Amorphous Calcium Phosphate Based Tooth Remineralization Systems in Dentistry – A Systematic Review of in-Vitro Studies

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Abstract

The aim of this study was to review the role of amorphous calcium phosphate (ACP) and ACP-based products in remineralization of enamel and dentin.

An automated search was conducted for articles and data published on ACP-based remineralization systems. References of the selected articles were also searched manually for related articles. In the present study, the focus was placed on in- vitro studies on ACP-based remineralization systems published from 2011 to 2020.

Out of the 600 articles, 11 articles were found to be suitable for our systematic review. These articles were analysed and together proved that ACP is a promising non – fluoride-based remineralizing agent, which furthermore can be combined with organic molecules, polymers, proteins, and ions for enhancing its stability and its remineralizing effect. ACP readily dissolves in saliva and acts as a reservoir of calcium and phosphate ions which are taken up by demineralized tissues; induce remineralization and balance the pH of the oral cavity.

ACP is turning out to be more and more essential in dentistry. It remineralizes enamel and dentin better than crystalline calcium phosphates, and the new mineral deposit is similar in structure and composition to the dental tissues. It is considered that ACP will be put into use even more comprehensively soon due to the rapid growth of tissue engineering and applied material science. However, more long-term clinical studies are essential to compare the remineralization efficacy of ACP compared to other remineralizing systems.

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Introduction

Dental enamel is a highly rigid tissue which forms the outermost surface of teeth.[1] It is made up of 96 wt % of inorganic matter, which is hydroxyapatite crystals (HA) ($Ca_{10}(PO_4)_6(OH)_2$). These crystals are 50 nm in width and are arranged parallel to one another, which form 5µm wide enamel rods.[2] During a lifetime, human enamel is subjected to a wide range of physical stresses (i.e., mastication) and chemical stresses (e.g., acidic attacks). Enamel is also exposed to oral fluids such as saliva and plaque fluid, which influence the pH of the oral cavity.[3] Below the enamel is a layer of less mineralized tissue called dentin, made up of 50 wt.% H.A. crystals, 30 wt.% collagen fibers, and 20 wt.% is fluids called dentinal fluid.[4]

In dentin, H.A. crystals are distributed as intrafibrillar matrix, located inside the spaced gaps in the collagen fibrils, as well as extrafibrillar matrix, which is present in the interstices between the fibrils.[5] H.A. of enamel and dentin is under a dynamic equilibrium with the ions present in the oral cavity, which could lean toward H.A. dissolution and mineral loss (demineralization) or recrystallization and mineral gain (remineralization). The components influence this dynamic equilibrium in saliva,

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gingival crevicular fluid, and plaque fluid. For example, dental caries occurs when the pro-demineralization factors such as acids, produced by the fermentation of carbohydrates by cariogenic bacteria, and alteration in salivary pH overcome the protective remineralizing factors such as the presence in the saliva of Ca^{2+} and PO_4^{3-} ions, or antibacterial agents.[6] Luckily, demineralization can be reversed by remineralization, which is the deposition of Ca^{2+} and PO_4^{3-} ions of saliva into the demineralized enamel, thus increasing the overall mineral gain.[7] However, saliva alone often cannot effectively counteract demineralization when bacterial colonization increases or when saliva production decreases, such as in Sjogren's syndrome, chemotherapy, and radiotherapy. Therefore, there is a need for external tooth remineralizing agents to prevent demineralization and enhance remineralization.[8]

Tooth remineralization has been studied extensively, and a lot of research has been conducted to develop technologies and substances for inducing remineralization. The tooth remineralizing systems/materials can be classified into two categories:

1. Remineralization through fluoride-based systems
2. Remineralization through non – fluoride-based systems

Fluoride was and still is considered the gold standard for preventing dental caries. Fluoride ions primarily work by topical mechanisms, which comprise of:

A. Improvement of remineralization of demineralized dentin at the H.A. crystal surface.
B. Prevention of demineralization at the tooth surface by converting H.A. crystals into fluorapatite crystals.
C. Inactivation of bacterial enzymes.

