

## Original Article

# Efficacy of casein phosphopeptide-amorphous calcium phosphate varnish in remineralizing white spot lesions: A systematic review and meta-analysis

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## ABSTRACT

**Background:** This systematic review aimed to evaluate the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACPF) varnish for remineralization of white spot lesions (WSLs) "in vitro" in human teeth.

**Materials and Methods:** Literature search included three databases, namely Medline (via PubMed), The Cochrane Controlled Clinical Trials Register, and Google Scholar from 2010 to January 2021. The studies assessing WSL depth, calcium, phosphate ion release, and microhardness due to artificial demineralization or remineralization were considered for review. Reference articles were retrieved, and a customized risk assessment tool was used. The Cochrane risk of bias assessment tool was used to generate the risk of bias summary graph. Meta-analysis was performed using RevMan 5.4. Heterogeneity was evaluated by Cochrane's test, and random effects model was used to pool estimate of effect and its 95% confidence intervals (CIs) for surface microhardness.

**Results:** Eighteen studies were selected for review based on the eligibility criteria. Four studies showed superior remineralizing effect of CPP-ACPF compared to fluoride varnishes. Four studies involving 120 human permanent teeth samples were included in the meta-analysis. Efficacy of CPP-ACPF varnish was equivalent to other fluoride varnishes in improving surface microhardness after remineralization during 7-day period (mean surface microhardness: 3.94, 95% CI [-9.08–1.21], I<sup>2</sup>: 75%, P = 0.13). Major risks of bias associated with the studies included in the review were inadequate sample size, improper sample preparation, and unexplained blinding.

**Conclusion:** CPP-ACPF varnish appears to be equally effective as other fluoride varnishes in remineralizing artificially induced WSLs, but quality of evidence is low.

**Key Words:** Casein phosphopeptide-amorphous calcium phosphate, meta-analysis, remineralization, varnish

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## INTRODUCTION

The development of caries involves a dynamic biological process where acids produced by bacterial glycolysis of dietary carbohydrates cause demineralization of dental hard tissues. Noncavitated white spot lesions (WSLs) are indicative of early stage of dental caries. These lesions are considered reversible if detected early.<sup>[1]</sup> Topical application of fluoride-releasing materials has been used over three decades to combat dental caries.<sup>[2,3]</sup> They are considered important adjuvants in clinical practice, particularly when the patient's level of cooperation is low.<sup>[4]</sup> The major mechanism of fluoride ions in preventing enamel demineralization is by promoting the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced by plaque bacterial organic acids.<sup>[5,6]</sup> Fluoride ions can also drive the remineralization of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied.<sup>[7]</sup> Therefore, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralization to occur.<sup>[7]</sup> To overcome the limited bioavailability of calcium and phosphate ions, calcium phosphate-based remineralization systems such as amorphous calcium phosphate (ACP), calcium sodium phosphosilicate bioactive glass, and casein phosphopeptide-stabilized ACP were developed. The remineralizing potential of casein phosphopeptide-ACP (CPP-ACP) in cariogenic studies on animals, was demonstrated by researchers in 1995, and only in 2009 was it used for the treatment of WSLs.<sup>[8]</sup> CPP-ACP nanocomplexes is a technology based on ACP stabilized by casein phosphopeptides (CPP).<sup>[7]</sup> CPPs stabilize high concentrations of calcium and phosphate ions, together with fluoride ions, at the tooth surface by binding to pellicle and plaque. These ions are freely bioavailable to diffuse down concentration gradients into enamel subsurface lesions, thereby effectively promoting remineralization *in vivo*.<sup>[7]</sup>

Varnishes allow for the delivery of high concentrations of fluoride in small amounts.<sup>[9]</sup> The effectiveness, the relative safety, high fluoride uptake, and the ease of application, offer significant advantages over other professionally applied topical fluoride treatments such as fluoride gels, foams, and mouthrinses.<sup>[9]</sup> CPP-ACPF varnish is unique in that it uses Recaldent (CPP-ACP) technology incorporated with 5% sodium fluoride. The

inclusion of CPP-ACP in MI varnish® (GC America Inc., Alsip, IL, USA) has been proven to inhibit enamel demineralization to a much greater extent than fluoride varnishes without CPP-ACP.<sup>[10,11]</sup> Literature search revealed three systematic reviews on CPP-ACP, with one focusing on effectiveness of MI paste and the other two reviewing effect of all formulations of CPP-ACP in human randomized clinical trials.<sup>[12-14]</sup> Review of *in vitro* studies comparing remineralizing potential of CPP-ACPF varnish with other fluoride varnishes has not been done. Hence, this systematic review aimed to evaluate the efficacy of CPP-ACPF varnish in remineralizing WSLs *in vitro* on human teeth.

## MATERIALS AND METHODS

The Cochrane Handbook for Systematic Reviews of Interventions<sup>[15]</sup> and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement guidelines were followed for review.<sup>[16]</sup> The Population, Intervention, Comparison, Outcome, and Study design method as applicable is presented in Table 1. The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021237900).

### Search strategy for article identification

An extensive electronic search for *in vitro* clinical trials via three databases, namely Medline (via PubMed), The Cochrane Controlled Clinical Trials Register, and Google Scholar, till January 29, 2021, was done. The outcomes of the search and Medical Subject Headings are summarized in Table 2. Hand searching was performed for relevant journals and the reference lists of all eligible studies for additional relevant studies. No restrictions on the language or date of publication were applied during the search. Reference articles were retrieved and exported to the Mendeley Desktop 1.13.3 software (Elsevier, Mendeley Ltd, London, United Kingdom).<sup>[17]</sup> The authors were not blinded to country or journal names.

