Enhanced Remineralisation of Tooth Enamel Using Casein Phosphopeptide-Amorphous Calcium Phosphate Complex: A Review

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The protective effects of milk and milk products against dental caries, due to micellar casein or caseinopeptide derivatives, have been demonstrated in various animal and human in situ studies. Among all the remineralising agents, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) technology has shown the most promising scientific evidence to support its use in prevention and reversal of carious lesions. CPP-ACP complex acts as a calcium phosphate reservoir and buffer the activities of free calcium and phosphate ions in the plaque. Calcium phosphate stabilized by CPP produces a metastable solution supersaturated with respect to the amorphous and crystalline calcium phosphate phases, thereby depressing enamel demineralisation and enhancing mineralisation. CPP-ACP provides new avenue for the remineralisation of noncavitated caries lesions. The objective of this article was to review the clinical trials of CPP-ACP complex and highlight its evidence based applications in dentistry.

Keywords: remineralisation, casein derivatives, casein phosphopeptide-amorphous calcium phosphate

Introduction

Recent scientific advances in restorative materials, techniques and better understanding of the etiology, pathogenicity and prevention of caries, have led to more efficient management of oral health. Dental caries is a pathological condition arising from an imbalance in the physiological process of remineralisation/demineralisation of the tooth structure and results in the formation of a subsurface lesion [1]. At an early stage, the caries lesion is reversible via remineralisation process involving the diffusion of calcium and phosphate ions into the subsurface lesion, to restore the lost tooth structure. Due to the presence of calcium and phosphate ions in supersaturated state, the whole human saliva has the potential to remineralise demineralised crystals of tooth structure, while preventing surface deposition in the form of calculus [2]. However, net remineralisation produced by saliva is small and a slow process, with a tendency for the mineral gain to be in the surface layer of the lesion due to the low ion concentration gradient from saliva into the lesion [3]. Thus, if the pH challenge overcome the physiological remineralisation process, a therapeutic approach using the new remineralisation system is necessary to achieve effective lesion regression.
Enamel Remineralising Systems

Fluoride has been known as a remineralising agent that interacts with the oral fluids on the interface of enamel and subsurface regions of teeth, and combines with the calcium and phosphate ions to form fluorapatite crystals [4]. Fluoride is the cornerstone of the non-invasive management of non-cavitated caries lesions, but its ability to promote net remineralisation is limited by the availability of calcium and phosphate ions in the saliva or dental plaque [5]. Various studies have shown that enamel remineralisation in situ and the retention of fluoride in plaque are dependent on the availability of calcium ions [5-8]. Hence, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for fluoride retention and net enamel remineralisation to occur, and this is highly exacerbated under hypo-salivation conditions [5].

Moreover, the topical fluoride applications (particularly high concentrations) promote mainly the surface remineralisation of enamel [9-12]. However, remineralising agents should supply stabilized bioavailable calcium, phosphate and fluoride ions that favor the subsurface remineralisation rather than merely promoting the surface remineralisation. Also, this element can cause fluorosis, and is toxic if administered in high dosages [13,14].

With the increasing focus on early detection and non-invasive management of caries, researchers have been testing new methods to enhance the remineralisation of enamel. The problem with applying crystalline calcium phosphate remineralising materials to promote enamel remineralisation in the oral cavity is the poor solubility of the calcium phosphate phases, such that the calcium and phosphate ions are unavailable for remineralisation at normal pH range of saliva [15]. Furthermore, localization of significant quantities of solid calcium phosphate phases at the tooth surface is problematic [16].

The unstabilized amorphous calcium phosphate (ACP) systems deliver calcium ions with phosphate ions that result in immediate precipitation of ACP or, in the presence of fluoride ions, amorphous calcium fluoride phosphate (ACFP). In the intra-oral environment, these phases (ACP and ACFP) are potentially very unstable and may rapidly transform into a more thermodynamically stable, crystalline phase (e.g., hydroxyapatite [HA] and fluorhydroxyapatite) [17]. Although some of the published papers suggest that the unstabilized ACP/ACFP technology may have efficacy in preventing caries progression, some authors have expressed concern with the unstabilized nature of the product that forms intra-ora-
pose a risk if the patient ingests a significant amount of fluoride [50].

