Review of Casein Phosphopeptides-Amorphous Calcium Phosphate

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Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) is a bioactive agent with a base of milk products, which has been formulated from two parts: casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP). CPP was produced from milk protein casein and has a remarkable ability to stabilize calcium phosphate in solution and to substantially increase the level of calcium phosphate in dental plaque. CPP-ACP buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel, reducing demineralisation and promoting remineralisation. The free calcium and phosphate ions move out of the CPP, enter the enamel rods and reform onto apatite crystals. Laboratory, animal and human studies have shown that CPP-ACP inhibits cariogenic activity. CPP-ACP is useful in the treatment of white spot lesions, hypomineralised enamel, mild fluorosis, tooth sensitivity and erosion, and prevents plaque accumulation around brackets and other orthodontic appliances. CPP-ACP also facilitates a normal post-eruptive maturation process and is ideal for protecting primary teeth at a time when oral care is difficult. CPP-ACP has commercial potential as an additive to foods, soft drinks and chewing gum, as well as additive to toothpastes and mouthwashes to control dental caries.

Key words: *CPP-ACP, demineralisation, remineralisation, caries prevention*

Dental caries is a major public health problem worldwide, and its incidence is rising in the developing world with the easy availability of refined carbohydrates. This highlights the requirements for the development of a non-toxic, anticariogenic agent that could be added to toothpaste, mouthwash and food in an approach to lower caries experience. It would be particularly useful if the anticariogenic agent was a natural food derivative, as then it would be considerably easier to obtain the appropriate regulatory approval for the agent as a food additive. With approval as a food additive, the agent could be incorporated into sugar-containing foods to target the high caries risk groups.

The food group that is most recognised as exhibiting anticaries activity is dairy products (milk, milk concentrates, milk powder, and cheeses)[1,2]. Dairy products have been shown to be anticariogenic in animal and human *in situ* caries models. The anticariogenic effect of milk products was not attributed to a change in level of infection of streptococci; but attributed to a direct chemical effect by cheese components. Using *in vitro*, animal and *in situ* caries models, the components largely responsible for this anticariogenic activity have been identified as casein, calcium and phosphate[2]. The bovine milk phosphoprotein, casein, is known to interact with calcium and phosphate and is considered to be largely responsible for this anticariogenic property[3]. The casein phosphopeptides (CPP) have a remarkable ability to stabilise calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque through their multiple phosphoeryl residues, which prevent their growth to the critical size required for nucleation and precipitation of calcium and phosphate[4].

Milk produces only a minimal drop in plaque pH in subjects who rinsed with milk for 30 sec after refraining from tooth brushing for 3 days. Milk reduces the

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dissolution of enamel. Casein products of milk are rapidly absorbed onto enamel surfaces and provide resistance to acid. The K casein fractions in milk can modulate adherence of a strain of cariogenic microorganism, S. mutans to hydroxyapatite[5,6]. Micellar casein selectively modifies the microbial composition of dental plaque, thus reducing its cariogenic potential[7]. The proposed mechanism of anticariogenicity for the CPP-amorphous calcium phosphate (ACP) is that they localize ACP in dental plaque, which buffers the free calcium and phosphate ions, thereby helping to maintain a state of supersaturation with respect to tooth enamel, depressing demineralisation and enhancing remineralisation[8].

ACP was first described by Aaron S Posner in the mid 1960s. It is the initial solid phase that precipitates from a highly supersaturated calcium phosphate solution and can convert readily to stable crystalline phases, such as octacalcium phosphate or apatitic products. ACP has been demonstrated to have better *in vivo* osteoconductivity than hydroxyapatite, better biodegradability than tricalcium phosphate, good bioactivity but no cytotoxicity. ACP has been widely applied in the biomedical field due to its excellent bioactivity, high cell adhesion, adjustable biodegradation rate and good osteoconduction[9].

Using a human intraoral caries model, it has been shown that the digestion of caseinate with trypsin did not destroy the proteins' ability to prevent enamel subsurface demineralisation. Tryptic peptides of casein were found incorporated into the intraoral appliance, plaque and were associated with a substantial increase in the plaque's content of calcium and phosphate[4].

