of fluoride and also exert an added caries preventive effect are now being investigated. Use of CPP-ACP, xylitol, bioactive glass and ozone in combination with topical fluoride (TF) is implicated in exerting an added caries preventive benefit over topical fluoride use alone, but conclusive evidence is still lacking. This study aimed to compare the remineralizing potential and caries preventive efficacy of combined therapy using CPP-ACP/bioactive glass/xylitol/ozone and TF versus TF mono-therapy on high-risk individuals.

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Materials and methods

This study is registered with PROSPERO (CRD42020150746). The study is in accordance with the PRISMA guidelines.

Search strategy

Embase, PubMed, Scopus, Web of Science and the Cochrane Library databases were searched. Grey literature (using OpenGrey and TRIP databases) and cross-references were screened to obtain additional records. The search strategy is summarised in Table 1.

All the search results obtained from various databases were exported to an EndNote library, and duplicates were removed. Two authors ('SS' and 'AG1') screened the titles and abstracts of all the records followed by full texts assessment of the shortlisted articles according to the eligibility criteria. Discrepancies, if any, were discussed and consensus was sought. The PICO (S) strategy is given in Table 2.

Inclusion criteria

All randomised controlled trials published till May 2020, in English language or with original translation to English Language, were screened for possible inclusion. The studies should have evaluated the efficacy of combined use of TF with CPP-ACP/xylitol/bioactive glass/ozone versus TF alone on the remineralizing potential and caries prevention.

Exclusion criteria

In-vitro studies; non-human trials; reviews; observational studies; trials with imbalanced co-interventions that can directly or indirectly influence the outcome variables.

Outcome variables

The primary outcome assessed was remineralization of an existing lesion measured through change in the laser fluorescence/lesion area and visual assessment of white spot lesions (WSLs). Additionally, DIAGNOdent scorings were used to assess change in the tooth demineralization status. The secondary outcome was the caries preventive efficacy assessed through mean dental caries increment/proportion of subjects with new carious lesions and post-intervention *S. mutans* colony count/proportion of subjects with high *S mutans* count.

Data extraction and quality assessment

Two authors ('AG1' and 'SS') separately collected relevant information from each study on various aspects such as country of origin, study design, study site, study population, treatment regimen, and the reported results.

The risk of bias for the included studies was assessed using the Cochrane's Collaboration tool for Systematic Reviews [12]. Two authors ('AG1' & 'SS') scaled the articles for selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. Discrepancies, if any, were discussed with the third author ('AG2') and a consensus was sought.

Quantitative analysis was performed using the Review Manager Version 5.4. For the evaluation of remineralization potential of an existing lesion as well as post-intervention *S mutans* colony count, studies with a minimum follow-up of one month were pooled, whereas, a minimum follow-up of one year was chosen to assess the mean dental caries increment. A minimum follow-up time period was specified for pooling of studies to prevent misleading results and to ensure homogeneity amongst the studies pooled. For the trials that reported the outcome variables (caries increment, *S mutans* and WSLs severity) as dichotomous data, the estimate of effect expressed as odds ratio (OR) along with 95% confidence interval (CI) was converted to standard mean difference (SMD) and standard error (SE) [13]. This was pooled with the SMD (SE) derived from trials that have reported the same outcome variable (caries increment and *S mutans* and WSLs severity) as continuous data (mean ± SD) using the random effect model and generic inverse variance method. Studies that had summarised the outcomes (DIAGNOdent values, delta F and lesion area) as continuous data (mean ± SD) were pooled using the Inverse Variance function. Because of the variability in the scales used to assess the outcomes, standardised mean difference (SMD) with 95% Confidence Interval (95% CI) statistic was used [14]. $p < .05$ was considered statistically significant. Due to the high clinical heterogeneity – variability in intervention regimen and study population, a random effect model was used. Subgroup analysis was carried out for individual agents. Statistical heterogeneity was quantified using the I^2 statistic, where an I^2 value greater than 50% was considered as moderate to high heterogeneity [12].

Besides, two authors ('AG1' and 'KG') used GRADE to evaluate the certainty of the evidence (GRADEpro, Version 20). The evidence was scaled using five categories (Risk of bias, imprecision, inconsistency, indirectness, and publication bias) of the GRADE framework used to assess the randomised controlled trials. Based on the assessment, the certainty in evidence/quality of evidence was graded as very low/low/moderate or high [15].

Results

Out of 6187 records identified *via* literature search in the five databases, 1730 duplicates were removed. The titles and abstracts of the remaining 4457 records were screened followed by full texts assessment of 65 shortlisted articles. Finally, 26 randomised trials were included for qualitative synthesis, and data from 16 trials was pooled. The PRISMA flow diagram is shown in Figure 1.

Characteristics of included studies

The characteristics of the studies included in the qualitative review are presented in Table 3. All studies were published between the year 1995 and 2020. The study population comprised of individuals at high risk of dental caries. One trial

Table 1. Search strategy in the five databases.