**Mechanism of Action of CCP-ACP**

1. **Buffering action by the calcium phosphate reservoir**

Reynolds [44] suggested that the mechanism of anticariogenicity for CPP-ACP is that it substantially localizes the calcium and phosphate ions in the plaque, which provides a reservoir of soluble calcium phosphate ions on the tooth surface. When the acidogenic bacteria present in the dental biofilm metabolize dietary sucrose, the pH rapidly decreases below the resting pH of 7.0. Once this acid decreases the pH surrounding the teeth past a critical level (pH=5.5), it has the potential to diffuse into enamel and dissolve calcium phosphate mineral [51]. Under these acidic conditions, the CPP-bound ACP breakdowns and dissociate to release calcium and phosphate ions. This mechanism is ideal for the prevention of enamel demineralization as there appears to be an inverse association between plaque calcium and phosphate levels and measured caries experience [43,45,49].

Recently, it has been shown, with confocal laser microscopy and fluorescently labeled anti-CPP antibodies, that CPP was present inside a CPP-ACP remineralised enamel subsurface lesion [18]. Once present in the enamel subsurface lesion, the CPP-ACP release the weakly bound calcium and phosphate ions [32,52,53] which would then deposit into crystal voids. Hence, the CPPs have a role in regulating the anisotropic crystal growth and also inhibiting crystal demineralisation in the enamel subsurface lesions [54]. In addition to it, the increased calcium phosphate in the plaque buffers free calcium and phosphate ion activities and maintains a state of super-saturation of the ions in close approximation with the tooth, thus enhancing the enamel remineralisation [37].

Numerous in vitro studies have highlighted that CPP has the potential to stabilize calcium phosphate in solution by forming colloidal CPP ACP complexes [32,43]. The CPP contains a cluster of phosphoseryl residues –Ser(P)–Ser(P)–Ser(P)–Glu-Glu, which stabilize calcium and phosphate ions under neutral and alkaline conditions forming metastable solutions, thereby preventing their growth to the critical size required for nucleation and precipitation [43].

2. **Inhibition of streptococci adhesion to tooth surface**

Schüpbach et al. [19] proposed that the CPP-ACP inhibits the adhesion of cariogenic streptococci to tooth surface, resulting in the formation of a less cariogenic plaque. The CPP-ACP nano-complexes have also been demonstrated to bind on to the adsorbed macromolecules on the tooth surface, components of the intercellular plaque matrix and the surface of bacterial cells [14]. The method of binding CPP-ACP into plaque has been hypothesized to be due to calcium cross-linking [14,16,55] and/or hydrophobic and hydrogen-bond-mediated interactions [16]. Rose [14] demonstrated that CPP-ACP competes with calcium for plaque calcium binding sites and thus reduce the degree of calcium binding between the pellicle and adhering cells and between the cells themselves. CPP-ACP incorporates into the pellicle and plaque and results in an ecological alteration of the bacterial population, which, together with the remineralising capacity of the CPP-ACP, modifies the plaque’s cariogenic potential, leading to the formation of a less cariogenic plaque. Rose [14] also proposed that CPP-ACP could have a bactericidal or bacteriostatic effect by maintenance of high extracellular free calcium concentrations.

In a study by Nyvad and Fejerskov [56], basing their study on earlier work by Weiss and Bibby [24] and Pearce and Bibby [23], used transmission and scanning electron microscopy to show that milk of various fat contents could substantially modify the structure of the pellicle formed in vivo. The pellicle formed was not a uniform protein layer, but rather had a distinct globular structure. They suggested that caseinglycomacropeptide and CPP adsorb to the surface of the pellicle and mask receptors on salivary molecules for these streptococci [56]. Rahiotis et al.
and fluoride, as found in Tooth Mousse Plus™ (MI Paste [59] described a synergistic effect of CPP-ACP those animals receiving either CPP-ACP or fluoride alone [31]. 500 ppm fluoride had significantly lower caries activity than 2% CPP-ACP nanocomplexes plus 1,100 ppm F (CPP-ACPF) has been shown to be superior (2.6 times) to a dentifrice containing only 1,100 ppm F in remineralisation of enamel subsurface lesions in situ with the 950 ppm fluoridated paste groups. In a study conducted by Cochrane et al. [34], concluded that remineralisation of the mineral that was more resistant to acid challenge [5]. The CPP-ACP nanocomplexes-plus-fluoride dentifrices resulted in significantly greater incorporation of fluoride into the subsurface enamel as fluorapatite. In a randomized controlled mouth rinse trial, a rinse containing 2.0% CPP-ACP nanocomplexes plus 450 ppm fluoride significantly increased supra gingival plaque fluoride ion content to 33.0±17.6 nmol F/mg dry weight (wt) of plaque when compared with 14.4±6.7 nmol F/mg dry wt of plaque attained by the use of a rinse containing the equivalent concentration of fluoride ions [5].