The tryptic peptides responsible for the anticariogenic activity are the calcium phosphate sequestering phosphopeptides. As the CPP are not associated with the unpalatability or antigenicity of the caseins and have the potential for a specific anticariogenicity of at least 10 times greater on a weight basis, then their potential as a food toothpaste additive is considerably better than that of the intact proteins[10].

Interaction of CPP with calcium phosphate

CPP contain the cluster sequence of Ser(P)-Ser(P)-Glu-Glu from casein. Through these multiple phosphoseryl residues, CPP have a marked ability to stabilise calcium and phosphate ions in solution to form CPP-ACP complexes; preventing their growth to the critical size required for nucleation, phase transformation and precipitation[11]. The ion activity products for the various calcium phosphate phases: hydroxyapatite (HA); octacalcium phosphate (OCP); tricalcium phosphate (TCP); ACP; and dicalcium phosphate dihydrate (DCPD) are determined from the free calcium, and phosphate concentrations at each pH are determined using a modified computer programme that calculates the ion activity coefficient through the use of the expanded logarithm. The only ion activity product that significantly correlated with calcium phosphate bound to the independently of pH was that corresponding to ACP in neutral and alkaline pH. In supersaturated calcium phosphate solutions, ACP nuclei form spontaneously[11,12]. Each molecule of CPP can bind up to 25 calcium ions, 15 phosphate ions and 5 fluoride ions. The calcium phosphate in these complexes is biologically available for remineralisation of subsurface lesions in tooth enamel[13].

Anticariogenic potential of CPP-ACP

CPP is believed to have an antibacterial and buffering effect on plaque, and interfere in the growth and adherence of streptococcus mutans and streptococcus sobrinus. The mechanism of anticariogenicity for the CPP-ACP is that they incorporate amorphous calcium phosphate in plaque, depressing enamel demineralisation and enhancing remineralisation. In plaque, CPP-ACP would act as a reservoir of calcium and phosphate, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel. The binding of ACP to CPP is pH responsive, with binding decreasing as the pH falls[14]. As pH increases, the level of bound ACP increases and stabilises free calcium and phosphate, so that spontaneous precipitation of calcium phosphate does not occur. This is also an inherently anti-calculus action[15].

Rose measured the affinity of streptococcus mutans to CPP-ACP[16]. It was demonstrated that CPP-ACP binds with affinity to bacterial cells. Hence CPP-ACP binds well to plaque, providing a large calcium reservoir within plaque and slowing the diffusion of free calcium. Additional evidence also reported by Rose indicates that CPP-ACP would compete with calcium for plaque calcium binding sites. As a result, this will reduce the amount of calcium bridging between the pellicle and adhering bacterial cells and between bacterial cells themselves[15,16]. It has been observed that CPP-ACP significantly reduced caries activity in a dose dependent manner, as 1% CPP-ACP produced about 55% reduction in smooth surface caries and a 46% reduction in fissure caries activity, which is similar to the effect produced by 500 ppm of fluoride.

Remineralisation of enamel lesions by CPP-ACP

An *in vitro* model system has been used to study the effect of CPP-ACP solutions on the remineralisation of artificial lesions in human third molars. The model involved the preparation of uniform reproducible subsurface enamel lesions, which were cut into halves; one half was used as a control to the other, which was exposed to remineralising solution. At the end of the treatment (10-day exposure), the lesions were sectioned and subjected to microradiography and mineral content determined by microdensitometry. The results showed that there was a substantial increase in mineral content of test enamel sections exposed to CPP-ACP solutions[4].