**Table 1: Population, Intervention, Comparison, Outcome, and Study design format of study**

Component	Description
Population	Human permanent and primary molars or premolars
Intervention	CPP-ACPF varnish
Comparison	fluoride varnishes other than CPP-ACPF
Outcome	Lesion depth, microhardness and calcium and phosphate release, and mineral loss/gain
Study design	<i>In vitro</i> studies

CPP-ACPF: Casein phosphopeptide-amorphous calcium phosphate fluoride

### Selection of studies

The review included *in vitro* studies on human teeth from 2010 to January 2021. The studies assessing depth of WSL, microhardness due to artificial demineralization or remineralization and calcium, phosphate ion release, and mineral loss/gain were considered for review. Case reports, abstracts, editorials, review articles, non-English articles, animal studies, and studies testing formulations of CPP-ACP, such as sugar-free gums, lozenges, fluoridated gels, mouth rinse, paste, and antibacterial gels, were excluded from review.

### Data extraction

One author searched the studies, screened the titles and abstracts of each study based on the criteria, and extracted data. Two authors independently rechecked the full text of the screened studies. Any disagreement between the two authors, was resolved by a third reviewer. Data collected for each study included information pertaining to year of publication, authorship, sample size, study characteristics like intervention, comparison, follow-up period, examination methods, and results.

### Assessment of risk of bias

A customized risk assessment tool was prepared using Office of Health Assessment and Translation risk-of-bias tool<sup>[18]</sup> and checklist for reporting *in vitro* studies guidelines.<sup>[19]</sup> The domains evaluated are listed in Figure 1. All included studies were assessed independently by two review authors who were not blinded to identifying details of articles. Each domain was classified as having a low, high, or unclear risk of bias. The Cochrane risk of bias assessment

tool was used to generate the risk of bias summary graph.<sup>[20]</sup>

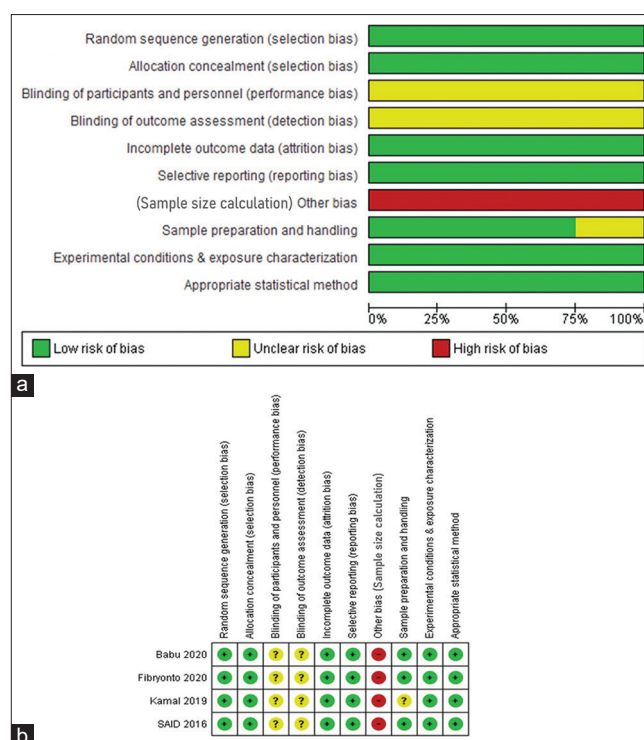
### Statistical analysis

Data were analyzed using RevMan 5.4.<sup>[21]</sup> Heterogeneity between the estimates was evaluated by Cochrane’s test ( $I^2$  test) at  $\alpha = 0.10$ .<sup>[22]</sup>  $I^2 > 50\%$  indicated a high heterogeneity.<sup>[22]</sup> Furthermore, the statistical significance for testing the hypothesis was set at  $P$  value (two-tailed)  $< 0.05$ . Studies assessing the effect of CPP-ACPF versus fluoride varnishes on surface microhardness of artificially created WSLs in permanent teeth over a period of 1 week were included in the meta-analysis. The treatment effect for each study was summarized using mean differences and standard deviations. Random effects model was used to combine the studies due to the clinical and methodological heterogeneity existing in the studies.<sup>[23]</sup> A pooled estimate of effect and its 95% confidence intervals (CIs) for surface microhardness was calculated. Data from eligible studies were extracted into RevMan software and forest plot was generated for graphic presentation. Meta-analysis was not performed for the parameters lesion depth and mineral loss/gain due to dissimilarity in outcome measurements.

**Table 2: Search strategy of the study**

Database	Search strategy	Articles retrieved
PubMed	(“Casein Phosphopeptide-Amorphous Calcium Phosphate nanocomplex”[Supplementary Concept] OR “Casein Phosphopeptide-Amorphous Calcium Phosphate nanocomplex”[All Fields] OR “CPP ACP”[All Fields]) AND (“paint”[MeSH Terms] OR “paint”[All Fields] OR “varnish”[All Fields]) AND Remineralization [All Fields] AND (Invitro[All Fields] AND Study[All Fields])	55
Cochrane Central Library	MI varnish “CPP ACP varnish” AND remineralization	16
Google Scholar	MI varnish “CPP ACP varnish” AND remineralization, MI varnish “CPP ACP varnish”	839