In a clinical trial, the animals receiving 0.5% CPP-ACP plus 500 ppm fluoride had significantly lower caries activity than those animals receiving either CPP-ACP or fluoride alone [31]. Sakaguchi et al. [59] described a synergistic effect of CPP-ACP and fluoride, as found in Tooth Mousse Plus™ (MI Paste Plus™), in remineralising subsurface enamel lesions in bovine teeth. Tooth Mousse Plus™ was compared with Tooth Mousse™, a placebo containing no CPP-ACP or fluoride, and a paste containing 950 ppm fluoride. The remineralisation potential of Tooth Mousse Plus™ (Tooth Mousse™ with 900 ppm fluoride) was greater than the additive effect of the Tooth Mousse™ and the 950 ppm fluoridated paste groups. A dentifrice formulation containing 2% CPP-ACP nanocomplexes plus 1,100 ppm F (CPP-ACPF) has been shown to be superior (2.6 times) to a dentifrice containing only 1,100 ppm F in remineralisation of enamel subsurface lesions in situ with mineral that was more resistant to acid challenge [5]. The CPP-ACP nanocomplexes-plus-fluoride dentifrices resulted in significantly greater incorporation of fluoride into the subsurface enamel as fluorapatite. In a randomized controlled mouth rinse trial, a rinse containing 2.0% CPP-ACP nanocomplexes plus 450 ppm fluoride significantly increased supra gingival plaque fluoride ion content to 33.0±17.6 nmol F/mg dry weight (wt) of plaque when compared with 14.4±6.7 nmol F/mg dry wt of plaque attained by the use of a rinse containing the equivalent concentration of fluoride ions [5].

In a study conducted by Papas et al. [60], to test the efficacy of ACFP-forming dentifrice in high-caries-risk patients, who underwent head and neck irradiation, the dentifrice forming ACFP was found to be superior in lowering the root caries incidence compared to the dentifrice having only fluoride.

Synergistic Effects of Fluoride and CPP-ACP

The additive anticariogenic effect of CPP-ACP and fluoride may be due to the localization of ACFP at the tooth surface which, in effect, would co-localize calcium, phosphate, and fluoride ions [43]. Electron microprobe analysis has shown that the mineral formed in the enamel lesion is consistent with HA, when CPP-ACP is provided with a low background of fluoride, and when fluoride is present, the mineral is consistent with fluorapatite or fluorhydroxyapatite [34]. The use of CPP-ACP alone, or in conjunction with fluoride, also reduces the amount of fluoride needed and thus reduces the amount of fluorosis [43].

In a study by Cochrane et al. [34], concluded that remineralisation of the subsurface lesions was observed at all pH values tested, with a maximum at pH 5.5. This study showed that CPP stabilizes high concentrations of calcium, phosphate and fluoride ions at all pH values (7.0-4.5). CPP-ACFP solutions produced greater remineralisation than the CPP-ACP solutions at pH 5.5 and below. The mineral formed in the subsurface lesions was consistent with HA and fluorapatite for remineralisation with CPP-ACP and CPP-ACFP, respectively.

The other reason for the additive effect of CPP-ACP and fluoride is that the plaque enzymes, such as phosphatases and peptidases partially degrade CPP-based products, consequently increasing pH due to the production of ammonia. Adding fluoride to CPP limits phosphatase action by extending the action of molecular complexes [58].