S. No.	Database	Search strategy	Number
1.	Embase	('cpp acp' OR 'casein phosphopeptide amorphous calcium phosphate'/exp OR 'casein phosphopeptide amorphous calcium phosphate' OR (('casein'/exp OR casein) AND ('phosphopeptide'/exp OR phosphopeptide) AND amorphous AND ('calcium'/exp OR calcium) AND ('phosphate'/exp OR phosphate)) OR 'ozone'/exp OR ozone OR healozone OR 'xylitol'/exp OR xylitol OR 'bioactive glass'/exp OR 'bioactive glass' OR (bioactive AND ('glass'/exp OR glass)) OR 'calcium sodium phosphosilicate'/exp OR 'calcium sodium phosphosilicate' OR (('calcium'/exp OR calcium) AND ('sodium'/exp OR sodium) AND phosphosilicate) OR 'novamin'/exp OR novamin OR recaldent OR 'tooth mouse' OR (('tooth'/exp OR tooth) AND ('mouse'/exp OR mouse))) AND ('fluoride'/exp OR fluoride) AND ('caries'/exp OR caries OR 'mutans streptococci' OR (mutans AND ('streptococci'/exp OR streptococci)) OR 'streptococcus mutans'/exp OR 'streptococcus mutans' OR (('streptococcus'/exp OR streptococcus) AND mutans) OR 'prevention'/exp OR prevention OR 'remineralization'/exp OR remineralization)	676
2.	PubMed	((((((((((("casein phosphopeptide amorphous calcium phosphate nanocomplex"[Supplementary Concept] OR "casein phosphopeptide amorphous calcium phosphate nanocomplex"[All Fields]) OR "cpp acp"[All Fields]) OR (((((("caseinate"[All Fields] OR "caseinates"[All Fields]) OR "caseine"[All Fields]) OR "caseins"[MeSH Terms]) OR "caseins"[All Fields]) OR "casein"[All Fields]) AND (("phosphopeptides"[MeSH Terms] OR "phosphopeptides"[All Fields]) OR "phosphopeptide"[All Fields]) AND ("amorphous calcium phosphate"[Supplementary Concept] OR "amorphous calcium phosphate"[All Fields]))) OR (((((((("ozonated"[All Fields] OR "ozonating"[All Fields]) OR "ozonation"[All Fields]) OR "ozonations"[All Fields]) OR "ozone"[MeSH Terms]) OR "ozone"[All Fields]) OR "ozone s"[All Fields]) OR "ozonization"[All Fields]) OR "ozonized"[All Fields]) OR "ozonizer"[All Fields])) OR "healOzone"[All Fields]) OR (("xylitol"[MeSH Terms] OR "xylitol"[All Fields]) OR "xylitols"[All Fields])) OR ((((((((("bioactivate"[All Fields] OR "bioactivated"[All Fields]) OR "bioactivates"[All Fields]) OR "bioactivating"[All Fields]) OR "bioactivation"[All Fields]) OR "bioactivations"[All Fields]) OR "bioactive"[All Fields]) OR "bioactives"[All Fields]) OR "bioactivities"[All Fields]) OR "bioactivity"[All Fields]) AND (((("eyeglasses"[MeSH Terms] OR "eyeglasses"[All Fields]) OR "glasses"[All Fields]) OR "glass"[MeSH Terms]) OR "glass"[All Fields]))) OR (((("calcium"[MeSH Terms] OR "calcium"[All Fields]) OR "calciums"[All Fields]) OR "calcium s"[All Fields]) AND ((((("sodium, dietary"[MeSH Terms] OR ("sodium"[All Fields] AND "dietary"[All Fields])) OR "dietary sodium"[All Fields]) OR "sodium"[All Fields]) OR "sodium"[MeSH Terms]) OR "sodiums"[All Fields]) AND "phosphosilicate"[All Fields])) OR (("novamin"[Supplementary Concept] OR "novamin"[All Fields]) OR "novamin"[All Fields])) OR (("casein phosphopeptide amorphous calcium phosphate nanocomplex"[Supplementary Concept] OR "casein phosphopeptide amorphous calcium phosphate nanocomplex"[All Fields]) OR "recaldent"[All Fields])) OR (((((("teeth s"[All Fields] OR "teeths"[All Fields]) OR "tooth"[MeSH Terms]) OR "tooth"[All Fields]) OR "teeth"[All Fields]) OR "tooth s"[All Fields]) OR "tooths"[All Fields]) AND (((("mice"[MeSH Terms] OR "mice"[All Fields]) OR "mouse"[All Fields]) OR "mouse s"[All Fields]) OR "mouses"[All Fields]))) AND ((((((((((("fluoridate"[All Fields] OR "fluoridated"[All Fields]) OR "fluoridating"[All Fields]) OR "fluoridation"[MeSH Terms]) OR "fluoridation"[All Fields]) OR "fluoridation s"[All Fields]) OR "fluoride s"[All Fields]) OR "fluorided"[All Fields]) OR "fluorides"[MeSH Terms]) OR "fluorides"[All Fields]) OR "fluoride"[All Fields]) OR "fluoridization"[All Fields]) OR "fluoridized"[All Fields])) AND ((((((("carie"[All Fields] OR "dental caries"[MeSH Terms]) OR ("dental"[All Fields] AND "caries"[All Fields])) OR "dental caries"[All Fields]) OR "caries"[All Fields]) OR (("mutan"[All Fields] OR "mutans"[All Fields]) AND "streptococci"[All Fields])) OR (("streptococcus mutans"[MeSH Terms] OR ("streptococcus"[All Fields] AND "mutans"[All Fields])) OR "streptococcus mutans"[All Fields])) OR (((((((((((((((("prevent"[All Fields] OR "preventability"[All Fields]) OR "preventable"[All Fields]) OR "preventative"[All Fields]) OR "preventatively"[All Fields]) OR "preventatives"[All Fields]) OR "prevented"[All Fields]) OR "preventing"[All Fields]) OR "prevention and control"[MeSH Subheading]) OR ("prevention"[All Fields] AND "control"[All Fields])) OR "prevention and control"[All Fields]) OR "prevention"[All Fields]) OR "prevention s"[All Fields]) OR "preventions"[All Fields]) OR "preventive"[All Fields]) OR "preventively"[All Fields]) OR "preventives"[All Fields]) OR "prevents"[All Fields])) OR ((("remineralization"[All Fields] OR "remineralize"[All Fields]) OR "remineralized"[All Fields]) OR "remineralizing"[All Fields]))	692
3.	Scopus	ALL (((cpp-acp) OR (casein AND phosphopeptide AND amorphous AND calcium AND phosphate) OR ozone OR (healozone) OR xylitol OR (bioactive AND glass) OR (calcium AND sodium AND phosphosilicate) OR novamin OR recaldent OR (tooth AND mouse)) AND (fluoride) AND ((caries) OR (mutans AND streptococci) OR (streptococcus AND mutans) OR prevention OR remineralization)) AND (LIMIT-TO (SUBJAREA , "DENT") OR LIMIT-TO (SUBJAREA , "MEDI"))	4106
4.	Web of Science Core Collection	(((CPP-ACP) OR (Casein Phosphopeptide Amorphous Calcium Phosphate) OR ozone OR (healOzone) OR xylitol OR (bioactive glass) OR (calcium sodium phosphosilicate) OR NovaMin OR Recaldent OR (tooth mouse)) AND (fluoride) AND ((caries) OR (mutans streptococci) OR (Streptococcus mutans) OR prevention OR remineralization))	480
5.	Cochrane Central Register for Controlled Trials	((CPP-ACP) OR (Casein Phosphopeptide Amorphous Calcium Phosphate) OR ozone OR (healOzone) OR xylitol OR (bioactive glass) OR (calcium sodium phosphosilicate) OR NovaMin OR Recaldent OR (tooth mouse)) AND (fluoride) AND ((caries) OR (mutans streptococci) OR (Streptococcus mutans) OR prevention OR remineralization) in All Text	233
Total (All databases)			6187

Table 2. PICOS strategy showing the inclusion criteria.

Population (P)	Individuals at high risk of dental caries
Intervention (I)	Combined therapy using CPP-ACP/ Xylitol/ Bioactive glass/ Ozone along with Topical Fluoride
Comparator (C)	Topical fluoride mono-therapy
Outcome (O)	Primary: Remineralizing potential Secondary: Caries preventive effectiveness
Study design (S)	Randomised Controlled Trials