CPP-ACP: Casein phosphopeptide amorphous calcium phosphate



**Figure 1: Cochrane risk of bias of the included studies (a) graph, (b) summary.**

## RESULTS

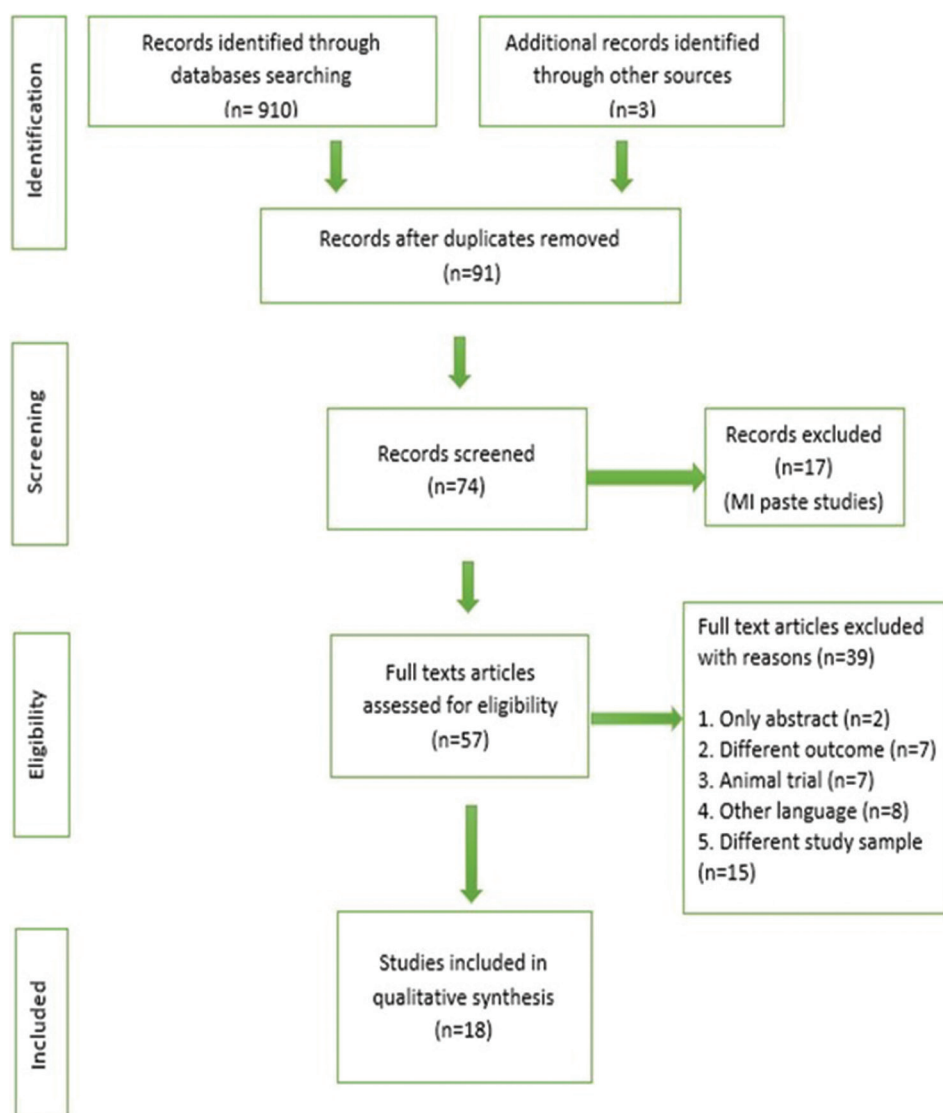
### Study selection and description

Through the literature search, 913 studies were identified, including 822 duplicates. Seventy-four articles were identified after excluding duplications. A total of 17 articles evaluating the efficacy of MI paste were excluded after reading abstract. Full-text articles were retrieved for 57 relevant studies. Thirty-nine articles were excluded after full-text reading. Finally, 18 studies which met the inclusion criteria were considered [Figure 2]. Review evaluated three different outcomes: lesion depth, surface microhardness, and mineral loss/gain. Studies assessing the remineralization effect of CPP-ACP varnish over a period of 7 days in comparison to other

fluoride varnish were pooled. Results of four studies measuring surface microhardness were synthesized using forest plot.

### Study characteristics

Eighteen relevant studies were found published from 2011 to 2021 (January). Ten studies were reported from Asia, three from the Middle East, two each from America and Australia, and one from Europe. All the studies were conducted on extracted human permanent teeth. These studies reported intervention on permanent molars, premolars, and anterior teeth. Examination methods for surface microhardness assessment included microhardness tester machine expressing Knoop hardness number and Vickers microhardness number. For evaluating lesion depth, polarized light microscope, FluorCam, transverse



**Figure 2:** Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of review methodology.

microradiography, stereomicroscope, micro-computed tomography analysis, and Diagnodent were used. To measure calcium and phosphate ion release, ion chromatography and scanning electron microscope were utilized and transverse microradiography was used for assessing mineral content. Test group considered was only ACP-CPPF varnish compared with one or more control groups such as no treatment, fluoride varnish, and other varnishes. The follow-up period ranged from 4 to 84 days [Table 3].