Sugar free lozenges containing CPP-ACP is effective in the remineralising of subsurface lesions in enamel. CPP-ACP can be found in plaque up to 3 hours after chewing of gum. CPP-bound ACP acts as a reservoir of calcium and phosphate ions, including the neutral ion $CaHPO_4$, which are formed in the presence of acid. The acid can be generated by dental plaque bacteria; under these conditions, the CPP-bound ACP would buffer plaque pH and in doing so would dissociate to calcium phosphate ions including $CaHPO_4$[17]. The increase in plaque calcium and phosphate ions and ion pairs would offset any fall in pH, thereby preventing enamel demineralisation. Acid is also generated in plaque but there is formation of HA in the enamel lesion during remineralisation. This explains why the CPP-ACP solutions are such efficient remineralising solutions, as they would consume the acid generated during enamel lesion demineralisation by generating more $CaHPO_4$, thus maintaining its concentration gradient into the lesions[18]. In a study conducted by Cai et al to study the remineralisation potential of CPP-ACP, the authors concluded that CPP-ACP produced better remineralisation even in presence of citric acid, thus conclusively proving that CPP-ACP can promote remineralisation in acidic environments[19]. Another study compared the efficacy of different remineralising products, the addition of CPP-ACP along with Xylitol in a chewing gum was found to be responsible for producing superior remineralisation[20]. In a study conducted to evaluate and estimate the salivary concentration of calcium after chewing gum containing CPP-ACP (Trident), it was noted that there was a mean 69.6% increase in calcium concentration of saliva after chewing the CPP-ACP containing gum for 10 minutes. This supply of relatively higher concentration of calcium in saliva and plaque ultimately reaches to the tooth surface and is responsible for remineralising tooth surfaces and increasing a cariostatic effect[21]. In a systematic review conducted to appraise whether CPP-ACP supplements can remineralise enamel subsurface lesions, all studies demonstrated a statistically significant increase in percentage of enamel subsurface remineralisation with use of chewing gum and lozenges supplemented with 18.8 mg CPP-ACP after 14 days[22]. Morgan et al determined that CPP-ACP chewing gum use over a period of 3 months can show a reversal of carious lesions, as determined by digital bitewing radiographs[23].

Inhibition of enamel demineralisation by CPP

Roberts reported that treating enamel slabs *in vitro* with a crude preparation of CPP at 0.5% w/v in water significantly inhibited acid demineralisation[24]. In experiments where enamel remineralisation has been measured by surface intra-lesion mineral deposition, CPP-ACP solutions substantially promoted remineralisation. The use of a crude CPP preparation at 5.0% w/w in a dentifrice was also reported by Roberts to significantly inhibit enamel demineralisation in a human intraoral caries model. The inhibition obtained in this study was similar to that obtained with a 500 ppm fluoride dentifrice[24].

A human *in situ* caries model has been used by Reynolds et al to study the ability of solution of 1% CPP, 60 Mm $CaCl_2$ and 36 mM sodium phosphate at pH 7 to prevent enamel demineralisation. Two exposures of CPP-ACP solution per day to one side of enamel slabs produced 51 ± 19% reduction in enamel mineral loss caused by frequent sugar solution exposure, compared to control side[25]. In a study by Rahiotis et al, the effect of a CPP-ACP agent was seen on the demineralisation and remineralisation of dentin. Surface analysis by Fourier Transformer micro multiple internal reflectance infrared spectroscopy (MIR-FTIR) was carried out on test samples prior to immersion in demineralising solution for 7 days. Tooth mousse was applied onto test samples prior to immersion in demineralisaing solution. A MIR-FTIR mineral matrix ratio was used to assess dentin demineralization. Tooth Mousse was applied onto test samples, which were then immersed in artificial saliva. Tooth mousse-treated samples showed both lower demineralisation when treated with demineralising solution and higher remineralisation when immersed in artificial saliva[26].

Potential of CPP to inhibit plaque formation

Guggenheim et al demonstrated that cariogenic diets containing micellar casein or CPP significantly reduce the number of streptococcus sobrinus colonising the teeth of experimental rats. The authors suggested that

this reduction in cariogenic streptococci is at least partly responsible for the substantial increase in caries prevention obtained by the CPP[7]. Milk and its derivatives have proven to be both anti-cariogenic, as well as substantially decreasing the quantity of plaque and changing its quality[27].