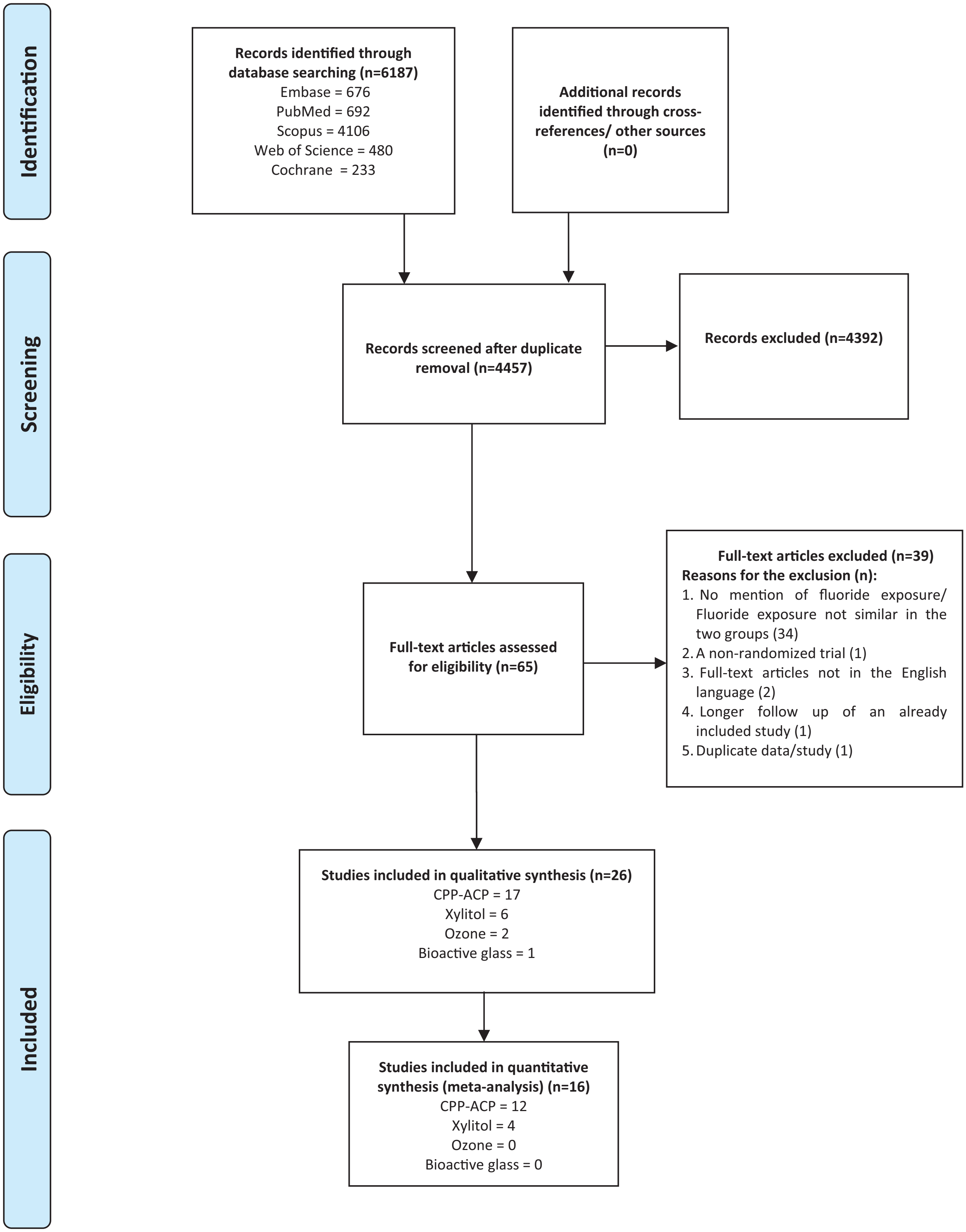

Figure 1. PRISMA flow diagram showing systematic review process.

was conducted each in Denmark [19], Italy [36], Romania [31], Sweden [34], Switzerland [40] and USA [37]; two in Brazil [28,41], Costa Rica [33,35], Germany [18,39] and Jordan [29,30]; three trials are reported from Thailand [20,24,38]; four from Australia [16,17,21,22] and five from Turkey [23,25–27,32]. Topical fluoride was administered as toothpaste [16–31,33–41], mouth-rinse [17,40] or varnish [26,32,37]. CPP-ACP was administered as 3% w/v gum [16], varnish [32] or as cream/paste [17–31] across the included trials. Trials have used xylitol in toothpaste [33–35,37] or gum form [36,38]. Ozone had been applied in the gaseous form [39,40] while the bioactive glass was used in the cream form [41].

Quality assessment using cochrane risk of bias assessment tool

Only one trial was assessed to have low risk of bias [17] while one was assessed at unclear risk of bias [16]. Rest all trials had a high risk of bias in one or more of the domains assessed. Most of the trials did not report the method of random sequence generation or allocation concealment and thus were graded at an unclear risk of selection bias [16,18,19,21–23,26,32,33,35,36,38,40]. The trials in which blinding of the participants/personnel was not done were graded to be at high risk of performance bias [18,19,21–23,26,27,29–31,37,38,40]. If the trials did not report the reason for loss to follow up or where the loss to follow up was expected to affect the effect of the outcome assessed, attrition bias was assessed to be high [21,22,33,35–37]. Details of the risk of bias are presented in Figure 2.

Primary outcome (remineralization potential)

For change in fluorescence and lesion area, three trials [19,24,30] with a total of 105 participants in CPP-ACP-TF combined therapy group and 118 participants in the control group were pooled together. No significant difference was observed for post-intervention ΔF [SMD −0.05, 95% CI (−0.40, 0.29), $p = .76$] (Figure 3) as well as for lesion area [SMD −0.19, 95% CI (−0.45, 0.08), $p = .16$] (Figure 4).

Two trials [25,27] that assessed the visual change in WSLs using the Gorelick criteria were pooled using the generic inverse variance method. The CPP-ACP-TF combined therapy was noted to perform better in regressing the WSLs as compared to the fluoride mono-therapy (SMD −0.60, 95% CI: [−1.07 to −0.14], $p = .01$] (Figure 5).

Three trials [26,27,31] with a total of 41 subjects in the experimental group and 44 subjects in the control group were pooled for post intervention DIAGNOdent values. The results significantly favoured CPP-ACP-TF combined therapy over fluoride mono-therapy [SMD −1.24, 95% CI (−1.96, −0.52), $p = .0007$] (Figure 6).

Secondary outcome (caries preventive efficacy)

Three trials for CPP-ACP [16,21,22] and two trials for xylitol [33,35] were pooled together using the generic inverse variance method for assessing the caries increment. The overall results significantly favoured the combined therapy over topical fluoride use alone [SMD −0.14, 95% CI (−0.19, −0.08), $p < 0.00001$]. However, the subgroup analysis showed no significant difference between the CPP-ACP-TF combined therapy and fluoride alone groups [SMD −0.21, 95% CI (−0.55, 0.13), $p = .23$]. The overall result is thus strongly influenced by the two xylitol studies [SMD −0.14, 95% CI (−0.21, −0.07), $p < .0001$] (Figure 7).

For post intervention *S mutans* count, five trials for CPP-ACP [21,22,27,29,32] and two trials [36,38] for xylitol were pooled using the generic inverse variance method. The overall results significantly favoured the combined therapy over topical fluoride use alone [SMD −0.28, 95% CI (−0.46, −0.10), $p = .003$]. However, the subgroup analysis showed no significant difference between the xylitol-TF combined therapy and fluoride alone [SMD −0.02, 95% CI (−0.27, 0.24), $p = .89$]. On the other hand, CPP-ACP combined therapy is seen to exhibit significantly better results than the control group [SMD −0.42, 95% CI (−0.62, − 0.23), $p < .0001$] (Figure 8).

Certainty of evidence

The certainty of the evidence for the remineralization potential assessed through post intervention DIAGNOdent values, Delta F, lesion area and regression of WSLs is graded as low. For the secondary outcome also, there is low quality evidence in favour of combined therapy with respect to caries increment and post intervention *S mutans* count (Table 4).

Discussion

This study significantly favours the use of CPP-ACP-TF combined therapy over TF monotherapy in remineralizing incipient lesions and in reducing the salivary *S mutans* load. Results also indicate the superiority of xylitol-fluoride combined therapy in preventing caries increment.