**Risk of bias assessment**

Score of 1 was given for each risk of bias item, if mentioned. The overall level of risk for each study was subsequently classified as low risk, “moderate risk/unclear risk,” and “high risk” (if score was 7 or more, 3 or more, and <3 out of the nine categories, respectively). The scores were averaged for each included study. Score was not given when individual risk of bias item was not mentioned clearly. For example: no mention of sample size calculation;<sup>[19]</sup> blinding and multiple samples prepared from the same specimen.<sup>[19]</sup> Risk of bias item randomization and allocation concealment were considered low risk as the samples (human teeth enamel) used were homogenous.<sup>[18]</sup> Overall, 7<sup>[26,31,32,34,38]</sup> of the 18 included studies had unclear risk of bias and rest 11<sup>[10,11,24,27,29,30,33,35-37,39]</sup> had low risk of bias. The risks of bias of the included studies are summarized in Table 4 [Figure 3]. Major risks of bias associated with these studies included inadequate sample size, improper sample preparation, and unexplained blinding.

**Synthesis of results – Effect of interventions**

*Surface microhardness*

Out of six studies, five studies<sup>[27,30-32,34]</sup> showed no significant difference in surface microhardness between the intervention and fluoride groups after an interval of 7 days. CPP-ACPF varnish groups

recorded significantly harder enamel than no treatment groups [Table 5].<sup>[24,27-29,31,32,34]</sup>

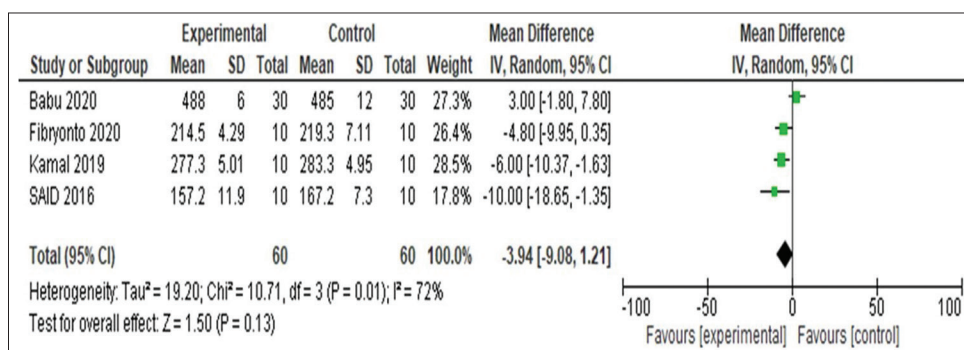
For meta-analysis, we pooled data of four studies<sup>[27,30,31,34]</sup> involving 120 human permanent teeth samples. CPP-ACPF varnish compared to fluoride varnish did not significantly favor improvement of surface microhardness after remineralization during 7-day period (SMH: 3.94, 95% CI [-9.08–1.21], *P* = 0.13), and there was substantial heterogeneity (tau = 19.2, Chi-squared = 10.7, *I*<sup>2</sup> = 72%) across the studies [Figure 1].

*Mineral loss/gain*

Three studies evaluated calcium and phosphate ions release. Two studies<sup>[11,36]</sup> reported calcium and phosphate ion release to be significantly higher in CPP-ACPF varnish group than fluoride group, whereas in one study, there was no significant difference.<sup>[32]</sup> The mean change in mineral gain after remineralization was superior in CPP-ACPF varnish group over control.<sup>[34-36]</sup> The mineral gain was highest in CPP-ACPF varnish than fluoride groups in a study,<sup>[36]</sup> but two studies did not report significant result.<sup>[34,35]</sup> Reduced mineral loss during demineralization was seen in CPP-ACPF group compared to fluoride varnish<sup>[11]</sup> [Table 5].

*Lesion depth*

Of the 12 studies, 2 studies<sup>[10,11]</sup> showed superior demineralizing inhibitory effect of CPP-ACPF varnish over fluoride varnish. CPP-ACPF varnish showed superior remineralizing effect when compared to no treatment.<sup>[11,24,25,26,28,34,36-39]</sup> When remineralizing effect of CPP-ACPF varnish was compared to fluoride varnish, superior efficacy was seen in four studies<sup>[11,33,36,39]</sup> while two studies<sup>[34,38]</sup> did not report a significant effect [Table 5].