In a clinical trial, the animals receiving 0.5% CPP-ACP plus 500 ppm fluoride had significantly lower caries activity than those animals receiving either CPP-ACP or fluoride alone [31]. Sakaguchi et al. [59] described a synergistic effect of CPP-ACP and fluoride, as found in Tooth Mousse Plus™ (MI Paste Plus™), in remineralising subsurface enamel lesions in bovine teeth. Tooth Mousse Plus™ was compared with Tooth Mousse™, a placebo containing no CPP-ACP or fluoride, and a paste containing 950 ppm fluoride. The remineralisation potential of Tooth Mousse Plus™ (Tooth Mousse™ with 900 ppm fluoride) was greater than the additive effect of the Tooth Mousse™ and the 950 ppm fluoridated paste groups.

Scientific Evidence for the Remineralisation Potential of CPP-ACP

There are numerous scientific studies highlighting the promotion of remineralisation of enamel subsurface lesions by CPP-ACP and CPP-ACFP. The mineral gained by the enamel subsurface lesions during in situ treatment with CPP-ACP has been acid-challenged to determine its relative solubility [5,61,62]. These results of these studies highlighted that the CPP-ACP produced mineral was more acid-resistant than the non-CPP-ACP-treated lesions. This is due to the production of a more stable mineral phase (e.g., HA or fluorapatite, as shown by electron microprobe analysis) that has a lower solubility than a calcium-deficient carbonated apatite of normal tooth enamel [62].

In a study by Morgan et al. [48], conducted a randomized, controlled caries clinical trial in 2,720 schoolchildren for a period of 24-month, to assess the impact of CPP-ACP in sugar-free gum relative to a control sugar-free gum. Participants were instructed to chew their assigned gum for 10 min three times per day. Standardized digital radiographs were taken at baseline and at the completion of the trial. The results showed that the CPP-ACP gum significantly slowed caries progression up to 18% and enhanced regression of baseline carious lesions up to 53% compared with the control sugar-free gum. Caruana et al. [63] performed a crossover study in which the plaque pH was measured on 15 subjects with and without prior application of the paste and it was observed that prior application of a CPP-ACP containing paste reduced the fall in plaque pH following a sucrose challenge.

The reduction of caries activity by CPP-ACP is dose-dependent [31,45,49,62]. Reynolds et al. [31] conducted a study in the specific-pathogen-free rats that were orally infected with...
Streptococcus sobrinus bacterium. CPP-ACP solutions applied twice daily, showed significant reduced caries activity, with 0.1% weight/volume (w/v) CPP-ACP producing a 14% reduction, and 1.0% w/v CPP-ACP producing a 55% reduction on smooth surfaces and 0.1% and 1.0% w/v CPP-ACP, respectively, produced a 15% and 46% reduction in fissure caries activity relative to the distilled water control.

Shen et al. [45] evaluated the effect of incorporating CPP-ACP into sugar-free gum on enamel remineralisation. The addition of CPP-ACP to either the sorbitol or xylitol-based gums at 10.0, 18.8, or 56.4 mg produced a significant increase in enamel remineralisation, with a 63%, 102%, and 152% average increase, respectively, relative to the sugar-free gum not containing CPP-ACP. Cai et al. [49] found that the used of sugar-free lozenges containing CPP-ACP significantly increased remineralisation of enamel subsurface lesions in situ, with 18.8 and 56.4 mg of CPP-ACP increasing remineralisation by 78 and 176% respectively. Manton et al. [64] observed significant greater remineralisation with the gum containing CPP (Trident White [18.4%]) than gum without CPP (Orbit [8.9%] and Orbit Professional [10.5%]).

The CPP-stabilised calcium phosphate solutions can remineralise enamel subsurface lesions at rates of 1.5 to 3.9×10^-8 mol HA m^-2 s^-1 [43]. The CCP can stabilize over 100 times more calcium phosphate than is normally possible in aqueous solution at neutral and alkaline pH before spontaneous precipitation [65]. ACP, crystalline phases dicalcium phosphate dehydrate, and octacalcium phosphate have been suggested as the intermediate structures in the formation of HA, depending on pH and degree of saturation. Assuming the deposited mineral in the remineralised lesions to be predominantly HA, the maximal average rate of remineralisation was 3.9±0.8×10^-8 moles HA/m² for the ten-day period. This value is equivalent to the maximal rate of remineralisation of enamel subsurface lesions obtained by de Rooij and Nancollas [66] using a constant-composition procedure. Another in vitro study demonstrated that after a ten-day period, 1.0% CPP-calcium phosphate (pH 7.0) solution promoted a 63.9%+20.1% of remineralisation of enamel subsurface lesion when compared to solutions with lower concentrations of CPP-stabilized free calcium and phosphate ions [43].