The primary outcome evaluated in this review is the remineralization potential assessed through quantitative light-induced fluorescence (QLF), visual inspection or laser fluorescence methods. QLF method provides the change in fluorescence (Delta F) and lesion area values that are considered reliable ways to quantify the demineralised tooth surface [42,43]. The conventional method of a direct visual examination of the incipient lesions using the Gorelick/Ekstrand criteria is well validated in literature [44]. Additionally, laser fluorescence (using DIAGNOdent) can be used to distinguish healthy tissue from diseased and is implicated to be an excellent early caries detection method with high sensitivity and specificity [43,45]. Nonetheless, the validity of the visual inspection method is similar to the objective means, and thus both ways can be used as reliable methods of non-cavitated lesion diagnosis [46]. For the secondary outcome, the caries preventive efficacy is assessed by the caries increment and *S mutans* count. It is well documented that assessment of *S mutans* is a clinically useful tool for identifying those at high risk of caries occurrence and progression [47–50]. Banas and Drake have also reported that although the pathogenesis of dental caries has shifted from the specific plaque hypothesis to ecological plaque hypothesis, the role of the *S mutans* in caries initiation and progression cannot be overlooked [51]. Out of the 26 trials included in this systematic review, data from ten studies could not be pooled due to the lesser follow-up period [18,41] or due to variability in the reporting of the outcome variable [17,20,23,28,34,37,39,40].

Table 3. Characteristics of included studies.

S. No.	Author, year; country	Study population (age)	Experimental group	Control group	Outcome variables	Follow-up period	Primary outcome	Secondary outcome
1.	Morgan et al. [16] 2008; Australia	11.5 – 13.5 years	$N = 892$; 1000 ppm F toothpaste; 3% w/w CPP-ACP chewing gum	CPP-ACP $N = 857$; 1000 ppm F toothpaste; Placebo chewing gum	Caries progression assessed through bitewing radiograph	2 years	Approximal caries progression (caries incidence/total surfaces assessed) Experimental: 785/14589 Control: 906/14017 $p < .05$	–
2.	Bailey et al. [17] 2009; Australia	12-18 years	$N = 23$; 1000 ppm F toothpaste; supervised 900 ppm F mouth-rinsing; 10% CPP-ACP cream;	$N = 22$; 1000 ppm F toothpaste; supervised 900 ppm F mouth-rinsing; Placebo cream	ICDAS II scorings	3 months	Active lesion: n/total Experimental: 35/207 Control: 69/201 $p < .05$	–
3.	Altenberger et al. [18] 2010; Germany	18 – 70 years	$N = 24$; 1450 ppm F toothpaste; 10% CPP-ACP paste	$N = 24$; 1450 ppm F toothpaste	DIAGNOdent; Ekstrand classification (score 2a)	21 days	DIAGNOdent readings (mean ± SD) Experimental: 8.4 ± 4.4 Control: 15.1 ± 4.5 $p < .05$ Proportion of subjects scored 2a/ total subjects assessed Experimental: 3/24 Control: 5/24 $p > .05$	–
4.	Brochner et al. [19] 2011; Denmark	13 – 18 years	$N = 22$; 1100 ppm F toothpaste; 10% CPP-ACP paste	$N = 28$; 1100 ppm F toothpaste;	Delta F (ΔF); Lesion area (A)	1 month	Experimental Control mean (SD) mean (SD) ΔF 4.45(1.82) 4.51(2.46) $p > .05$ A, mm^2 0.05(0.09) 0.14(0.31) $p > .05$	–
5.	Sitthisettapong et al. [20] 2012; Thailand	2.5-3.5 years	$N = 117$; 1000 ppm F toothpaste; 10% CPP-ACP paste	$N = 112$; 1000 ppm F toothpaste; Placebo paste	ICDAS II scorings	1 year	Experimental Control mean (SD) mean (SD) $Ds_{1\text{-}3}$ 17.0(8.1) 18.3(9.3) $Ds_{4\text{-}6}$ 12.7(13.2) 13.1(14.9) $Ds_{5-6}mfs$ 13.4(13.8) 13.5(15.9) $p > .05$	–
6.	Plonka et al. [21] 2013; Australia	Recruited at 44 days after birth	$N = 171$; 0.304% F toothpaste; 10% CPP-ACP cream	$N = 188$; 0.304% F toothpaste	Caries incidence; Salivary *S mutans*	2 years	No. of new carious teeth at 2 years (mean ± SD) Experimental: 1.5 ± 0.7 Control: 1.3 ± 0.58 $p > .05$	Number of *S mutans* positive children / Total children: Experimental: 50/171 Control: 34/188 $p < .05$

(*continued*)

Table 3. Continued.

S. No.	Author, year; country	Study population (age)	Experimental group	Control group	Outcome variables	Follow-up period	Primary outcome	Secondary outcome
7.	Pukallus et al. [22] 2013; Australia	Recruited a few days after birth;	$N = 66$; 400ppm F toothpaste; 10% CPP-ACP cream	$N = 58$; 400ppm F toothpaste	Caries incidence; Salivary *S mutans*	2 years	Number of children with new carious lesions/ total no. of children assessed: Experimental: 1/ 66 Comparator: 4/58 $p > .05$	Number of MS positive children/total: Experimental: 17/66 Comparator: 27/58 $p < .05$
8.	Aykut-Yetkiner et al. [23] 2014; Turkey	Mean age: 13 ± 0.68 years	$N = 27$; 1450ppm F toothpaste; 10% CPP-ACP cream	$N = 29$; 1450ppm F toothpaste	DIAGNOdent; Salivary *S. mutans*	3 months	No significant difference in between the groups.	Statistically significant reduction in salivary *S mutans* count.
9.	Sitthisettapong et al. [24] 2015; Thailand	2.5-3.5 years	$N = 40$; 1000 ppm F toothpaste; 10% CPP-ACP paste	$N = 39$; 1000 ppm F toothpaste; Placebo paste	Delta F (ΔF) Lesion area (A)	1 year	Post intervention ΔF (mean ± SD) Experimental: -12.39 ± 4.26 Control: -11.97 ± 4.03 $p > .05$ Post intervention A (mean ± SD) Experimental: 3.01 ± 2.03 Control: 3.16 ± 2.29 $p > .05$	–
10.	Esenlik et al. [25] 2016; Turkey	Mean age (experimental): 16.9 years Mean age (control): 17.1 years	$N = 20$; F toothpaste; CPP-ACP cream	$N = 20$; F toothpaste	DMFT; DMFS; WSL	Experimental group: 24.9 months Control group: 25.1 years	Post intervention DMFT (median): Experimental: 3.0 Control: 4.5 $p > .05$ Post intervention DMFS (median): Experimental: 3.0 Control: 5.5 $p > .05$ Post intervention WSL (mean±SD) Experimental: 1.9 ± 1.5 Control: 4.1 ± 4.0 $p < .05$	–
11.	Guclu, Alacam and Coleman [26] 2016; Turkey	8 – 15 years	$N = 22$; 1450 ppm F toothpaste; 23.2% xylitol chewing gum; 0.12% CHX mouthwash; 5% NaF varnish; 10% CPP-ACP paste	$N = 28$; 1450 ppm F toothpaste; 23.2% xylitol chewing gum; 0.12% CHX mouthwash; 5% NaF varnish	Ekstrand criteria; DIAGNOdent	3 months	Post-treatment VAS: median (inter-quartile range) Experimental: 1 (1–2) Control: 2 (2–3) $p < .05$ Post-treatment DIAGNOdent measurements (mean ± SD) Experimental: 3.95 ± 2.6 Control: 6.18 ± 3.0 $p < .05$	–

(*continued*)

Table 3. Continued.