**Figure 3:** Forest plot summary of included studies.

**Table 3: Characteristics of included studies**

Author/Year/Country	Sample teeth/surface	Intervention group (n)	Comparison group (n)	Follow-up period	Examination methods
Attiguppe <i>et al.</i> , 2019, India <sup>[10]</sup>	Premolars/buccal and the lingual surfaces	Group 1 - CPP-ACPF varnish (24)	Group 2 - Fluor protector varnish (24)	1. Lesion depth - 30 days 2. F release (2 and 4 weeks)	1. Polarized light microscope - Lesion depth 2. Fluoride-specific ion electrode - Fluoride release
Abufarwa <i>et al.</i> , 2019, Texas <sup>[24]</sup>	Premolars and Molars/buccal surface	Group 1 - CPP-ACPF varnish (21)	Group 2 - Laser (21) Group 3 - Control group (21)	10 days	1. KNH number - surface microhardness 2. FluoreCam - lesion depth
Abufarwa <i>et al.</i> , 2019, Texas <sup>[25]</sup>	Premolars and Molars/buccal surface	Group 1 - CPP-ACPF varnish (40)	Group 2 - Control group (40)	2, 4, 8, and 12 weeks	1. FluoreCam system - area 2. Polarized light microscope - lesion depth
Girish Babu <i>et al.</i> , 2018, India <sup>[26]</sup>	Premolars/buccal surface	Group 1 - CPP-ACPF varnish (25)	Group 2 - Fluor protector (25) Group 3 - Control window - no varnish	6 days	1. Polarized light microscope - Lesion depth
Babu <i>et al.</i> , 2020, India <sup>[27]</sup>	Premolars/buccal surface	Group 1 - CPP-ACPF varnish (30)	Group 2 - Fluor protector (30) Group 3 - No varnish (30)	8 days	1. Microhardness tester machine - Surface microhardness
Bakry and Abbassy 2018, Saudi Arabia <sup>[28]</sup>	Third molars/buccal and lingual surfaces	Group 3 - CPP-ACPF varnish (25)	Group 1 - MI paste (25) Group 2 - MI paste+bond (25) Group 4 - Control (25)	7 days	1. Vickers microhardness - surface microhardness 2. Transverse microradiography analysis - lesion depth, mineral loss
Bapat <i>et al.</i> 2020, India <sup>[29]</sup>	Premolars/buccal surface	Group 1 - CPP-ACPF varnish (10)	Group 2 - Embrace Varnish (10) Group 3 - Control (10)	10 days	1. Vickers microhardness method - surface microhardness
Fibryanto <i>et al.</i> , 2020, Indonesia <sup>[30]</sup>	Premolars/buccal surface	Group 1 - CPP-ACPF varnish (10)	Group 2 - Bifluorid (10) Group 3 - Clinpro XT varnish (10)	7 <sup>th</sup> and 14 <sup>th</sup> days	1. Vickers microhardness method - surface microhardness
Kamal <i>et al.</i> , 2020, Egypt <sup>[31]</sup>	Molars/buccal surface	Group 1 - CPP-ACPF varnish (10)	Group 2 - Control (10) Group 3 - Fluoride (10)	1 <sup>st</sup> and 4 <sup>th</sup> weeks	1. Vickers microhardness method - Surface microhardness
Majithia <i>et al.</i> , 2016, Malaysia <sup>[32]</sup>	Premolars/buccal surface	Group 4 - CPP-ACPF Varnish (10)	Group 1 - No varnish (10) Group 2 - Flor-Opal® Varnish White (10) Group 3 - Premier Enamel Pro Varnish (10)	5 days	1. Vickers microhardness - surface microhardness 2. SEM-EDAX testing - Calcium/phosphorus ratio
Ramadevi <i>et al.</i> , 2020, India <sup>[33]</sup>	Anteriors/buccal surface	Group 2: CPP-ACPF Varnish (10)	Group 1: NaF (10)	5 days	1. Polarized light micro-scope - lesion depth
Mohd Said <i>et al.</i> , 2017, China <sup>[34]</sup>	3rd molars/buccal surface	Group 2: CPP-ACPF Varnish (10)	Group 0: Control (no varnish), (10) Group 1: Duraphat, (10) Group 3: Embrace Varnish, (10) Group 4: Enamel Pro-Varnish, (10) Group 5: Clinpro-White Varnish. (10)	8 days	1. Knoop surface microhardness - surface microhardness 2. Transverse microradiography - lesion depth and change in mineral loss

Contd...

Table 3: Contd...

Author/Year/Country	Sample teeth/surface	Intervention group (n)	Comparison group (n)	Follow-up period	Examination methods
Shen <i>et al.</i> , 2016, Australia <sup>[11]</sup>	3rd molars/ buccal and lingual surfaces	Group 3 - CPP-ACPF varnish (12)	Group 1 - Enamel Pro (12) Group 2 - Clinpro (12) Group 4 - Duraphat (12) Group 5 - Profluorid (12) Group 6 - Control (12)	4 days	1. Transverse microradiography - lesion depth 2. Ion chromatography-ions release
Sleibi <i>et al.</i> , 2018, UK <sup>[35]</sup>	Premolar roots/ buccal surface	Group 1 - CPP-ACPF varnish (12)	Group 2 - Bioglass (12) Group 3-5% NaF (12) Group 4 - Control (12)	5 days	1. XMT - change in mineral concentration
Shen <i>et al.</i> , 2020, Australia <sup>[36]</sup>	3rd molars/ gingival or occlusal	Group 1 - CPP-ACPF varnish (10)	Group 2 - Duraphat varnish (10) Group 3 - Control group (10)	14 days	1. Ion chromatography - Ions release 2. Transverse microradiography - mineral content
Thakkar <i>et al.</i> , 2017, India <sup>[37]</sup>	Molars/ buccolingually, and mesiodistally (4 sections)	Group 3 - CPP-ACPF varnish (20)	Group 1 - CPP-ACP paste (20) Group 2 - Tooth Mousse plus (20) Group 4 - Control group (20)	Demineralization=12 days Remineralization=19 days	1. Stereomicroscope -lesional depth
Üstün and Aktören 2019, Istanbul <sup>[38]</sup>	3rd Molars/ buccal and lingual surfaces	Group 1 - CPP-ACPF varnish (8)	Group 2 - Duraphat varnish (8) Group 3 - Artificial saliva (8)	30 days	1. Diagnodent - lesional depth 2. µCT analysis - Lesion depth, surface area
Varma <i>et al.</i> , 2019, India <sup>[39]</sup>	Premolars/ buccal surface	Group 1 - CPP-ACPF varnish (10)	Group 2 - Clinpro XT varnish (10) Group 3 - Control group (10)	7 days	1. Diagnodent - lesional depth.

