CPP-ACP Complex as a New Adjunctive Agent for Remineralisation: A Review

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Summary: In addition to regular professional oral hygiene visits and the application of appropriate preventive medications, successful preventive strategies involve oral health promotion, patient education and patient compliance. The Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex has been shown to remineralise tooth surfaces *in situ* when delivered in oral care products. This complex has a unique ability to deliver bio-available calcium and phosphate when they are needed most. The effectiveness of casein derivatives, specifically CCP-ACP, in caries prevention and lesion reversal has been supported by many controlled clinical studies. This review summarises the research on Casein phosphopeptide-amorphous calcium phosphate complex and provides information related to its benefit in dentistry. Further research is required to provide a scientifically supported recommendation for other clinical applications.

Key words: *amorphous calcium phosphate, casein phosphopeptide, remineralisation, review*

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Better understanding of the aetiology and pathogenicity of caries and the decline of the disease in recent years, especially in children and adolescents, have led to the use of more medial management and conservative measures in accordance with minimally invasive intervention criteria. However, several risk factors for dental caries are still present which include the frequent consumption of medicines affecting salivary flow, especially in older people, and erosive lesions especially in young people by high consumption of soft drinks and by dietary disorders.

Caries initiation is associated with demineralisation of subsurface tooth enamel. Calcium and phosphate are lost from the subsurface enamel, resulting in the formation of a subsurface lesion. At this early stage, the caries lesion is reversible via a remineralisation process involving the diffusion of calcium and phosphate ions into the subsurface lesion to restore lost tooth structure. Since several studies had demonstrated that milk-based products appeared to have anticariogenic properties in animal models, attention was focused on identifying the specific milk-based agents that were responsible for the 'anticaries effect' (Mellanby et al, 1924; Hubbell and Bunting, 1932; Bunting, 1934; Schweigert et al, 1946; Schweigert et al, 1946; Smith et al, 1948; Anderson et al, 1948; Zita et al, 1959; Bibby and Averill, 1963; Bibby et al, 1980; Bowen et al, 1980, Bibby et al, 1980; Navia and Lopez, 1983; Harper et al, 1986; Reynolds and Black, 1987b; Guggenheim et al, 1999).

Casein is the predominant phosphoprotein in bovine milk and accounts for almost 80% of its total protein, primarily as calcium phosphate stabilised micellular complexes. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes are derived from bovine milk protein, casein, calcium and phosphate. The concept of CPP-ACP as a remineralising agent was first postulated in 1998 (Reynolds, 1998). A number of subsequent studies have demonstrated CPP-ACP to have anticariogenic activity in laboratory, animal, and human *in situ* experiments (Reynolds et al, 1999; Reynolds et al, 2003; Shen et al, 2001). This has led to the incorporation of CPP-ACP into

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dental products and food products as a new tool in the fight against caries (Reynolds, 2003; Huq et al, 2004).

HISTORICAL BACKGROUND

Mellanby (1930) proposed milk as an important nutritional factor affecting pre-eruptive tooth mineralisation and post-eruptive caries resistance (Schweigert et al, 1946; Shaw, 1950; Bavetta and McClure, 1957; Holloway et al, 1961; Reynolds and Storey, 1979; Reynolds et al, 1982). The reduction in enamel solubility and adsorption of milk proteins into the enamel were reported when this was treated topically with milk and it was attributed to milk's relatively high calcium and phosphate content. Nyvad and Fejerskov (1984) 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*.

They suggested that the pellicle is not a uniform protein layer, but rather has a distinct globular structure. This was confirmed by Rolla and Rykke (1994), who found that the surface of a recently formed pellicle was composed of micelle-like protein globules formed in the presence of saliva or milk. It was suggested that the globules were salivary micelles, which were closely related to the casein micelles in structure. Reynolds (1987) using an *in situ* caries model, showed that exposure of inset-enamel plaque to solutions containing tryptic peptides of casein significantly reduced enamel subsurface demineralisation. The casein peptides were incorporated into the inset-enamel plaque and were associated with an increase in the plaque's content of calcium and phosphate (Reynolds and del Rio A, 1984). It was concluded that the tryptic peptides responsible for caseinate's anticariogenic activity were the calcium-phosphate-stabilising casein phosphopeptides (Reynolds et al, 1995).