S. No.	Author, year; country	Study population (age)	Experimental group	Control group	Outcome variables	Follow-up period	Primary outcome	Secondary outcome
12.	Karabekiroğlu et al. [27] 2017; Turkey	14-20 years	$N = 18$; 1450 ppm F toothpaste; 10% CPP-ACP paste	$N = 16$; 1450 ppm F toothpaste	DIAGNOdent DMFT; Gorelick Index; Salivary *S mutans*	3 years	Post intervention DIAGNOdent resdings (mean ± SD) Experimental: 4.76 ± 2.48 Control: 8.20 ± 4.38 $p < .05$ Post intervention DMFT scores (mean ± SD) Experimental: 8.1 ± 4.0 Control: 7.4 ± 3.4 $p > .05$ WSL severity [OR (95% CI)] 1.54 (0.86, 2.74) $p > .05$	No. of subjects/total subjects $> 10^5$ CFU/mL *S mutans* count post intervention Experimental: 2/16 Control: 6/18 $p < .05$
13.	Mendes et al. [28] 2018; Brazil	5 – 13 years	$N = 20$ teeth; 1450 ppm F toothpaste; 10% CPP-ACP paste	$N = 20$ teeth; 1450 ppm F toothpaste; Placebo paste	DIAGNOdent	3 months	DIAGNOdent readings at 3 month follow up [median (1st;3rd quartiles)]: Experimental: 18 (13; 24) Control: 14 (7;19) $p > .05$	–
14.	Al-Batayneh O.B, Al-Rai S.A. and Khader YS. [29] 2020; Jordan	4-5 years	$N = 46$; 500 ppm F toothpaste; 10% w/v CPP-ACP cream	$N = 37$; 500 ppm F toothpaste	Salivary S. mutans	6 months	–	Number of *S mutans* positive children/ total Experimental: 21/ 46 Control: 17/ 37 p .05
15.	Al-Batayneh, Hmood and Al-Khateeb [30] 2020; Jordan	4-5 years	$N = 41$; 500 ppm F toothpaste; 10% w/v CPP-ACP cream	$N = 35$; 500ppm F toothpaste	Delta F (ΔF) Lesion area (A)	6 months	Post intervention ΔF: Experimental: −6.26 ± 1.43 Control: −6.43 ± 1.6 $p > .05$ Post intervention Lesion Area: Experimental: 31.25 ± 35.9 Control: 38.93 ± 49.5 $p > .05$	–
16.	Bobu et al. [31] 2019; Romania	21 – 26 years	$N = 20$; 1450 ppm F toothpaste; 10% CPP-ACP paste	$N = 20$; 1450 ppm F toothpaste;	DIAGNODdent	3 months	DIAGNOdent scores (mean ± SD) Experimental: 8.18 ± 0.7 Control: 13.25 ± 0.97 $p < .05$	–
17.	Almaz & Oba [32] 2020; Turkey	3-5 years	$N = 20$; Dental treatment under general anaesthesia followed by 5% NaF + CPP-ACP varnish	$N = 20$; Dental treatment under general anaesthesia followed by 5% NaF varnish application	Salivary *S mutans*	3 months	–	No. of children with high MS count/ Total Experimental: 11/20 Control: 11/20 $p > .05$

(*continued*)

Table 3. Continued.

S. No.	Author, year; country	Study population (age)	Experimental group	Control group	Outcome variables	Follow-up period	Primary outcome	Secondary outcome
				Xylitol				
18.	Sintes et al. [33] 1995; Costa-Rica	8-10 years	$N = 840$; 1100 ppm F + 10% xylitol toothpaste	$N = 837$; 1100 ppm F toothpaste	DFS increment	3 years	DFS increment (mean ± SD): Experimental: 5 ± 3.7 Control: 5.7 ± 4.1 $p < .05$	–
19.	Twetman and Petersson [34] 1995; Sweden	3-6 years	$N = 33$; 250 ppm F + 9.7% xylitol toothpaste	$N = 32$; 250 ppm F toothpaste	Salivary *S mutans* and *Lactobacillus*	3 months		No significant difference within the groups or in-between the groups at 3 months follow-up.
20.	Sintes et al. [35] 2002; Costa Rica	7-12 years	$N = 1280$; 1100 ppm F + 10% xylitol toothpaste	$N = 1259$; 1100 ppm F toothpaste	DFS increment	30 months	DFS increment (mean ± SD): Experimental group = 1.3 ± 1.89 Control group = 1.51 ± 2.0 $p < .05$	–
21.	Campus et al. [36] 2009; Italy	7-9 years	$N = 80$; Xylitol chewing gum + 1450 ppm F toothpaste	$N = 85$; Placebo chewing gum + 1450 ppm F toothpaste	Salivary *S mutans*	3 months	–	Post intervention *S mutans* (log CFU/ml mean ± SD) Experimental group:5.28 ± 5.37 Control group: 5.36 ± 1.84 $p < .05$
22.	Chi et al. [37] 2014; USA	4-5 years	$N = 95$ 1400 ppm F +31% xylitol toothpaste; 5% NaF varnish	$N = 101$ 1,400 ppm F toothpaste; 5% NaF varnish	Caries increment; Salivary *S mutans*	6 months	Mean dmfs increment (mean ± SD): Experimental group = 2.5 ± 2.8 Control group = 1.4 ± 2.5 $p > .05$	*S mutans* levels reduction (mean ± SD): Experimental group = 0.98 ± 0.8 Control group = 0.9 ± 0.9 $p > .05$
23.	Mitrakul et al. [38] 2017; Thailand	18-23 years	$N = 29$; 1000 ppm F toothpaste ; 7.3 g/day xylitol chewing gum	$N = 26$; 1000 ppm F toothpaste	Salivary *S mutans* (CFU/ml)	1 month	–	Post intervention *S mutans* (mean ± SD) Experimental: $3.46 \times 10^4 \pm 6.37 \times 10^4$ Control: $3.50 \times 10^4 \pm 7.89 \times 10^4$ $p > .05$
				Ozone				
24.	Huth et al. [39] 2005; Germany	Mean age: 7.7 ± 2.2. years	$N = 41$; 500 ppm fluoride toothpaste for age < 6 years and 1000 ppm fluoride toothpaste for age ≥ 6 years; 2100 ppm Ozone delivered for 40 s;	$N = 41$; 500 ppm fluoride toothpaste for age < 6 years and 1000 ppm fluoride toothpaste for age ≥ 6 years;	DIAGNOdent	3 months	Wilcoxan test for DIAGNOdent values: Overall: $p > .05$ High caries risk: $p = .035$	
25.								–

(*continued*)

Table 3. Continued.