**CPP-ACP Complex-Evidence Based Clinical Applications**

The various recommended professional applications of CCP-ACP complex are white spot lesions prevention/reversion, topical use following professional tooth cleaning and root smoothing, after application of topical fluoride, bleaching and in patients suffering from erosion, caries, dentin hypersensitivity and conditions arising from xerostomia.

1. **Prevention of erosive/abrasive wear**

Erosive tooth wear has become a focus of attention for the dental researchers and practitioners. During the last decade, the use of CPP-ACP to enhance the remineralisation of carious lesions and reduce the erosive potential of acidic drinks has been reported [45,46]. Ramalingam et al. [46] immersed human enamel specimens in an erosive sports drink (Powerade [World of Coca-Cola, Atlanta, GA, USA] alone [0.063%, 0.09%, 0.125%, and 0.25%] and double deionized water as the placebo). Scanning electron microscopic examination of the specimens showed that the erosive lesions that developed in specimens immersed in Powerade, were eliminated with the addition of CPP-ACP at all concentrations except 0.063%. It was concluded that adding CPP-ACP to the sports drinks, soft drinks and other frequently consumed acid products significantly reduce the beverages erosivity without affecting the product’s taste.

It has been suggested that the erosion inhibiting potential of CPP-ACP probably involves remineralisation by deposition of mineral into the porous zone of the eroded enamel [67-70]. Furthermore, studies by Ranjitkar et al. [68-70] showed statistically significant protective effects of CPP-ACP containing cream against erosive/abrasive tooth wear. This effect, however, was not shown in other studies dealing with CPP-ACP containing products [71,72]. Their studies elicited erosion using solutions with pH-values below the pH measured in the oral cavity during reflux events. During reflux events, intra-oral pH remains above 5.5 most of the time. Thus information is lacking as to whether the use of CPP-ACP is able to minimize erosively induced hard tissue loss in patients suffering from gastro-oesophageal reflux disease.

2. **Prevention and reversion of white spot lesions**

Many a times, the orthodontic treatment is tarnished by the appearance of white spot lesions on the facial surface of teeth, after removing the fixed appliances. White spot lesion is an area of demineralised enamel that usually develops because of prolonged plaque accumulation. If these early lesions are left untreated, further decalcification may lead to development of cavitations, requiring tooth reduction or permanent restoration [73]. Prevention of the white, opaque areas throughout orthodontic treatment is essential to providing the patient with the most esthetic outcome. Reynolds et al. [5,16] concluded that CPP-ACP promotes remineralisation of enamel subsurface lesion, restoring the white opaque appearance of the lesions to translucency, even in the presence of fluoride.
Andersson et al. [74] conducted a randomized, controlled clinical trials on 26 individuals who developed 152 visible white-spot lesions on 60 incisors and canines immediately after the orthodontic debonding. After bracket removal, professional tooth cleaning and drying, visual scoring (0-4) and laser fluorescence assessment were performed. The participants were randomly assigned to two different treatment protocols with the aim of remineralising the lesions. One treatment modality was the daily topical application of a dental cream containing CPP-ACP for 3 months, followed by a 3-month period of daily tooth brushing with a fluoride dentifrice. The other treatment protocol was daily topical application of a 0.05% sodium fluoride mouthwash combined with the use of a fluoride dentifrice for 6 months. Follow up clinical examinations were repeated after 1, 3, 6, and 12 months, and data were compared with baseline measurements. The study showed that 63% of white spots regressed in the CPP-ACP group compared with 25% in the fluoride group, which was significantly different (p<0.01).

In a study by Ardu et al. [75], concluded that enamel micro-abrasion together with prolonged use of a CPP-ACP based paste is useful for treating white spot enamel lesions. Kumar et al. [76] observed the effect of CPP-ACP on remineralisation of artificial caries-like lesions. They found that the CPP-ACP containing Tooth Mousse remineralised initial enamel lesions and has a higher remineralising potential when applied as a topical coating after the use of a fluoridated tooth paste, as compared to either of them alone.