Schupbach et al demonstrated that incorporation of CPP into salivary pellicles *in vitro* substantially inhibited the adherence of streptococcus mutans and streptococcus sobrinus. They concluded that the incorporation of CPP into the pellicle will not only increase its remineralisation potential but also inhibit incorporation of cariogenic streptococci[28]. Yamanaka et al demonstrated that the incorporation of caseino glycopeptides and casein-phosphopeptides into salivary pellicles inhibits the adherence of mutans streptococci to teeth[29].

Rahiotis et al proved that CPP-ACP interacts with calcium binding sites and masks the bacterial receptors on salivary molecules. It thus reduces bacterial colonisation as shown with CPP-ACP germanium-treated surfaces. Thus, there is reduced plaque formation with CPP-ACP use[30].

Thus, the use of CPP-ACP products changes the plaque composition, as well as leading to a reduction in the amount of plaque, which will lead to reduced caries risk.

Interaction of CPP-ACP with fluoride

The additive anticariogenic effect of the 1.0% CPP-ACP and 500 ppm F in the rat caries experiments led to the investigation of the potential interaction between the CPP-ACP and F. Analysis of the solution containing 1.0% CPP, 60 mM CaCl2, 36 mM sodium phosphate and 500ppm F (26.3 mM NaF) pH 7.0 after ultrafiltration, revealed that nearly half of the fluoride ion had incorporated into the ACP phase stabilised by the CPP to produce a novel amorphous calcium fluoride phosphate phase. The identification of this novel amorphous calcium fluoride phosphate phase (ACFP) led to the proposition that the formation of this phase is responsible for the observed additive anticariogenic effect of CPP ACP and F[31]. The proposed anticariogenic mechanism of the CPP-ACP is the localisation of ACP at the tooth surface so that in the presence of acid, the ACP dissociates to release calcium and phosphate ions increasing the degree of saturation with respect to HA, preventing enamel demineralization and promoting remineralisation[32]. The anticariogenic mechanism of fluoride is now proposed to be the localisation of the fluoride ion at the tooth surface particularly in plaque, in the presence of Ca and phosphate ions. This localisation increases the degree of saturation with respect to fluoride (FA) thus promoting remineralization of enamel with FA[33,34]. The advantage of CPP-ACFP is the availability of calcium, phosphate and fluoride in one product. Each molecule of CPP can bind upto 25 calcium ions, 15 phosphate ions, and 5 fluoride ions. The calcium phosphate in these complexes is biologically available for remineralisation of subsurface lesions in tooth enamel[35].

Reynolds et al compared the enamel remineralisation ability of mouth rinse containing CPP-ACP and fluoride, with that of fluoride mouth rinse. They demonstrated that 0.4% CPP-ACP and 220 ppm F produce 19% enamel subsurface remineralisation, compared to 8% remineralisation with 220 ppm F and 14% by 0.4% CPP-ACP alone[35].

Combined with fluoride, CPP-ACP has an additive effect on caries activity. The use of CPP-ACP, along with fluoride containing dentifrice, has proved to be beneficial in reducing the demineralisation around orthodontic brackets[36]. In a study conducted to find out the efficacy of CPP-ACP and CPP-ACFP in remineralising enamel surfaces on which artificial caries lesions had been created, both CPP-ACP and CPP-ACFP showed remineralisation, with CPP-ACFP perfoming better than just CPP-ACP alone[37].

Thus CPP-ACP can add into current fluoride containing dentifrices as a toothpaste additive to improve efficacy[38]. Kumar et al proved that CPP-ACP creams are very efficient in remineralising initial enamel lesions. CPP-ACP shows a greater remineralising potential when applied as a topical coating after using topical fluoride toothpaste containing 1,100 ppm F than when used alone[39]. Owing to its additive effect with fluoride, it can be recommended that CPP-ACP should be used as a self-applied topical coating after teeth have been brushed with a fluoridated toothpaste by children who have a high caries risk[40].

Applications for CPP-ACP

Remineralisation of white spot lesions

Andersen et al conducted a study to determine the effect of dental cream containing cream phosphate complexes on remineralising white spot lesions. Using LASER fluorescence, regression of white spot lesions was seen following the application of CPP-ACP cream[40].