At concentration levels as low as 1ppm, oral fluids such as saliva and plaque are supersaturated in fluoride compared to the mineral phase called fluorapatite; thus, even in low concentrations, they enhance mineral deposition on teeth averting demineralization and increasing remineralization. At high fluoride concentration, a reaction between the tooth minerals and available fluorides leads to calcium fluoride-like deposits that act as reservoirs for further supply of fluoride ions. This fluoride deposits in hydroxyapatite form fluorapatite, which increases the tooth's resistance to caries and dental erosion. [9]

A drastic decline in dental caries was observed. Thanks to the pervasive use of fluoride-containing dental care products.[10]However, there have been reports of the ill effects of excessive use of fluoride.[11] Dental fluorosis is one significant ill effect of fluoride, which is a progressive disruption of tooth enamel triggered by consecutive exposures to high fluoride levels throughout tooth development, causing the formation of enamel with low mineral content and high porosity.[12]

It has been observed that fluoride products more efficiently reduce smooth surface caries, and their limited effect is observed in reducing pit caries. This can be justified by facts that fluoride ions easily penetrate the plaque on smooth surfaces and kill the cariogenic microorganisms.[13] It was reported that there is plateauing in the caries experience despite increasing fluoride in toothpaste and dentifrices.[14] This can be explained by the fact that fluoride has little effect on the caries-causing factors, such as biofilm formation and sugar consumption. Fluoride's antibacterial activity, limited to doses below ten ppm in the oral cavity, does not affect bacterial metabolism.[15] Moreover, fluoride has recently been classified as a neurotoxin, which has raised safety concerns.[16]According to a report published by National Toxicology Program (NTP), fluoride ions significantly impaired learning and memory in experimental animals at a concentration of 2.5 to 4 mg/L in drinking water. (Table 1) Though there were many confounding factors affecting the outcomes of this report, it could be assumed that there is a probability of the possible incidence of neurodevelopmental effects in humans due to excessive fluoride ingestion.17

FLUORIDE RECOMMENDATION FOR BASED ON THE AGE OF THE CONSUMER			
AGE	FLUORIDE ION CONCENTRATION IN DRINKING WATER		
	<0.3 PPM	0.3-0.6 PPM	>0.6 PPM
≤6 months	NONE	NONE	NONE
6months-3 years	0.25MG/DAY	NONE	NONE
3-6 years	0.50 MG/DAY	0.25MG/DAY	NONE
6-16 years	1MG/DAY	0.50MG/DAY	NONE

1ppm= 1 mg/L, Ppm- Parts per million, Source- Reference 23

Table 1. Fluoride Dose Recommendation for Different Age Groups according to ADA.

Considering these issues, it can be said that an ideal tooth remineralizing agent is still needed. This agent should promote calcium and

phosphate deposition in the demineralized subsurface region of enamel and should be a more efficient remineralizing agent than the product with only fluoride.[18] As a result, various alternative non-fluoride-based remineralizing agents were developed and marketed in the last decade, such as calcium phosphate-based systems, polyphosphate systems, and naturally occurring plant products.

Amorphous calcium phosphate (ACP) is a volatile compound which crystalizes to octacalcium phosphate or H.A. crystals in water. But for the tooth remineralization, ACP is needed in the ionic form of calcium ions and phosphate so that the enamel rods can take up Ca^{+2} and PO_4^{-3} ions and strengthen the enamel. However, incorporating Ca^{2+} and PO_4^{3-} ions in H.A. crystals of enamel and dentin through other calcium phosphate-based remineralizing agents and their natures are not similar. In this regard, there is a high interest in amorphous calcium phosphate as a remineralizing agent, and its popularity has surged in the last 15 years. (Figure 1) The present review focuses on the use of ACP in remineralizing tooth enamel and dentine and the various modifications of ACP that have been developed to enhance its remineralization effect.