S. No.	Author, year; country	Study population (age)	Experimental group	Control group	Outcome variables	Follow-up period	Primary outcome	Secondary outcome
	Kronenberg et al. [40] 2009; Switzerland	Mean age: 15 years	$N = 20$ (one quadrant/ patient); F toothpaste and 250 ppm F mouth rinse; 2100 ppm Ozone delivered for 30 s;	$N = 20$ (two quadrants/ patient); F toothpaste and 250 ppm F mouth rinse	Gorelick criteria; DIAGNOdent	For at least 16 months or until the end of MB therapy	New WSLs (Percentage of quadrant area) Experimental: 3.2% Control: 2.1% $p > .05$ Quantitative Light Induced Fluorescence Index (Percentage of quadrant area) Experimental: 2.1% Control: 1.9% $p > .05$	
				Bioactive glass/ NovaMin				
26.	Alexandrino et al. [41] 2017; Brazil	18 – 26 years	$N = 17$ 1450 ppm F toothpaste – thrice a day; Bioactive glass applied for 5 min after each bleaching session (3 bleaching sessions 1 week apart)	$N = 17$ 1450 ppm F toothpaste – thrice a day (3 bleaching sessions one week apart)	Colour change assessed using spectro-photometer	21 days	Color change (mean ± SD) Experimental: 9.6 ± 3.24 Control: 10.46 ± 1.6 $p > .05$	–

Level of significance set at $p < .05$.
CPP-ACP: Caesin Phospho Peptide – Amorphous Calcium Phosphate.
CHX: Chlorhexidine.
Delta F (ΔF): Change in fluorescence.
dmfs: decayed missing filled surfaces (deciduous).
DMFT: Decayed Missing Filled Teeth (permanent).
DMFS/ DFS: Decayed Missing Filled Surfaces (permanent).
F: Fluoride.
ICDAS II: International Caries Detection and Assessment System II.
NaF: Sodium Fluoride.
ppm: parts per million.
S mutans: Streptococcus mutans.
VAS: Visual Analog Scale.
WSL: White Spot Lesion.

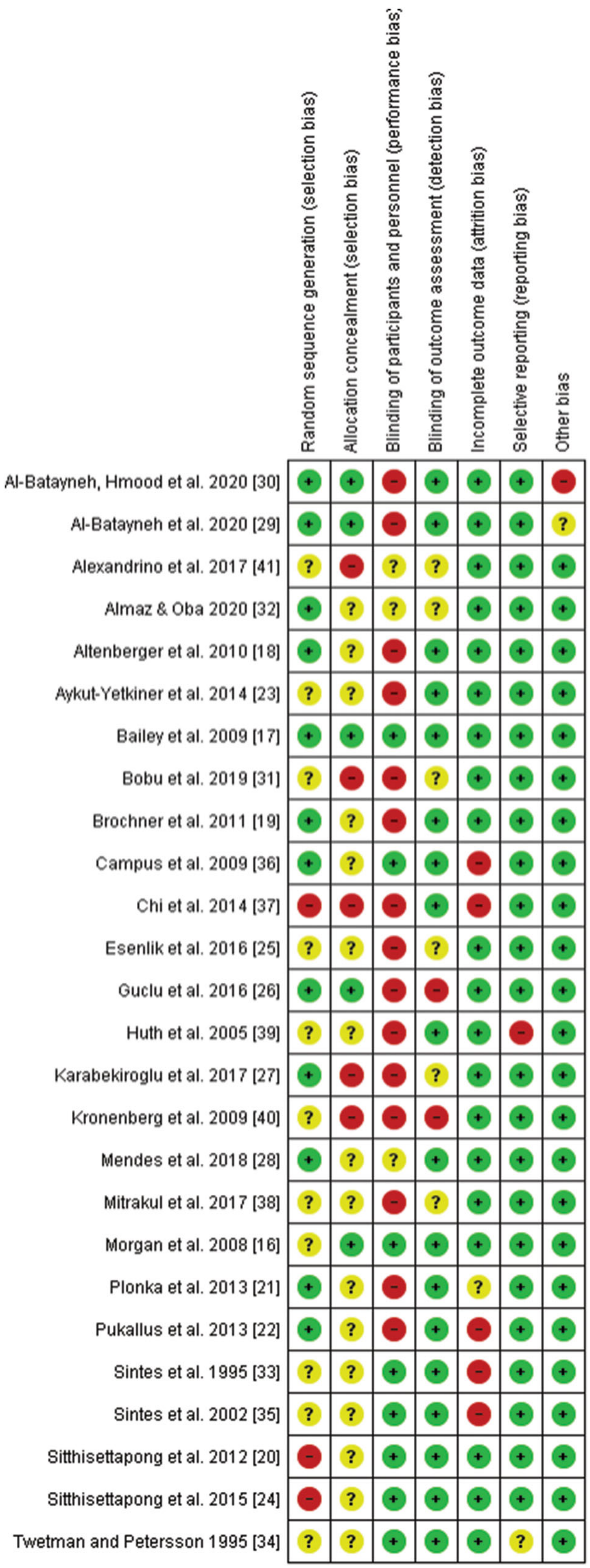

Figure 2. Risk of Bias assessment of the included studies.

CPP-ACP, a milk derivative, acts as a reservoir of bio-available calcium and phosphate and is reported to strengthen and remineralize the tooth structure [52]. CPP-ACP is also believed to exert an antibacterial effect as it prevents adherence of the cariogenic bacteria on the surface of the teeth[53]. In the present study, the combined effect estimate of three trials [19,24,30] pooled to assess the remineralization potential of CPP-ACP-TF versus TF monotherapy, measured through QLF, reported no significant difference in between the two groups. On the other hand, for the visual assessment of WSLs regression using Gorelick Index, the pooled analysis of two studies [25,27] significantly favoured the combined therapy. Guclu et al. [26] assessed the visual change in WSLs using Ekstrand criteria and reported significant difference in between the groups in favour of CPP-ACP-TF combined therapy. However, this study could not be pooled because of differences in the reporting of outcome variable. With respect to the DIAGNOdent scorings, four included trials favour the use of CPP-ACP-TF combined therapy [18,26,27,31] while two trials [23,28] reported no added benefit of the combined therapy. The current pooled analysis of three studies [26,27,31] favours the combined therapy over fluoride monotherapy in remineralizing early lesions with respect to post-intervention DIAGNOdent values. This is in line with the result of the previous meta-analysis on in-situ trials and in-vitro studies that also favoured the use of CPP-ACP in remineralizing early lesions and preventing caries when compared to control/no intervention [54,55]. However, the pooled results are in contrast with Tao *et al.* [56] that compared concluded that the CPP-ACP-TF combined therapy is as efficient as fluoride mono-therapy in remineralizing early caries lesions on smooth surfaces. The authors have included studies having different fluoride exposure in the experimental and control group [56]. However, the eligibility criterion in the current meta-analysis mandates similar fluoride exposure in the two groups to ascertain the effect of the added agent.

Results of a few trials significantly favour the caries preventive efficacy of CPP-ACP-TF combined therapy over fluoride mono-therapy assessed through change in caries status [16,17]. On the contrary, the results of other trials show no significant difference between the two groups [20–22,25,27]. In the current study, the subgroup analysis of the three studies [16,21,22] pooled to assess the mean caries increment also concludes no considerable difference between the combined versus mono-therapy. With respect to post-intervention *S mutans* colony count, few trials significantly favour the use of CPP-ACP-TF combined therapy [21–23,27] while others have shown no significant difference [29,32]. Results of the sub-group pooled analysis driven by two trials [21,22], however, favours the use of combined therapy over fluoride alone in reducing the salivary *S mutans* load.