In another study to evaluate the effect of 3 months application of CPP-ACP versus NaF rinse with a follow up of 12 months, the CPP-ACP cream was found more effective in the reducing number of white spot lesions[41].

Regular application of Topical CPP-ACP cream in orthodontic patients prevented white spot lesions[42]. In another study, application of CPP-ACP tooth cream when compared with placebo showed regression of white spot lesions by 4 and 8 weeks and reversal was substantial by 12 weeks[43].

Post bleaching

Bleaching procedures often lead to decreased hardness, decreased fracture toughness, decreased flexural strength and other changes in mechanical properties. CPP-ACP application, subsequent to both 9.5% and 38% hydrogen peroxide bleaching, results in compensation for decreased flexural strength of bleached enamel dentin complex[44]. Bleaching brings about the desiccation of enamel, making teeth more susceptible to stain absorption. Surface treatment of freshly bleached enamel with CPP-ACP leads to a reduction in stain absorption[45].

Dentin hypersensitivity

CPP-ACP is useful in reducing dentinal hypersensitivity by occluding dentinal tubules. According to Poitevin et al, tooth mousse containing CPP-ACP has proven to be effective in treating dentinal hypersensitivity. This was evaluated using a visual analogue scale (VAS). Tooth mousse was used every day for 3 minutes for 21 days after brushing. At the end of this period VAS was again used and an improvement was noted[46]. Azarpazhhooh and Limeback found CPP-ACP cream useful in treating dentin hypersensitivity and dry mouth but found insufficient evidence for efficacy[47].

Erosion

Dental erosion in seen very commonly in swimmers and athletes who consume sports energy drinks, such as gatorade. Erosion is also seen in teenagers who consume excessive aerated drinks and in patients who suffer from acid-reflux problems. Ramalingam et al concluded that the addition of CPP-ACP to sports drink eliminated *in vitro* erosion[48]. Fizzy cola drinks are known to decrease enamel hardness. In a study to evaluate the effect of CPP-ACP, Panich et al proved that cola-affected teeth showed a decrease in enamel hardness. When exposed to CPP-ACP, these teeth showed an improvement in surface hardness[49].

Incorporation of CPP-ACP into glass ionomer restorative material

Glass ionomer cement (GIC) has excellent dynamic adhesion to enamel and dentin. Mazzoui et al determined the effect of incorporation of CPP-ACP (1.56% w/v) into a self-cured GIC, Fuji IX. This GIC demonstrated a significant increase in microtensile bond strength to dentin by 33% and compressive strength by 23% and significantly enhanced the release of calcium, phosphate, and fluoride ions at neutral and acidic pH. The release of CPP-ACP and fluoride from CPP-ACP containing GIC as the acid erodes the cement was associated with enhanced protection of the adjacent dentin during acid challenge *in vitro*. It was concluded that 1.56% CPP-ACP containing GIC might be a superior restorative/base with an improved anticariogenic potential[50]. Furthermore, CPP has been shown to keep calcium, phosphate and fluoride as ions in solution, thereby enhancing the efficacy of fluoride as a remineralising agent[51].

Sealants

The addition of ACP to resin sealants has made them comparable to glass ionomer-based sealants. The solubility of ACP enables it to release supersaturating levels of calcium and phosphate ions in proportion that is favourable for hydroxyapatite formation. These fortified sealants have a higher remineralising capacity with the potential to remineralise enamel subsurface lesions[52-54].

Addition with milk

A study by Walker et al found that although milk contains casein phosphate, the addition of CPP-ACP results in enhanced remineralisation. A dose of 5 gm of CPP-ACP produced 148% more remineralisation compared to 2 gm of CPP-ACP per litre of milk[55].

Bonding

Tooth mousse influences resin adhesion to dentin. Tooth mousse may compromise bonding effectiveness of etch and rinse adhesives. It is beneficial to dentin bonding of self-etch systems, as chemical interaction between calcium and functional monomers of adhesives may be enhanced to some degree[56].