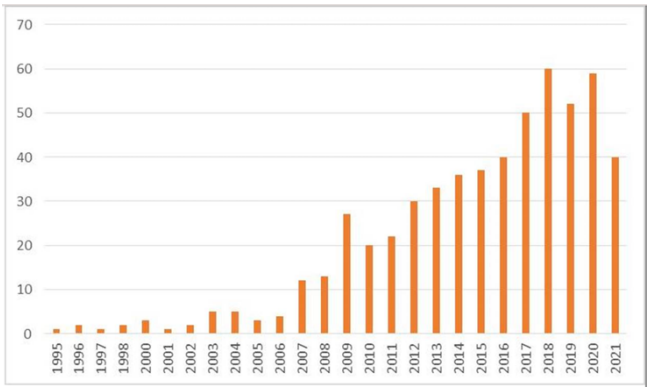

Figure 1. Articles published onremineralization of teeth using Amorphous Calcium Phosphate in PubMed from 1995 to 2021.

Materials and methods

Search Strategy

A systematic literature search was conducted in English and with time constraints, i.e., in-vitro studies on the role of ACP in the remineralization of tooth enamel and dentin published from 2010 to 2020 across the following electronic databases:

•PubMed: The national library of medicines online search interface for Medline and preMEDLINE (https://www.ncbi.nlm.nih.gov/Entrez/query.fcgi).

•Scopus: Scopus is Elsevier's abstract and citation database launched in 2004 (https://www.scopus.com/home.uri).

•Google India http://www.google.co.in.

Additional records were discovered by looking through the bibilography of the included studies. Medical Subject Headings (MeSH) and key words were formed in the course of the search. (Table -2).

Database	Search string	Hits	Selected article
Scopus	(Amorphous Calcium Phosphate) (Human Teeth) (surface remineralization) (remineralization) (in-vitro study)	44	2
Pub Med	(Amorphous Calcium Phosphate) (enamel rods) (surface remineralization) (dentin) (remineralization) (in-vitro study)	15	5
Google Scholar	(Amorphous Calcium Phosphate) (enamel rods) (dentin) (human teeth) (remineralization) (demineralization) (in-vitro study)	55	2
Additional records identified through other sources	(Amorphous Calcium Phosphate) (enamel rods) (dentinal tubules) (remineralization) (in-vitro study)	37	1

Table 2. Search Results

The search focused on systematic reviews (level Ia evidence) and experimental trials (evidence levels IIb). The Oxford Center for Evidence-Based Medicine (http://www.cebm.net/levels of evidence. asp) guidelines was used to categorize the level of evidence for articles.[19]

Selection Criteria

All citations were aggregated and uploaded to EndNote v.X9.3.3 (Clarivate Analytics, PA, USA) to eliminate duplicate articles. Two reviewers segregated all the titles and abstracts of the studies selected for full-text review. Two reviewers independently evaluated the full text of the reference chosen articles contrary to the proposed inclusion criteria. In the final systematic review, the search results were reported in detail and summarized in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.[20]

Inclusion Criteria

In- Vitro, prospective, and retrospective studies with blank control or placebo control and parallel-group design were integrated in the study with the following criteria:

1. Subjects- for in – vitro studies, extracted human teeth were used with no enamel defects, caries, or micro cracks.
2. Interventions-
   a. any remineralizing agent that contained ACP or a modified ACP
3. Control group-
   a. non- ACP remineralization agents applied
   b. blank (no treatment)
   c. negative (such as placebo treatment or deionized water)
   d. positive (other treatments such as fluoride toothpaste).
4. Outcomes- tests performed to assess the efficacy of remineralization (e.g., micro hardness test)

Exclusion Criteria

1. Studies with no quantitive outcomes but descriptive analysis
2. Irrelevant studies
3. Studies published in other languages
4. In vitro studies performed on non-human teeth
5. Systematic reviews, Randomized controlled trials, In vivo studies
6. Unpublished studies
7. Studies with no sample size mentioned
8. RCT studies

Quality Assessment

Risk of Bias in every selected study was using Cochrane Review Manager Version 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark). The risk of bias was classified as low or high risk of bias.

Data Extraction

Two authors obtained the data from the filtered studies in terms of year of publication, name of the author, study design, tissue examined, the type of ACP used for remineralization, the experimental groups in the study, a sample size of each group, tests conducted on the samples and outcomes.