Control group - Untreated, (n) - sample size. CPP-ACPF: Casein phosphopeptide-amorphous calcium phosphate fluoride, F: Fluoride, KNH: Knoop hardness, XMT: X-ray microtomography, SEM-EDAX: Scanning electron microscopy with an energy dispersive X-ray analysis attachment, NaF: Sodium fluoride

## DISCUSSION

This systematic review and meta-analysis underline the significant remineralizing effect of CPP-ACPF varnish compared to fluoride varnishes. Majority of *in vitro* studies<sup>[11,33,36,39]</sup> have shown pronounced remineralizing effect of CPP-ACPF varnishes compared with other fluoride varnishes. However, few *in vitro*<sup>[34,38]</sup> studies have shown contradictory results. Meta-analysis showed no significant difference between CPP-ACPF varnish and other fluoride varnishes in improving the surface microhardness after remineralization during 7-day period.

Fluoride varnishes are more effective compared to other forms of delivery because of long contact periods resulting in high fluoride uptake and the formation of CaF<sub>2</sub> deposits that act as fluoride reservoirs.<sup>[40]</sup> The CPP-ACP has been shown to interact with fluoride ions to produce an additive anticariogenic effect through the formation of CPP-stabilized amorphous calcium fluoride phosphate (ACFP) phase.<sup>[41-44]</sup> The *in situ* study by Reynolds<sup>[45]</sup> showed that CPP-ACP plus fluoride

formulation not only increased fluoride incorporation into plaque but also in subsurface enamel and substantially increased remineralization of subsurface lesions of enamel compared with fluoride alone. An *in vitro* study by Duraisamy *et al.* reported that demineralization inhibitory potential on the additive use of F – varnish + CPP-ACP was superior to fluoride varnish or CPP-ACP applied alone on the enamel of young permanent teeth.<sup>[46]</sup> The presence of the CPP-ACFP nanocomplexes in MI varnish has shown to be superior to fluoride alone in inhibiting enamel demineralization and promoting remineralization in a number of *in situ* and *in vivo* randomized controlled clinical trials.<sup>[8,45,47-49]</sup>

An *in vitro* study by Thakkar *et al.* found that CPP-ACPF varnish had significant demineralization inhibitory property compared to CPP-ACP paste and CPP-ACPF paste plus. Remineralizing effect did not differ significantly compared to both groups.<sup>[37]</sup> A previous study, which evaluated remineralizing efficiency of CPP-ACPF varnish and paste groups, showed increased values of calcium, phosphate contents, higher percent surface

Table 4: Summary of risk of bias of the included studies

Author/year/country	Sample size calculation	Sample preparation and handling - S/MS <sup>†</sup>	Randomization	Allocation concealment	Blinding (M/NM)	Experimental conditions and exposure characterization similar (yes/no)	Outcome data - attrition/exclusion (M/NM)	Reported - measured outcome (Yes/No)	Appropriate statistical method (Yes/No)	Risk of bias score
Attiguppe et al., 2019, India <sup>[10]</sup>	M	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	7/9
Abufarwa et al., 2019, Texas <sup>[24]</sup>	M	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	7/9
Abufarwa et al., 2019, Texas <sup>[25]</sup>	M*	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	6/9
Girish Babu et al., 2018, India <sup>[26]</sup>	NM	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	6/9
Babu et al., 2020, India <sup>[27]</sup>	NM	S	NM	NM	NM	Yes	M	Yes	Yes	7/9
Bakry and Abbassy 2018, Saudi Arabia <sup>[28]</sup>	M*	MS <sup>†</sup>	M	NM	NM	No	M	Yes	Yes	5/9
Bapat et al., 2020, India <sup>[29]</sup>	NM	S	M	NM	NM	Yes	M	Yes	Yes	7/9
Fibryanto et al., 2020, Indonesia <sup>[30]</sup>	NM	S	M	NM	NM	Yes	M	Yes	Yes	7/9
Kamal et al., 2020, Egypt <sup>[31]</sup>	NM	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	6/9
Majithia et al., 2016, Malaysia <sup>[32]</sup>	NM	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	6/9
Ramadevi et al., 2020, India <sup>[33]</sup>	NM	MS <sup>†</sup>	NM	NM	M	Yes	M	Yes	Yes	7/9
Mohd Said et al., 2017, China <sup>[34]</sup>	NM	MS <sup>†</sup>	M	M	NM	Yes	M	Yes	Yes	6/9
Shen et al., 2016, Australia <sup>[11]</sup>	NM	MS <sup>†</sup>	M	M	M	Yes	M	Yes	Yes	7/9
Sleibi et al., 2018, UK <sup>[35]</sup>	M	MS <sup>†</sup>	M	M	NM	Yes	M	Yes	Yes	7/9
Shen et al., 2020, Australia <sup>[36]</sup>	NM	MS <sup>†</sup>	M	NM	M	Yes	M	Yes	Yes	7/9
Thakkar et al., 2017, India <sup>[37]</sup>	M	MS <sup>†</sup>	M	NM	M	Yes	M	Yes	Yes	8/9
Üstün and Aktören 2019, Istanbul <sup>[38]</sup>	M*	MS <sup>†</sup>	M	NM	NM	Yes	M	Yes	Yes	6/9
Varma et al., 2019, India <sup>[39]</sup>	M	S	NM	NM	NM	Yes	M	Yes	Yes	8/9

Unclear/moderate risk - Yellow, Low risk - Green. M: Mentioned; NM: Not mentioned; M\*: Mentioned but less samples used, MS<sup>†</sup>: Multiple sample from same specimen, S: Single sample from the specimen



**Table 5: Summary of findings of review related to surface microhardness, mineral loss/gain, ion release, and lesion depth**