Comparison of CPP-ACP with other similar products

Calcium sucrose phosphate: Anticay

There are some similarities between CPP-ACP and calcium sucrose phosphate (CaSP). CaSP is a mixture of calcium sucrose mono and diphophate, disucrose monophosphate and inorganic calcium phosphate that contains approximately 11% w/w calcium and 7.6% w/w inorganic phosphate[57]. CaSP has been shown to decrease tooth enamel demineralisation, promote enamel remineralisation and inhibit the formation of plaque similar to the proposed mechanism of CPP-ACP. In a 2-year double blind clinical trial in the USA, CaSP incorporated into chewing gum was shown to reduce decayed, missing, filled tooth surfaces (DMFS) by 39% relative to a control gum. CPP-ACP contains 18% w/w Ca and 30% w/w PO_4 in the form of amorphous calcium phosphate so that the CPP have specific conformations that allow them to stabilise ACP and to interact and localise ACP at the tooth and mucosal surfaces. Finally, plaque phosphate and peptidase activity, when degrading the CPP, ultimately results in a pH rise through ammonia release, further leading to an anticaries effect[57].

Calcium sodium phosphosilicate: NovaMin

The compound is a bioactive glass composed of minerals that naturally occur in the body and reacts when it comes into contact with water, saliva, or other body fluids. This reaction releases calcium, phosphorus, sodium and silicon ions in a way that results in the formation of new hydroxycarbonateapatite (HCA) crystals. This can be added to toothpaste and prophylactic pastes[58].

Unstabilised amorphous calcium phosphate: Enamelon

Enamelon has proven to be effective in the treatment of root caries. It has anticariogenic potential. It is used as an additive in cements and sealants[54,59].

FluorideTriCalciumPhosphate (Ftcp): ClinPro tooth crème

This is another calcium-based remineralising product available on market. It is based on tricalcium phosphate technology to which fluoride is added. Bioavailable fluoride levels are 950 ppm derived from 0.21% NaF. It is used as a toothpaste for 2 minutes and then expectorated. It is found to be better than conventional 1,000 ppm fluoridated toothpaste in the reversal of white spot lesions[60].

Conclusion

The anticariogenic potential of the CPP-ACP has been demonstrated in the rat caries model, *in situ* human caries model, *in vitro* remineralisation models and human trials. Calcium and phosphate are essential components of enamel and dentin and form highly insoluble complexes, but in the presence of CPP they remain soluble and biologically available. This bioavailable calcium and phosphate and fluoride buffer plaque pH, depress enamel demineralisation and enhancing remineralisation. The CPP-ACP complex is applied to teeth by means of chewing gum, toothpaste, lozenges, mouth rinses or sprays. These adhere to dental biofilm, preventing colonisation of streptococci and providing supersaturated environment of calcium and phosphate.

Anticariogenic activity is greatest when the peptides are delivered at the same time as the cariogenic challenge and the CPP-ACP are a natural derivate of milk, therefore unlike fluoride they could be added to sugar-containing foods. Preliminary results indicate that the CPP-ACP can be incorporated into confectionery without adverse organoleptic effects. CPP-ACP therefore could have an important role as a food additive for the control of dental caries.

References

1. Reynolds EC, Johnson IH. Effect of milk on caries incidence and bacterial composition of dental plaque in rat. Arch Oral Biol 1981;26:445–451.
2. Rosen S. Effect of cheese, with and without sucrose, on dental caries and recovery of streptococcus mutans in rats. J Dent Res 1984;63;894–896.
3. Bowen WH, Pearson SK. Effect of milk on cariogenesis. Caries Res 1993;27:461–466
4. Reynolds EC. Remineralisation of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587–1595.
5. Scholz-Arens KE, Schrezenmeir J. Effects of bioactive substances in milk on mineral and trace element metabolism with special reference to casein phosphopeptides. Br J Nutr 2000;84:147–153.
6. Vacca-Smith AM, Van Wuychhuyse BC, Tabak LA, Bowen WH. The effect of milk and casein proteins on the adherence of streptococcus mutans to saliva coated hydroxyapatite. Arch Oral Biol 1994;39:1063–1069.
7. Guggenheim B, Schmidt R, Aeschlimann JM, et al. Powdered milk miscellar casein prevents oral colonization by S. sobrinus and dental caries in rats: a basis for caries protective effect of dairy products. Caries Res 1999;33:446–454.
8. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intraoral model. J Dent Res 1987;66:1120–1127.
9. Boskey AL. Amorphous calcium phosphate: the contents of bone. J Dent Res 1997;76:1433–1436.
10. Reynolds EC. Production of phosphopeptides. Patent application PK 5706, 199.