Results

Results of the search

A total of 186 studies were at first collected using our retrieval search approach with the help of the reference manager End Note v.X9.3.3. As a result, 90 duplicate records were deleted from this set. After reading the titles and abstracts of these retrieved data, another 46 unrelated research were deleted. We could add one publication to our records through reference reading. Of the remaining 50 studies, ten were eventually chosen for the systematic review based on full-text screening—many of the studies were eliminated due to multiple exclusion criteria.

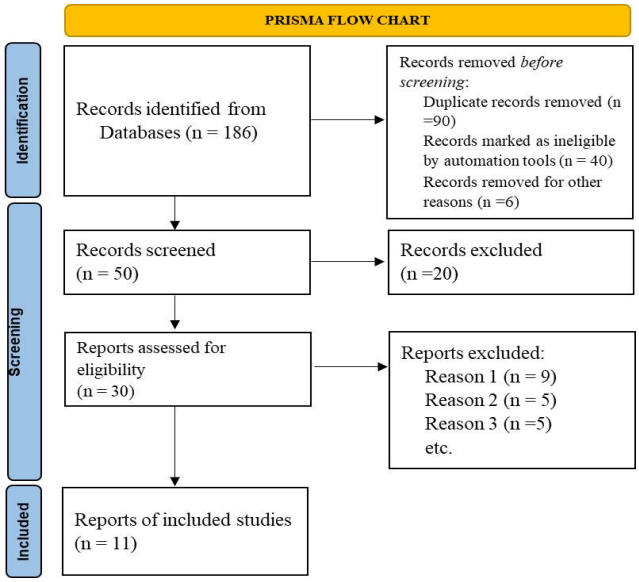

Figure 2. prisma flow chart.

Figure 2 depicts a flow chart of the studies screened, identified, evaluated for eligibility, included, and eliminated in this systematic review.

Characteristics of the selected studies

Table 3 gives more information about the features of the ten in-vitro studies—the years in which these studies were published range from 2010 to 2020. In the in vitro studies selected for the review, human primary and permanent teeth were tested, including incisors, premolars, and molars.

The sample sizes ranged from 5 to 123 in different studies. In addition, the effects of ACP-based remineralization of artificial dental caries were explored using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) to evaluate the average of the enamel rods, dentinal tubules, or surface roughness.

Discussion

ACP is the first calcium phosphate phase precipitates into a supersaturated solution of Ca^{+2} and PO_4^{-2} ions. It was discovered by Aaron Posner, who first studied its structure. Morphologically, it has a short-range periodic regularity[32] and is composed of random aggregates of spherical ionic clusters, called Posner clusters. In the presence of H_2O, ACP can readily convert into a crystalline phase, such as octacalcium phosphate or H.A. ACP has good bioactivity, biodegradability, and osteoconductivity, which makes it a material that is successfully used in orthopedics, dentistry, and medicine.[33]

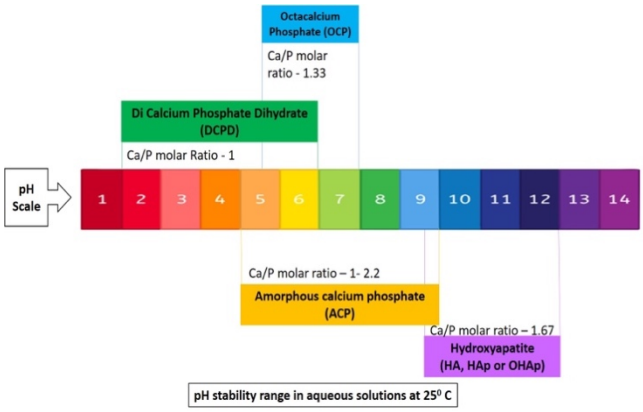

Figure 3. pH stability.