Author	Summary of findings
<b>Surface microhardness</b>	
Babu <i>et al.</i> <sup>[27]</sup>	There was statistically significant difference between control and experimental windows (P<0.001). No significant difference was seen between CPP-ACPF varnish and fluoride group
Abufarwa <i>et al.</i> <sup>[24]</sup>	The fluoride group showed statistically significant harder enamel than the control at 20, 40, and 60 µm depths
Bakry and Abbassy <sup>[28]</sup>	Percent surface microhardness recovery for CPP-ACPF varnish group was significantly higher compared to control
Bapat <i>et al.</i> <sup>[29]</sup>	The CPP-ACPF Varnish group showed significantly high value of enamel surface microhardness as compared to pulp dent embrace group and control group
Kamal <i>et al.</i> <sup>[31]</sup>	No statistically significant difference was found between fluoride and CPP-ACPF
Majithia <i>et al.</i> <sup>[32]</sup>	Microhardness values were statistically significant from those of the control group. But no significant difference was seen between varnish groups
Mohd Said <i>et al.</i> <sup>[34]</sup>	Varnish groups post remineralization microhardness values and (% SHR) were statistically significant from those of the control group. No significant difference was reported between varnishes groups
Fibryanto <i>et al.</i> <sup>[30]</sup>	Day seven remineralization showed no significant difference in microhardness between CPP-ACP and 5% calcium fluoride group
<b>Mineral loss/gain and Ions release from varnish</b>	
Majithia <i>et al.</i> <sup>[32]</sup>	Varnish groups post remineralization Ca and P values, Ca/P ratio were statistically significant from those of the control group. No significant difference was seen between the different varnish groups
Mohd Said <i>et al.</i> <sup>[34]</sup>	Mean change in mineral loss in CPP-ACPF group was significantly higher when compared with the control group
Shen <i>et al.</i> <sup>[11]</sup>	CPP-ACPF Varnish released the highest levels of calcium, phosphate fluoride ions. Showed significantly lower reduction in mineral loss compared to fluoride alone varnishes
Sleibi <i>et al.</i> <sup>[35]</sup>	The mineral gain was superior in fluoride groups than CPP-ACPF and there was no significant difference
Shen <i>et al.</i> <sup>[36]</sup>	Mineral gain was significantly higher in CPP-ACPF varnish than fluoride group. Significantly higher levels of all ions (Ca, PO <sub>4</sub> , F) in the CPP-ACPF varnish group than fluoride varnish group
<b>Lesion depth</b>	
Attiguppe <i>et al.</i> <sup>[10]</sup>	Deminerlization inhibitory effect was higher in CPP-ACPF varnish grp and showed a high statistically significant difference between the groups
Girish Babu <i>et al.</i> <sup>[26]</sup>	There was statistically significant difference between control and experimental windows in both groups. No significant difference was seen between varnish groups
Abufarwa <i>et al.</i> <sup>[24]</sup>	The area of enamel deminerlization in the CPP-ACPF group was significantly smaller than control
Abufarwa <i>et al.</i> <sup>[25]</sup>	Deminerlization inhibitory effect was higher in CPP-ACPF varnish group than control

**Table 5: Contd...**

Author	Summary of findings
<b>Lesion depth</b>	
Bakry and Abbassy <sup>28</sup>	CPP-ACPF varnish groups recorded significant decrease in lesion depth than control group
Mohd Said <i>et al.</i> <sup>[34]</sup>	Significant difference in lesion depth postremineralization was seen between different varnishes and control. No difference was reported between the varnish groups
Shen <i>et al.</i> <sup>[11]</sup>	The CPP-ACPF varnish inhibited deminerlization significantly better than when compared with the Duraphat fluoride-alone control
Thakkar <i>et al.</i> <sup>[37]</sup>	Difference in the mean lesional depth of sections from CPP-ACPF varnish was significantly lower compared to control group after deminerlization cycle. Samples from CPP-ACPF showed significant remineralization effect when compared to control group
Üstün and Aktören <i>et al.</i> <sup>[38]</sup>	There was no significant difference between CPP-ACPF/NaF for all time intervals. Statistically significant differences were found for varnish groups when compared to the control group
Varma <i>et al.</i> <sup>[39]</sup>	CPP-ACPF varnish had significantly better remineralization effect than others
Shen <i>et al.</i> <sup>[36]</sup>	The CPP-ACP/F varnish promoted significantly greater remineralization than the fluoride-alone Duraphat varnish
Ramadevi <i>et al.</i> <sup>[33]</sup>	CPP-ACPF varnish had statistically significant reduction in the mean lesion depth compared to 5% sodium fluoride varnish alone

%SHR: Percent surface hardness recovery, F: fluoride, NaF: Sodium fluoride, Ca/P: Calcium/phosphorus, PO<sub>4</sub>: Phosphate, CPP-ACPF: Casein phosphopeptide - amorphous calcium phosphate fluoride

microhardness recovery, decrease in lesion depth, and mineral loss compared to paste groups.<sup>[28]</sup>

In the studies considered for review, fluoride varnishes contained a variety of combinations, which accounted for substantial clinical heterogeneity. CPP-ACPF varnish showed significantly higher deminerlization inhibitory effect and greater amount of fluoride release as compared to fluor protector for a period of 1 month.<sup>[10]</sup> An *in vitro* study compared calcium phosphate-containing varnishes such as enamel pro, Clinpro, and CPP-ACPF varnish with other fluoride varnishes. The results showed that only CPP-ACPF varnish significantly inhibited deminerlization than fluoride alone varnishes such as Duraphat and Profluorid.<sup>[11]</sup> Furthermore, CPP-ACPF varnish provided 130% greater inhibition when compared with the Duraphat fluoride-alone control.<sup>[11]</sup> The presence of the CPP-ACPF nanocomplexes and combination with fluoride would explain the superior ability of CPP-ACP varnish in inhibiting deminerlization.