Xylitol, sugar alcohol, has been suggested to exert an antimicrobial effect against the cariogenic bacteria [57]. Also, owing to its sweetness, xylitol stimulates salivation, facilitating a high mineral content salivary bath that makes physiologic remineralization possible [58]. Previously published meta-analyses [57,59] suggest that using a xylitol-fluoride containing toothpaste can significantly reduce tooth decay as compared to a fluoride-only toothpaste. The current pooled analysis has also shown similar results where the use of xylitol has an added benefit over fluoride use alone in inhibiting caries increment. However, these results are driven

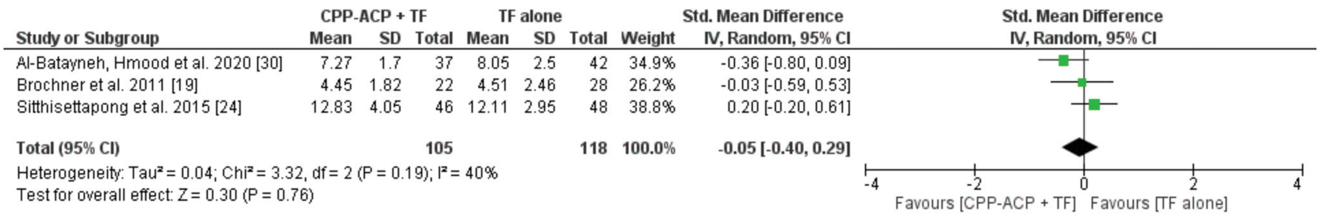

Figure 3. Meta-analysis on post-intervention Delta F values.

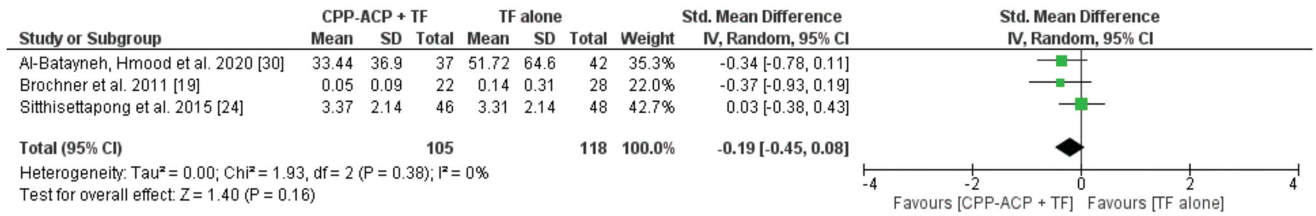

Figure 4. Meta-analysis on post-intervention lesion area values.

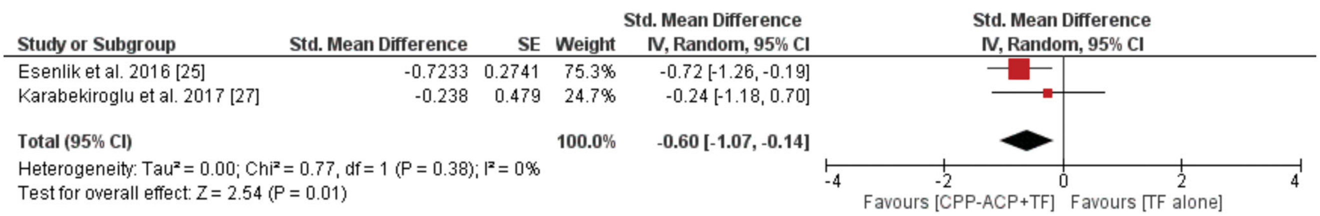

Figure 5. Meta-analysis on regression of WSLs severity.

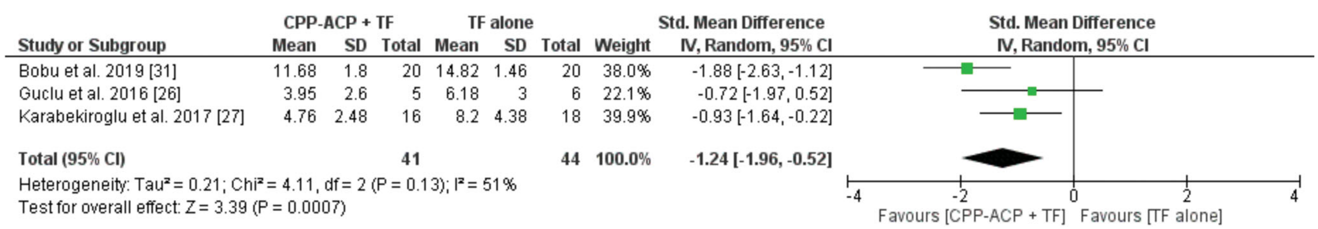

Figure 6. Meta-analysis on post-intervention DIAGNOdent values.

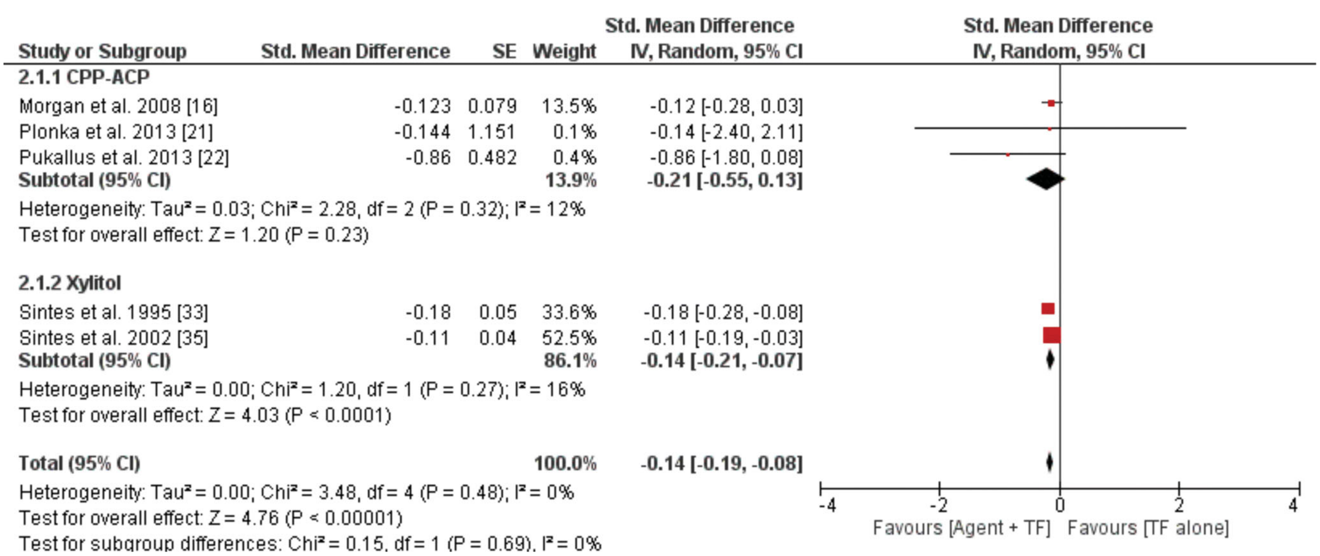

Figure 7. Meta-analysis on mean caries increment.

by two trials by the same authors on similar population [33,35]. On the contrary, results from a cluster-randomised trial reported no added advantage of xylitol-TF combined therapy in preventing caries incidence among 4–5-year-old children [37]. This may be due to the sub-optimal dose of xylitol administered as children are advised to use only a pea-sized amount of toothpaste. Unfortunately, this study could not be pooled due to the cluster randomised study