11. Huq NL, Cross KJ, Reynolds EC. Molecular modeling of the multiphosphorylated casein phosphopeptides alpha s1, casein (59-79) based on NMR constraints. J Dairy Res 2004;71:28–32.
12. Cross KJ, Huq NL, Palmar JE. Phsicochemical characterization of CPP-ACP nanocomplexes. J Biol Chem 2005;280:15362–15369.
13. Karlinsey RL, Mackey AC. Solid-state preparation and dental application of an organically modified calcium phosphate. J Mater Sci 2009;44:346–349.
14. Reynolds EC, Cai F, Shen P, Walker GD. Retention of plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinses or sugar free chewing gum. J Dent Res 2002;82:206–211.
15. Rose RK. Effects of anticariogenic casein phosphopeptides on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol 2000;45:569–575.
16. Rose RK. Binding characteristics of streptococcus mutans for calcium and casein phosphopeptides. Caries Res 2000;34:427–431.
17. Cai F, Shen P, Morgan MV, et al. Remineralisation of enamel subsurface lesions in situ by sugar –free lozenges containing CPP-ACP. Aus Dent J 2003;48:240–243.
18. Iijima Y, Cai F, Shen P et al. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38:551–556.
19. Cai F, Shen P, Walker GD. Remineralization of enamel subsurface lesions by chewing gum with added calcium. J Dent 2009;37:763–768.
20. Manton DJ, Walker GD, Cai F, et al. Remineralisation of enamel subsurface lesions in situ by use of three commercially available sugar-free gums. Int J Ped Dent 2008;18:284–290.
21. Shanmukha G, Santosh BP, Jethmalani P, et al. Evaluation of changes in salivary concentration of calcium by CPP-ACP containing chewing gum - a clinical trial. Int J Adv Res Oral Sci 2012;1:1–7.
22. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: a systematic review of literature. J Am Dent Assoc 2008;139:915–924.
23. Morgan MV, Adams GG, Bailey DL, et al. The anticariogenic effect of sugar-free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiography. Caries Res 2008;42:171–184.
24. Robert AJ. Role of models in assessing new agents for caries prevention-non fluoride system. J Dent Res 1995;9:304–311.
25. Reynolds EC, Black CL, Cai F. Advances in enamel remineralization: casein phosphopeptides amorphous calcium phosphate. J Clin Dent 1999;10:86–88.
26. Rahiotis C, Vououklakis G. Effect of CPP-ACP agent on demineralization and remineralisation of dentine in-vitro. J Dent Res 2007;35:695–698.
27. Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr 2004;134:989–995.
28. Schupbach P, Neeser JR, Golliard M, et al. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of of mutans streptococci. J Dent Res 1996;75:1779–1788.
29. Sato T, Yamanaka K, Yoshii E. Caries Prevention Potential of a Tooth-coating Material Containing Casein Phosphopeptide-Amorphous Calcium Phosphate (CCP-ACP). [81st General Session and Exhibition of the IADR, June 2003, Gothenburg]. Gothenburg: IADR, 2003.
30. Rahiotis C, Vougiouklakis G, Elaides G. Characterisation of oral films formed in presence of CPP-ACP agent: an in situ study. J Dent 2008;36:272–280.
31. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in rat. J Dent Res 1995;74:1272–1279.
32. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dent 1998;18:8–16.
33. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptides in oral health – chemistry and clinical applications. Current Pharm Des 2007;13:793–800.
34. Shen P, Cai F, Nowicki A ,et al. Remineralisation of enamel subsurface lesions by casein phosphopeptide - amorphous calcium phosphate. J Dent Res 2001;80:2066–2070.
35. Cochrane NJ, Saranathan S, Cai F, et al. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res 2008;42:88–97.