Remineralization potential on artificial enamel caries-like lesions in permanent teeth was significantly better with CPP-ACPF varnish compared to 5%

*Contd...*

sodium fluoride varnish<sup>[33,36]</sup> and Clinpro varnish.<sup>[33]</sup> However, CPP-ACPF varnish did not achieve better remineralization of artificial enamel carious lesions when compared with Duraphat varnish.<sup>[34,38]</sup> In a clinical trial by Obradović *et al.*, CPP-ACPF varnish showed better remineralization of initial smooth surface caries lesions of primary teeth compared to conventional varnish with fluoride.<sup>[50]</sup> A systematic review and meta-analysis on randomized controlled trials by Tao *et al.* concluded that fluorides combined with CPP-ACP treatment produce significantly better remineralization of occlusal early caries lesions. For lesions on smooth surfaces, the meta-analysis showed no significant difference between using fluorides with CPP-ACP and using fluorides alone.<sup>[13]</sup> The CPP-ACPF are amorphous electroneutral nanocomplexes and their hydrodynamic radius allows rapid diffusion out of the varnish and is reported to enter the porosities of an enamel subsurface lesion through intraprismatic spaces favoring remineralization.<sup>[51]</sup>

Most varnishes in the market contain 5% NaF with an alcohol- and resin-based solution. The evaporation of the alcohol makes the system fast drying, and the resin functions to allow the fluoride to adhere to the teeth.<sup>[52]</sup> The most common resin additive is colophony, a resin derived from pine tree sap.<sup>[52]</sup> The main complaint about varnish, “stickiness,” and a noticeable film on the teeth is due to the carrier. The resin base in fluoride varnishes allows them to stick to tooth surfaces and stay for up to 24 h whereby fluoride is gradually released from the varnish and is taken up by the tooth enamel and dentin.<sup>[52]</sup> The strongly bound fluoride, incorporated onto the surface of the crystals of apatite, can reduce the solubility of the tooth mineral and hence inhibit demineralization due to acids generated by plaque bacteria.<sup>[53]</sup> Varnishes are preferred over other topical forms since they are user friendly and requires less handling and application time, When compared to gels, they are less likely to be ingested by young children. Varnishes are a successful strategy of prevention of caries in individuals with special requirements, such as those with developmental disabilities, because they stick to the tooth surface for longer lengths of time and requires less patient compliance.<sup>[54]</sup> A randomized trial by Huang *et al.* assessed the effectiveness of MI Paste Plus and PreviDent fluoride varnish for treatment of WSLs. The results showed no significant difference between two groups compared to a standard oral hygiene and toothpaste. This result can be attributed

to the presentation of material in the form of varnish and not in toothpaste form.<sup>[4,55]</sup>

A Cochrane review in 2016 suggested the superiority of resin-based fissure sealants over fluoride varnish applications for preventing occlusal caries in permanent molars, but the quality of evidence was low.<sup>[56]</sup> Fluoride varnish was shown to be less expensive than fissure sealant in a randomized clinical trial that used mobile dental clinics to target children with high caries risk. However, caries prevention was not significantly different when either strategy was used twice a year.<sup>[57]</sup> A cost-effectiveness analysis based on a clinical decision tree found the application of fluoride varnish as a cost-effective community strategy to prevent early childhood caries among rural children in nonfluoridated areas.<sup>[58]</sup> Cost-effectiveness of varnish against sealant was assessed over a period of 4 years in a school-based setting. Varnish appeared to be more cost-effective than sealants as the latter required expertise in application.<sup>[59]</sup> The application of varnish requires very little infrastructure, and it can be more readily applied in nontraditional settings (dental chair with illumination and fluid evacuation to maintain a dry field).<sup>[59]</sup> Even health-care providers with minimal training can also apply varnish.<sup>[59]</sup>

*In vitro* studies make possible the inexpensive and rapid yet sensitive assessment of any new inventions in a highly controlled environment representing a key component of product activity confirmation. However, major limitation is their inability to simulate the complex biological processes involved in caries. And, also the oral conditions that prevail in the mouth.<sup>[60]</sup> In in-situ and in -vitro investigations, quantitative measurements of mineral loss and lesion depth are regarded as standard primary outcomes for evaluating re and demineralization.<sup>[60]</sup> Surface microhardness is indirect technique which complements direct measures of mineral gain and loss.<sup>[60]</sup> There were very few studies which assessed mineral loss/gain in the present review.<sup>[11,34-36]</sup>

Lesion depth defines the magnitude of penetration and damage from acid caused by varnishes. Transverse microradiography and polarized light microscopy are desirable methods<sup>[61]</sup> for direct assessment of lesion depth which was followed in only a few studies. Some studies had a small sample size. There was heterogeneity in the method of outcome assessment for lesion depth and mineral loss/gain, which limited meta-analysis for these parameters. Future research

would benefit from a uniform method of assessment for mineral loss/gain and lesion depth using these products. Being cognizant about heterogeneity in reporting of methodology and evaluation, including the duration of follow-up and assessment, the evidence should be considered with caution. Future studies should be planned overcoming these limitations and dental association bodies should frame guidelines for reporting of efficacy of caries remineralizing agents in scientific studies which will help in review of articles and framing of clinical practice guidelines.

## CONCLUSION

CPP-ACPF varnish appears to be equally effective as other fluoride varnishes in remineralizing WSLs, but quality of evidence is low.

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