Krobicka et al (1987) reported a topical anticariogenic effect of cheddar cheese in rats with their submandibular/sublingual glands surgically removed and parotid ducts tied. The effect could not be attributed to a change in level of infection of *Streptococcus sobrinus* and so was attributed to a direct chemical effect by cheese components (Rosen et al, 1984). Similarly, Harper et al (1986) tested the anticariogenic potential of four types of cheese with different levels of fat, protein, calcium, and phosphate in the rat model. They concluded that, of the cheese components, the protective effect was best attributed to the casein and/or calcium phosphate contents. A similar conclusion was reached by Silva and others using a human intraoral caries model (Silva et al, 1987). These authors showed that a water extract of cheddar cheese significantly reduced enamel softening without affecting resting or minimum plaque pH values. The cheese extract significantly increased the level of calcium in the experimental plaque, and it was concluded that the protective effect was associated with a decrease in demineralisation and/or enhancement of remineralisation. Most type of cheese contains around 20% weight per weight (w/w) casein in various stages of proteolysis. Since casein is rapidly degraded by intraoral bacterial proteolytic activity and the phosphopeptides released are relatively stable to further degradation (Reynolds and Black, 1987a; Reynolds et al, 1994), it is very likely that calcium phosphate complexes of these peptides are at least partly responsible for the cariostatic activity of cheese and other dairy products.

The casein phosphopeptides are approximately 10% w/w of casein, are tasteless, and can be easily and relatively cheaply purified from a tryptic digest of casein by selective precipitation, iron exchange, or ultrafiltration (Holt et al, 1996). The use of casein has not been implemented because of its adverse organoleptic properties and large amount required for its efficacy. In contrast, CPP does not have these limitations. The potential for a specific anticariogenic activity is at least 10 times greater on a weight basis for CPP than it is for casein (Reynolds, 1998). The phosphopeptides are solely responsible for the previously reported anticariogenicity of caseinate (Reynolds and Black, 1987a; Reynolds and Johnson, 1981). The 0.5% to 1% weight per volume (w/v) solution of CPP-ACP nanocomplexes was comparable with a 500 ppm fluoride solution in reducing caries activity (Reynolds et al, 1995).

MECHANISM OF ACTION

The anticariogenic potential of CCP-ACP can be explained as follows.

Calcium phosphate reservoir

According to Reynolds and co-workers, the anticariogenic potential of CCP-ACP has been attributed to the ability of CCP to localise ACP at the tooth

surface, increasing the level of calcium phosphate in plaque. In this way, the CCP-ACP may act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth mineral depressing enamel demineralisation and enhancing remineralisation (Reynolds, 1999; Huq et al, 2000). It has been shown that casein phosphopeptide (CPP) has the ability to stabilise calcium phosphate in solution by forming colloidal casein phosphopeptide amorphous calcium phosphate complexes (Reynolds, 1997; Cross et al, 2005). The CPP molecules contain a cluster of phosphoseryl residues –Ser(P)–Ser(P)–Ser(P)-Glu-Glu-, which markedly increase the apparent solubility of calcium phosphate by stabilising amorphous calcium phosphate ($Ca_3(PO_4)_2$-nH_2O) under neutral and alkaline conditions. The multiple phosphoseryl residues of the CPP bind to nanoclusters of ACP in supersaturated solutions, thereby preventing growth to the critical size required for phase transformations (Reynolds, 1997).

Dose-dependent response

CPP-ACP reduces caries activity in a dose-dependent mechanism and the subsequently formed mineral is more resistant to acid attack (Shen et al, 2001; Reynolds et al, 1995; Cai et al, 2003; Iijima et al, 2004) CPP-ACP solutions, applied twice daily to the teeth of specific-pathogen-free rats orally infected with *Streptococcus sobrinus*, a bacterium that causes tooth decay in humans, significantly reduced caries activity with 0.1% 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 (Reynolds et al, 1995).