It is widely reported that when calcium and phosphate ions are mixed at high concentration, there is a spontaneous formation of a phase of non-crystalline calcium phosphate, i.e., ACP, which has a calcium-phosphate molar ratio (Ca/P) of 1.50. Subsequently, in physiological conditions, this non-crystalline phase converts into poorly crystalline apatite and then transforms into crystalline H.A. with a Ca/P molar ratio of 1.67. This slow conversion occurs by autocatalytic mechanism.[34] However, pH and temperature also have an important role in the crystallization of ACP and can induce the formation of other crystalline phases apart from H.A.[35] (Fig. 3). ACP plays a significant role as a predecessor in H.A. formation.[36] The initially obtained ACP transforms into a crystalline phase which gets deposited in the intrafibrillar gaps of collagen fibrils and acts as extrafibrillar particles that are the reservoirs of Ca^{+2} and PO_4^{-}3ions.[37] Numerous proteins like DMP1 were detected to be implicated in converting ACP to HAP and have been called biomineralization proteins.[38] MP1 induces the restructuring of amorphous calcium phosphate into an organized crystalline state, i.e., HAP.[39]

In the present review, we observed that ACP is a volatile compound. Compounds like casein-phosphopeptide, citrate, and xylitol have been used to stabilize it during storage.[40]As discussed below, various modifications have been made to stabilize ACP and enhance its remineralizing effect on teeth. (Table 4).

Form of ACP	Active ingredients in remineralizing agents	Commercial names
Unstabilized	amorphous calcium phosphate	Enamel Pro Varnish (5% NaF + ACP)
Stabilized	a. CaseinPhosphoPeptide-Amorphous Calcium Phosphate	i. Tooth Mousse crème ii. MI Paste iii. Tooth Mousse Plus crème (0.09% NaF + CPP-ACP) iv. MI Paste Plus (0.09% NaF + CPP-ACP)
	b. citrate-stabilized fluoride doped amorphous calcium phosphate	i. Biosmalto tooth mousse
	c. nanoparticles of amorphous calcium phosphate (NACP)	n.a.
	d.Chlorhexidine-loaded amorphous calcium phosphate nanoparticles	n.a.

Table 4. Various forms of ACP and their commercial names.

CITRATE-STABILIZED FLUORIDE-DOPED AMORPHOUS CALCIUM PHOSPHATE NANOPARTICLES

In a study by Iafisco et al., fluoride-doped ACP particles (FACP) were stabilized using citrate ions. The fluoride ions incorporated into FACP enhanced the anti-caries effect, induced remineralization of dental hard tissues, and reduced hypersensitivity by occluding the dentinal tubules.[31] Citrate stabilization of FACP nanoparticles prevented crystallization of FACP and maintained it in amorphous form for several years at room condition. Citrate also increased the specific surface area of FACP, leading to rapid release of F^- and Ca^{2+} and conversion into H.A. [31]

SILVER NANOPARTICLES – LOADED ACP MICROSPHERES

Silver has been reported to have significant antibacterial effectiveness.[41] According to a study published by Keskar et al., silver nanoparticles were loaded in ACP microspheres and incorporated into commercially available dental adhesives at 2 %, 5 %, and 10 % by wt. The samples were subjected to artificial cariogenic

conditions and examined for release of Ca^{2+}, $PO_4^{3-,}$ and Ag^{2+}. A sustained ion release in the aqueous medium brought the antibacterial and remineralization effects of silver nanoparticle-loaded ACP microspheres. [42]

CHLORHEXIDINE-LOADED ACP NANOPARTICLES

Demineralization of dentin is a phenomenon occurring in the pathophysiology of dental caries and treatment procedures in adhesive dentistry. An etchant is applied to uncover collagen fibrils and permit the monomer to permeate inside the dentin and create resin tags. However, the resin monomer fails to replace water completely inside the extrafibrillar and intrafibrillar regions of demineralized collagen matrix; thus, the resin cannot entirely infiltrate into the collagen framework.[43] Furthermore, the use of etchant during the procedure also stimulates the cysteine cathepsins and matrix metalloproteinases (MMPs), which cause collagen degradation.[43] These effects lead to failure in the formation of resin-dentin bond formation. To overcome this problem, compounds like chlorhexidine have been employed in combination with ACP to protect collagen, inhibit MMPs and enhance biomimetic remineralization.[43] According to a study published by Cai et al., chlorhexidine-loaded ACP nanoparticles were synthesized, and their role in the degradation and mineralization of dentin collagen fibrils was observed. It was reported that chlorhexidine-loaded ACP nanoparticles not only inhibited the degradation of dentinal collagen by the persistent release of chlorhexidine but also induced remineralization of dentinal collagen fibrils. The particles of ACP measured 40 nm, which can penetrate the 40nm gaps and later crystallize into apatite while releasing chlorhexidine. The penetration into the gaps allowed intrafibrillar mineralization of dentine, followed by interfibrillar mineralization. [44]