36. Sudjalim TR, Wood MG, Manton DJ, et al. Prevention of demineralization around orthodontic brackets in vitro. Am J Orthod Dentofacial Orthop 2007;131:705.e1–9.
37. Jayarajan J, Janardhanam P, Jaykumar P, Deepika. Efficacy of CPP-ACP and CPP-ACFP on enamel remineralization. An in-vitro study using SEM and Diagnodent. Ind J Dent Res 2011;22:77–82.
38. Reynolds EC. Calcium phosphate based remineralization systems: scientific evidence? Aus Dent J 2008;53:268–273.
39. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Aus Dent J 2008;82:207–211.
40. Andersson A, Skold- Larsson K, Hallgren A, et al. Effect of dental cream containing cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. Oral Health Prev Dent 2007;5:229–233.
41. Bergstrand F, Twetman S. A review on prevention and treatment of post – orthodontic white spot lesions – evidence-based methods and emerging technologies. Open Dent J 2011;5:158–162.
42. Nasab NK, Kajan ZD, Baladil A. Effect of topical C-5 on enamel adjacent to orthodontic brackets. An in vitro study. Aust Orthod J 2007;23:46–49.
43. Bailey DL, Adams GG, Tsao CE, et al. Regression of post-orthodontic lesions by a remineralising cream. J Dent Res 2009;88:1148–1153.
44. Khroushi M, Mazaheri H, Manoochehrri AE. Effect of CPP-ACP application on flexural strength of bleached enamel and dentin complex. Operative Dent July/Aug 2011;36:372–379.
45. Singh RD, Ram SM, Shetty O, et al. Efficacy of CPP-ACP to prevent stain absorption on freshly bleached enamel. An in-vitro study. J Conser Dent 2011;13:76–79.
46. Poitevin A, Peumans M, De Munck J, Braem M, Van Meerbeek B. Clinical effectiveness of a CPP-ACP Crème for Tooth Hypersensitivity Treatment. EADR Istanbul, 2004, Abstract 0136.
47. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: a systematic review of literature. J Am Dent Assoc 2008;139:915–924.
48. Ramalingam L, Messer LB, Reynolds EC. Adding casein phosphopeptides amorphous calcium phosphate to sports drink to eliminate in vitro erosion. Pediatr Dent 2005;27:61–67.
49. Panich M, Poolthong S. Effect of CPP-ACP and cola soft drink on in vitro enamel hardness. J Am Dent Assoc 2009;140:455–460.
50. Mazzoui SA, Burrow MF, Tyas MJ, et al. Incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass-ionomer cement. J Dent Res 2003;82:914–918.
51. Walsh LJ. Preventive dentistry for general dental practitioner. Aus Dent J 2000;45:76–82.
52. Sharma S, Kugel G. ACP sealants – the potential to remineralize. Inside Dent 2009;5:78–80.
53. Silva KG, Pedrini D, Delbem AC, et al. In situ evaluation of remineralising capacity of P & F Sealants containing ACP and/or fluoride. Acta Odontol Scand 2010;68:11–18.
54. Zhao J, Liu Y, Sun WB, et al. Amorphous calcium phosphate and its application in dentistry. Chemistry Central J 2011;5:40.

55. Walker G, Cai F, Shen P. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. J Dairy Res 2006;73:74–78.
56. Adebayo OA, Burrow MF, Tyas MJ. Resin-dentine interfacial morphology following CPP-ACP treatment. J Dent 2010;38:96–105.
57. Craig GG. The use of calcium sucrose phosphates - calcium orthophosphate complex as a cariostatic agent. Br Dent J 1975;25:1–8.
58. Burwell AK, Muscle D. Sustained calcium ion and ph release from calcium phosphate-containing dentifrices. [IADR/AADR/CADR 87th General Session and Exhibition; April 3, 2009; Miami, Florida, USA.
59. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J 2008;53:268–273.
60. Walsh LJ. The current status of tooth crèmes for enamel remineralization. Dental Inc 2009;2:38–42.