Inhibition of bacterial adhesion

Another suggested mode of action is the CPP-ACP inhibition of cariogenic streptococci adhesion to tooth surface inducing the formation of a non-cariogenic plaque (Schupbach et al, 1996). The immunolocalisation studies have revealed that CPP-ACP can be incorporated into supragingival dental plaque by binding to the surfaces of bacterial cells to components of the intercellular plaque matrix and to adsorbed macromolecules on the tooth surface. All these interactions may then lead to the formation of a less cariogenic plaque (Rose, 2000a). Rose (2000a) demonstrated that CPP-ACP competes with calcium for plaque calcium binding sites and this will reduce the degree of calcium binding between the pellicle and adhering cells and between the cells themselves as supposed by Schupbach et al (1996). It was suggested that caseinglycomacropeptide (CGMP) and CPP adsorb to the surface of the pellicle and mask receptors on salivary molecules for these streptococci (Nyvad and Fejerskov, 1984). The high extracellular free calcium concentrations may have bactericidal or bacteriostatic effects. Rose (2000a) also suggested that by forcing the maintenance of high free calcium, CPP-ACP could have an additional antiplaque effect. Rose (2000b) in a streptococcal model of plaques observed that 0.1% CCP-ACP provides a large number of possible binding sites for calcium diffusion coefficient by about 65% at pH 7 and 35% at pH 5. The experimental rat caries studies showed that the caries-protective effect of milk is dependent on the presence of micellar casein. Cariogenic diets containing micellar casein or active fragments significantly reduce the numbers of *S. sobrinus* colonising the oral cavities of the test animals. In addition, *in vitro* experiments had shown that soluble caseinopeptides adsorbed to saliva-coated hydroxyapatite beads release albumin in exchange (Neeser et al, 1994). The micellar casein or the active sequences were incorporated into the salivary pellicle, reducing the adherence properties for these streptococci (Ung et al, 2004). Rahiotis and others (2008) observed that the presence of CPP-ACP agent (Tooth Mousse®) delays the biofilm formation and favoured the nucleation and crystallisation of calcium phosphates, possibly in apatitic form, in matured biofilms. Plaque pH was measured on 15 subjects in a crossover study 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 (Caruana et al, 2009).

Rate of remineralisation

The CPP-stabilised calcium phosphate solutions can remineralise enamel subsurface lesions at rates of 1.5 to 3.9 x 10^{-8} mol hydroxyapatite m^{-2} s^{-1} (Reynolds, 1997). The CCP can stabilise over 100 times more calcium phosphate than is normally

possible in aqueous solution at neutral and alkaline pH before spontaneous precipitation (Holt and van Kemenade, 1989). In the process of mineralisation, ACP and the crystalline phases dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) have been implicated as intermediates in the formation of hydroxyapatite (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 $3.9 \pm 0.8 \times 10^{-8}$ moles HA/m^2 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 (1984) using a constant-composition procedure.