CASEIN PHOSPHOPEPTIDE – ACP

ACP is a volatile compound. Compounds like casein phosphopeptide, citrate, and xylitol have been used to stabilize it during storage.[45] Casein phosphopeptide (CPP) is a compound that stabilizes ACP and maintains the calcium and phosphate ions in the bioavailable form at a high concentration at the surface of demineralized enamel by binding to plaque and facilitating deeper infusion of these ions into the demineralized tissue.[46] Sir Eric Reynolds discovered it in Australia at the school of Dental Science at the University of Melbourne. [47]

Gjorgievska et al. conducted a study to test the ability of two kinds of toothpaste, one based on Recalden (CPP- ACP) and the other on NovaMin (Calcium-sodium-phosphosilicate), to remineralize enamel. It was found that toothpaste containing Recalden, particularly NovaMin, can remineralize enamel.[21] (Table-3)

In another study, Hegde et al. showed that in vitro, CPP-ACP paste could considerably remineralize artificial enamel subsurface lesions. Using energy dispersive X-ray analysis to quantify changes in mineral content during demineralization and in vitro remineralization processes is very efficient.[22] (Table 3)

In a similar study conducted by Elkassas et al., the effects of different calcium phosphate and fluoride administration strategies on enamel remineralization were compared on 115 extracted human teeth. Compared to the control group, remineralizing treatments comprising various calcium-phosphate formulae and fluoride had a higher remineralization potential. ClinproTM varnish had the most increased remineralization tendency and the best acid resistance.[23] (Table-3)

In an in vitro study by Memapour et al., the remineralization potential of CPP-ACP, functionalized tri-calcium phosphate, 5% sodium fluoride varnish, and 500 ppm fluoridated toothpaste were tested on extracted and artificially demineralized primary molars. It was observed that CPP-ACP had better potential to remineralize the eroded primary teeth than the other remineralizing agents.[24]

In another study, Thakkar et al. examined the amount of demineralization inhibition and enhancement of remineralization of enamel in permanent molars with and without the administration of three remineralizing agents. It was observed that CPP-ACPF varnish caused substantial prevention of demineralization. All three medications remineralized previously demineralized lesions significantly. Nevertheless, CPP-ACPF varnish demonstrated significant remineralization, followed by CPP-ACPF paste and CPP-ACP paste.[26]

Several *in situ* studies have also been conducted on the remineralization effect of CPP-ACP. In a survey piloted by Shen *et al.*, human enamel samples with subsurface carious lesions were prepared and implanted into intra-oral

appliances worn by the study volunteers. The remineralization efficacy of tricalcium phosphate with 950 ppm F, CPP-ACP, CPP-ACPF (casein phosphopeptide- ACP with fluoride), 1000 ppm F, and 5000 ppm F were compared. Each volunteer rinsed the slurry of each product for 60 s, four times a day for ten days. Ions levels were measured post rinsing using ion chromatography, and the mineral content was analyzed using transverse microradiography. It was observed that CPP-ACP and CPP-ACPF achieved the highest level of mineralization compared to tricalcium phosphate. [48]

According to a systematic review of Imani et al. on 13 randomized controlled trials done using CPP-ACP, both CPP-ACP and CPP- ACPF exhibited remineralization of white spot lesions, thus confirming that the prevalence and incidence of dental caries can be reduced by regular use of CPP-ACP and CPP-ACPF.[49]

CARBOXYMETHYL CHITOSAN – ACP

Complex carbohydrates that stabilize ACP are a potential route for developing new biomimetic-remineralizing agents. Carboxy

supersaturated pool for calcium and phosphate ions from ACP that partially remineralized the demineralized dentin along with preserving the dentinal tissue. Thus CMC-ACP showed promising effects of remineralization.[53]