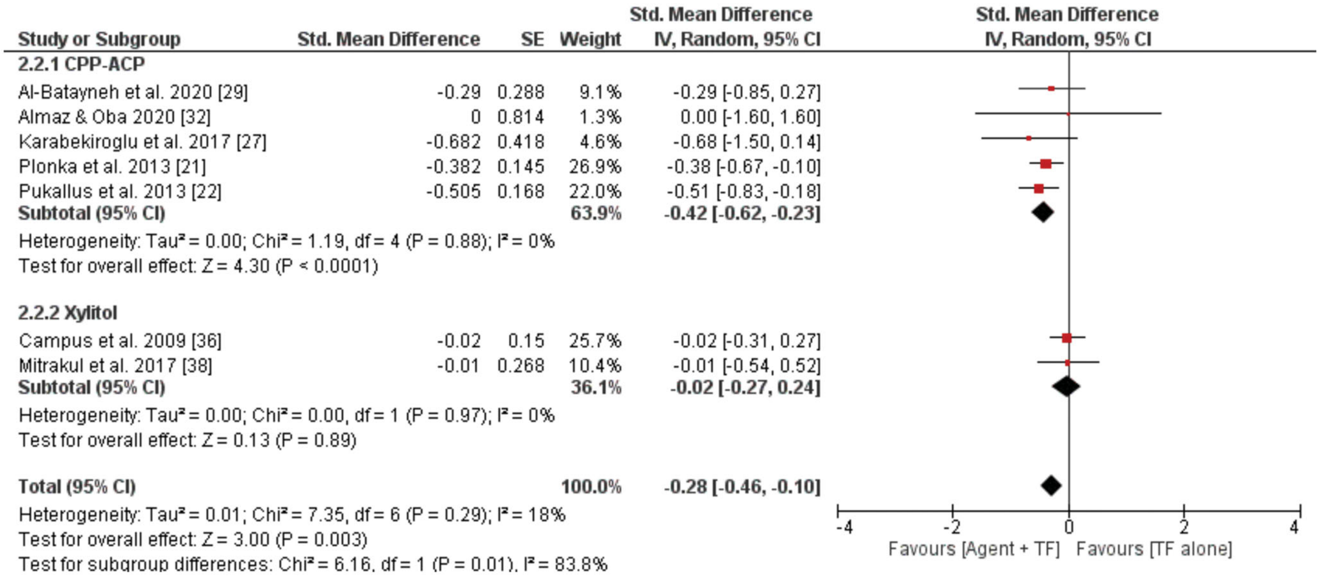

Figure 8. Meta-analysis on post-intervention *S mutans* count.

design that might lead to artificially narrow confidence intervals and p values thereby giving false positive conclusions [60]. It is to be noted that the daily recommended dose of xylitol for appropriate caries preventive action is $7-10$ g daily [61]. The antibacterial effect of xylitol is attributed to the interference of carbohydrate metabolism by xylitol. Three months follow up trial by Campus et al. [36] have reported a significant reduction of salivary *S mutans* colony count among children administering 10 g xylitol per day *via* chewing gum. On the other hand, Mitrakul et al. [38] showed no added benefit after daily exposure for four weeks to 7.3 g xylitol among the adult population. Twetman and Petersson [34] also showed no significant difference between the two groups in reducing the bacterial load at a 3 months follow up period among 3–6 year old children. Exposure to the recommended dose of xylitol over a more extended period may exert notable caries preventive effect.

The potential for the use of calcium sodium phosphor-silicate (Bioactive glass/NovaMin) for caries prevention is attributed to the release of Ca^{2+}, Na^{+}, PO_4^{3-} and Si^{4+} ions resulting in a rise in local pH and osmotic pressure [62]. The only clinical trial that could be included in this review showed no significant difference between the bioactive glass containing toothpaste versus fluoride only toothpaste in the post-intervention visual change assessed using a spectrophotometer [41]. The paucity of clinical trials makes it difficult to comment on the added caries preventive benefit of bioactive glass over fluoride alone.

Recent advancements in dentistry have made use of the sterilising effect and bactericidal potential of ozone in caries arrest and prevention [63,64]. Besides, the oxidising nature of ozone helps in removing proteins from the demineralised tooth structure thus enabling the diffusion of calcium and phosphate ions that ultimately leads to remineralization [65]. The one trial included in this review showed no added remineralization potential of ozone therapy over fluoride use alone [40]. However, more trials are needed to generate reliable evidence on the efficacy of ozone-fluoride combined therapy in caries prevention and tooth remineralization.

The certainty of evidence with respect to remineralization potential assessed through post-intervention DIAGNOdent values, Delta F, lesion area and regression of WSLs was downgraded for imprecision (due to the small number of study participants) and high risk of bias.

For the caries increment and post intervention *S mutans* count also, the evidence was downgraded for the high risk of bias. Moreover, for the xylitol trials pooled for mean caries increment, the certainty of effect obtained was downgraded as the studies were conducted by same authors in similar population. In addition, the seven trials pooled to estimate the effect on post intervention *S mutans* count had a wide variability in the time of follow up and thus downgrading the evidence.

Limitations and recommendations

Very few studies could be pooled together for each outcome assessed. In this study, to minimise the impact of clinical heterogeneity amongst the include trials, random effect model using inverse (generic) variance method was used. However, such heterogeneity might still project some uncertainties in the results. The quality of evidence generated is assessed to be low, further limiting the certainty of the effect estimates. In particular, there is a paucity of randomised controlled trials for ozone-TF combined therapy, and bioactive glass-TF combined therapy.

Good-quality clinical trials should be conducted to evaluate the efficacy of various agents that are expected to exhibit an added advantage over fluoride use alone. The trials should also take into account the multiple concentrations, delivery vehicles and application regimen that could be followed for each of these agents.

Table 4. Certainty of evidence according to GRADE.

Quality Assessment		Summary of Findings					
		No. of participants		Effect			
					Absolute		
No. of studies	Design	Combined therapy	Topical fluoride mono-therapy	Relative (95% CI)	Risk with TF mono-therapy	Risk difference with combined therapy	Quality
Post Intervention DIAGNOdent values							
3	Randomised Controlled Trials	41	44	–	–	**SMD 1.24 lower** (1.96 lower to 0.52 lower)	⊕⊕OO **Low**[a,b]
Post-intervention Delta F values							
3	Randomised Controlled Trials	105	118	–	–	**SMD 0.05 lower** (0.4 lower to 0.29 higher)	⊕⊕OO **Low**[a,b]
Post-intervention Lesion Area values							
3	Randomised Controlled Trials	105	118	–	–	**SMD 0.19 lower** (0.45 lower to 0.08 higher)	⊕⊕OO **Low**[a,b]
Regression of WSLs severity							
2	Randomised Controlled Trials	38	36	–	–	**SMD 0.6 lower** (1.07 lower to 0.14 lower)	⊕⊕OO **Low**[a,b]
Mean caries increment							
5	Randomised Controlled Trials	3249	3199	–	–	**SMD 0.14 lower** (0.19 lower to 0.08 lower)	⊕⊕OO **Low**[a,c]
Post-intervention *S mutans*							
7	Randomised Controlled Trials	425	428	–	–	**SMD 0.28 lower** (0.46 lower to 0.1 lower)	⊕⊕OO **Low**[a,c]

[a]Downgraded due to high risk of bias.
[b]Downgraded on Imprecision (small number of study participants/ wide confidence interval).
[c]Downgraded because same authors did study on similar population/ wide variability in follow up.

Conclusion

CPP-ACP-fluoride combined therapy exhibits superior remineralization potential and antibacterial effect but not more effective in preventing new lesions over fluoride use alone. Xylitol, on the other hand, is shown to exert an added benefit over fluoride in preventing caries increment. However, the effect estimate is graded to be of low certainty. Thus, future trials addressing the same research question may have a substantial impact on the results obtained.

Disclosure statement

The authors declare no potential conflicts of interest.

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