In another study conducted by Bailey et al. [77] in a post-orthodontic population of 45 individuals, with 408 white-spot lesions, results revealed that 92% of white spot lesions regressed or stabilized after using a remineralising cream (Tooth Mousse™) containing 10% w/v CPP-ACP. Over 12 weeks, significantly more post-orthodontic white spot lesions (31%) regressed with the remineralising cream compared with an identical cream not containing CPP-ACP. Zhou et al. [78] showed that the mean reduction in demineralised enamel white spot lesions size after treatment was 4.89% for one month, and 8.36% for two months. They concluded that CPP-ACP can effectively remineralise the long-standing post-orthodontic demineralised enamel white lesion.

3. Decrease postoperative dentin sensitivity

CPP-ACP may enhance remineralisation and decrease post-operative sensitivity following tooth whitening and micro-abrasion procedures in hypomineralised teeth [79]. Manton et al. [64] concluded that Tooth Mousse may be applied concurrently with the bleach without reducing bleaching effectiveness. In a study by Giulio et al. [80], proposed that the topical applications of CPP-ACP could be effective in promoting enamel remineralisation after interdental stripping.

The reduction in postoperative dentin sensitivity is due to the fact that mineralisation of the dentinal tubule openings recloses the tubules and rapidly reduces the dentine hypersensitivity.

4. Treating dry mouth

Clinical trials with CCP-ACP preparations have revealed positive results in terms of caries prevention [81] and mouth moistening [81,82], in patients with severe xerostomia. Hay and Morton [82] administered a self evaluation survey to 38 patients in the original sample. They were asked to compare the casein derivatives coupled with calcium phosphate (CD-CP) mouth rinse with their usual mouth moistening strategies (for e.g., sipping water, chewing gum, and using artificial saliva). The authors concluded that the CD-CP mouth rinse, when used as an atomized spray in the mouth, provide good moistening and lubrication.

The CCP-ACP preparations have a clear advantage over the fluoride based preparations because it could be swallowed instead of spat out as CPP-ACP formulations are nontoxic. However, the potential side effects from ingestion of casein derivative protein in people with immunoglobulin E allergies to milk proteins should be considered before administration.

5. Transport medium for the avulsed teeth

Cehreli et al. [83] suggested that highly diluted CPP-ACP preparation may be used as a transport medium for the avulsed teeth, as it preserves L929 cell viability in the short term without inducing apoptosis.

6. Improve the properties of other dental materials

A study by Mazzaoui et al. [84] used 1.56% w/w CPP-ACP in glass-ionomer cement (GIC). The in vitro study found that the new cement has higher compressive strength (23%) and higher microtensile bond strength (33%). This is due to incorporation of the CPP-ACP nanoparticles into the cross linked matrix of the GIC. The investigators also noted significant and enhanced release of fluoride from the cement with CPP-ACP. Matsuya et al. [85] suggested that the CPP-ACP nanoparticles become physically encapsulated into the set GIC and are released as the acid erode the cement during the acid challenge, causing protection of the adjacent dentin.

In a study by Vanthana et al. [86], proposed that CPP-ACP complex may be beneficial to the dentine bonding of self-etching adhesive systems, as the chemical interactions between calcium and functional monomers of the adhesives might be enhanced to some degree.
Conclusion

Concept of minimal intervention dentistry has evolved as a consequence of increased understanding of the carious process and the advances in dental materials and techniques. The ultimate goal of the modern dentistry is the non-invasive management of non-cavitated carious lesions through remineralisation in an attempt to prevent caries progression and improve aesthetics, strength, and function. Steps should be taken to prevent the onset of caries in individuals who are at high risk, and the initial carious lesions should be treated non-invasively by remineralisation agents, in those in whom disease is already evident. The ability to stabilize calcium phosphate and promote mineral solubility and bioavailability confers upon the CPP-ACP the potential to be biological delivery vehicles for calcium and phosphate. Of the various remineralisation technologies currently available in the market, the CPP-ACP and CPP-ACFP technology has the most promising evidence to support its use in caries prevention and lesion reversal. Further scientific research and long term studies are necessary to provide definite and evidence based clinical recommendations of novel remineralisation treatment.

References

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