Conclusions

ACP is a significant metastable product related to apatite precipitation. Numerous ions, small organic molecules, and proteins were proved to have the ability to enhance the stability of ACP. ACP has gradually become more critical in dentistry due to its excellent mechanical properties and remineralization potential

The ACP-based remineralization systems have proven to be a necessary adjunctive therapy to fluoride therapy for remineralization and in the treatment of carious lesions and white spot lesions (WSLs). In addition, biomimetic methods used for stabilizing calcium, phosphate and fluoride ions in fluoridated ACP and applying these ions to non-cavitated carious lesions for measured remineralization have shown to be beneficial for the non-invasive treatment of dental caries.

Methyl chitosan (CMC), derived from chitosan functionalized with carboxyl groups, is one such compound that can retard the crystallization of ACP.[50]

CMC is a biocompatible, biodegradable, non-toxic, antibacterial, and water-soluble compound that can probably facilitate the formation of ACP nano precursors in solution by chelation.[51] In a study by Santoso *et al.*, CMC-ACP was prepared and intended to be used in bio-remineralization of dentin in molars with deep caries. An in vitro analysis using micro-CT was done. It was reported that CMC-ACP might induce dentin remineralization due to an increase in the grey level values in the radiograph.[52]

In another comparable study it was reported by Chen et al., that scaffolds of CMC/ACP nanocomplexes were used to remineralize demineralized dentine in an in-vitro tooth model of deep caries. It was reported that CMC stabilized ACP forming CMC/ACP nanocomplexes and prevented the crystal growth from growing into critical size. CMC also acted as a

It is considered that Amorphous Calcium Phosphate will be utilized more comprehensively in the upcoming days because of the rapid development of tissue engineering procedures and applied material science.

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Declaration of Interest

The authors report no conflict of interest.

Sl. No	Year	Author	Study Design	Tissue examined	Type of ACP used for remineralization	Sample size	Experimental Groups	Tests conducted	Conclusion
1	2010	Gjorgievska et al[21]	In vitro Pilot study	Human Enamel from permanent molars	i. CPP-ACP ii. Novamin	15 human permanent molars	i. Group I- Control group ii. Group II- CPP-ACP iii. Group III- Novamin	SEM-EDX	Tooth pastes containing Bio-active glass particles and cpp-acp showed enhanced potential to remineralize enamel or dentin
2	2012	Hegde et al[22]	In vitro study	Human Enamel from permanent teeth	i. CPP-ACP	90 human permanent molars	i. Group I- control group ii. Group II- CPP-ACP based remineralization for 7 days iii. Group III- CPP-ACP based remineralization for 14 days iv. Group IV- CPP-ACP based remineralization for 21 days v. Group V- CPP-ACP based remineralization for 28 days vi.Group VI- CPP-ACP based remineralization for 35 days	SEM-EDX	i. CPP-ACP paste could significantly remineralize the artificial enamel subsurface lesions in vitro: the remineralizing rates increasing with the time for which the samples were kept in the remineralizing paste.
3	2014	Elkassas et al[23]	In- vitro study	Human Enamel from permanent teeth	i.amorphous calcium phosphate containing 1100 ppm fluoride (ACPF) iii. CPP-ACPF	115 human molars	i. Group I- Control ii. Group II- fTCP iii. Group III- ACPF iv. Group IV- CPP-ACPF v. Group V- RMGI	i. Viker's microhardness test ii. SEM iii. Surface Roughness Test	i. Calcium phosphate based remineralizing agents provide superior remineralization effects and greater resistance to acid softening ii. functionalized tri-calcium phosphate containing 22,600 ppm fluoride (fTCP) proved to have the highest remineralization tendency
4.	2015	Memarpour et al[24]	In- vitro study	Human enamel from primary teeth	i. CPP- ACP	90 human primary canine	i. Group I- Control ii. Group II- Fluoride varnish iii. Group III- Fluoridated Toothpaste iv. Group IV- CPP-ACP v. Group V- fTCP varnish	i. Microhardness test ii. AFM	AFM scans revealed that fTCP treatment reduced enamel roughness the most, followed by CPP-ACP, toothpaste, and fluoride varnish. CPP-ACP cream outperformed fluoride toothpaste, fluoride varnish, and fTCP varnish in remineralizing enamel.
5	2016	Tuloglu et al[25]	In vitro study	Human enamel from primary teeth	i. MI varnish + 1–5 % CPP-ACP ii. Clinpro White iii. Duraphat	40 primary incisors and 40 primary molars	i. Group I- Control ii. Group II- MI varnish (1–8 %) sodium fluoride and 1–5 % CPP-ACP) iii. Group III- Clinpro White (1–5 % sodium fluoride and <5 % modified tricalcium phosphate) iv. Group IV- Duraphat (<5 % sodium fluoride)	i. Vicker's microhardness test ii. Lesion depth using polarized light microscope iii. SEM	i. fluoride varnish containing CPP-ACP was more effective in increasing the acid resistance of primary enamel than other fluoride varnishes
6	2017	Thakkar et al[26]	In vitro study	Human Enamel from permanent teeth	i. CPP-ACP paste ii. CPP-ACPF paste	40 human permanent molars	i. Group I- CPP-ACP paste ii. Group II- CPP-ACPF paste iii. Group III- 5% NaF varnish+ CPP-ACP varnish iv.Group IV- negative control	Steriomicroscope	5% NaF varnish+ CPP-ACP varnish significantly prevented demineralization followed by CPP-ACPF paste and CPP-ACP respectively.
7	2017	Xiao et al[27]	In vitro study	Human enamel	i.nanocomplexes of CMC/ACP ii.Chimaeric-peptide-guided CMC/ACP	10 human third molars	i. Group I- anocomplexes of CMC/ACP ii. Group II- Chimaeric-peptide-guided CMC/ACP	i. SEM ii. TEM iii. XRD iv. Confocal laser scanning microscopy	This study shows that a combination of CMC/ACP nanocomplexes, NaClO, and a chimaeric peptide that resembles amelogenin is a successful method for enamel remineralization.
8	2018	Lafisco et al[28]	In vitro study	Human enamel and dentin from permanent molars	Fluoride -doped ACP	5 human permanent molars	i. positive control group ii. ACP group iii. F- ACP group	i. TEM ii. SEM	F-ACP showed good ability to occlude the dentinal tubules and remineralize the demineralized dentin.
9	2019	Thierens et al[29]	In vitro study	Human Enamel from permanent teeth	1. CPP-ACP paste 2. CPP-ACPF paste	123 premolars (246 specimen)	i. Group I- CPP- ACP ii. Group II- CPP- ACPF iii. Group III- CONTROL GROUP A (brushed with fluoridated toothpaste) iv. Group IV- CONRTOL GROUP B	Transverse Micro Radiography	I. mineral content significantly increased for CPP- ACP group after 6 weeks. ii. Long term use of CPP-ACP and CPP-ACPF with conventional toothpaste remineralizes the initial carious lesion.
10	2019	Varma et al[30]	In- vitro study	Human enamel from permanent teeth	MI Varnish with CPP-ACP	30 Human premolars	i. Group I—MI varnish with CPP–ACP ii. Group II—Clinpro XT varnish with durable fluoride-releasing coating iii. Group III—Control group	Diagnodent	i. MI varnish containing CPP–ACP had the highest release of fluoride as compared to the Clinpro fluoride releasing varnish.
11	2019	Aras et al[31]	In- vitro study	Human enamel from permanent teeth	i. CPP- ACPF ii. NovaMin+ iii. Remin Pro	50 Human third molars	i. group I- Positive control ii. group II- Negative control iii. Group III- CPP-ACPF iv. Group IV- NovaMin+ v. Group V- ReminPro	i. Vicker's Microhardness test ii. ICP- AES	CPP-ACPF and Novamin+ exhibited better surface hardness.

Table 3. Studies conducted on ACP for bio-remineralization of enamel.
* References – 21-31
Abbreviations- CPP-ACP*- Casein phosphopeptide-amorphous calcium phosphate.
SEM-EDX*- Scanning Electron Microscope- Energy.

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