Prevention of tooth erosion

Erosive tooth wear is a growing concern in clinical dentistry. CPP-ACP might prevent tooth erosion by suppressing demineralisation, enhancing remineralisation or a combination of these two processes. The presence of CPP-ACP might permit a rapid return to resting calcium concentrations and allow earlier remineralisation of the enamel substrate. *In vitro* studies have shown lower enamel erosion due to citric acid when enamel is previously treated with a CPP-ACP paste (Rees et al, 2007). Iijima and coworkers observed that the addition of CPP-ACP to sugar-free gum containing citric acid negated the effect of the citric acid and produced a remineralising effect greater than the neutral sugar-free gum without citric acid (Iijima et al, 2004). ACP is completely released from the gum in eight minutes (Shen et al, 2001) and localised on the surface of teeth and not found in the saliva (Reynolds et al, 2003). The bound CPP-ACP, unaffected by the citrate in the bulk saliva, then provides calcium ions, phosphate ions and hydroxide ions to diffuse into the enamel subsurface lesion. Likewise, enamel treated with cola derivatives, which reduces its hardness, subjected to the action of a CPP-ACP paste showed a significant increase in hardness independent of the presence of fluoride (Tantbirojn et al, 2008; Panich and Poolthong, 2009; Willershausen et al, 2009). Both the remineralising and lubricating properties of the paste containing CPP-ACP appear to contribute to wear reduction in enamel, although lubrication appears to have a more pronounced effect (Ranjitkar et al, 2009). Reduced erosive tooth wear was observed using Tooth Mousse, involving toothbrush abrasion, with each regime involving erosion in 0.3% citric acid (pH 3.2) for 10 minutes followed by toothbrush abrasion in a slurry of fluoride-free toothpaste and artificial saliva (1:3 ratio by weight) under a load of 2N for 200 cycles (Ranjitkar, 2009). Tooth Mousse may have significant role in the management of wine erosion (Piekarz et al, 2008). Adding CPP-ACP to energy drinks reduces their erosive capacity with no change in flavour when added in a proportion of over 0.09% (Ramalingam et al, 2005).

Interaction of CCP-ACP with fluoride

The role of CPP-ACP complex in decreasing the incidence of dental caries in the community is anticipated to be adjunct to the beneficial effects of topical fluoride. 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 (Vitorino et al, 2005). The adjunct anti-cariogenic effect obtained with CPP-ACP plus fluoride could relate to fluoride also being incorporated into the CPP-ACP complex. The casein phosphopeptide-amorphous calcium phosphate interacts with fluoride ions to produce an amorphous calcium fluoride phosphate stabilised by the CPP at the tooth surface. Casein phosphopeptide with amorphous calcium fluoride phosphate (CPP-ACFP) provides all the elements necessary for dental remineralisation on the tooth surface and in the dental biofilm. This provides soluble calcium, fluoride and phosphate ions to promote remineralisation with fluorapatite that is more resistant to future acid challenge. CPP can adhere to 25 calcium ions, 15 phosphate ions and five fluoride ions per molecule and can stabilise calcium phosphate in solution (Cross et al, 2005). In this way the CPP could act as an efficient delivery system, not only for amorphous calcium phosphate, but also for fluoride (Holler et al, 2002). 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 (Reynolds et al, 1995). Combining fluoride and ACP with CPP-ACP can give a synergistic effect on enamel remineralisation (Elsayad et al, 2009). Sudjalim also observed the benefits of combining CPP-ACP with sodium fluoride (Sudjalim et al, 2007). Remineralisation of the subsurface lesions was observed at all pH values tested with a maximum at

pH 5.5 (Cochrane et al, 2008). This study shows that CPP stabilises 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 hydroxyapatite and fluorapatite for remineralisation with CPP-ACP and CPP-ACFP, respectively. Reynolds observed that a dentifrice containing 2% CPP-ACP plus 1100 ppm F was superior to all other formulations of mouthrinses and dentifrices containing CPP-ACP and fluoride (Reynolds et al, 2008).

MI paste Plus is a recently introduced product that contains 900 ppm fluoride. Its *in vivo* efficacy has not been established. This product is not considered ingestible and therefore, children younger than 6 years should not use it. Fluoride also has a tendency to interact with the ACP component of the casein complex and may precipitate out as calcium fluoride, rendering both inorganic components ineffective (Azarpazhooh and Limeback, 2008). But this only holds true for a massive excess of fluoride, which is not relevant to the product MI Paste, which has only 900 ppm fluoride. Moreover, development of the carrier for calcium and phosphate in the Recaldent technology (as casein in MI Paste Plus) has greatly reduced this problem (Anderson et al, 1948).

Prevent demineralisation and improve remineralisation

A 1.0% w/v CPP solution can stabilise 60 mM $CaCl_2$, and 36 mM sodium phosphate at pH 7.0 to form colloidal amorphous calcium phosphate-CPP nanocomplexes (Reynolds, 1997). The formation of hydroxyapatite in the lesion leads to the generation of acid and phosphate, which diffuse out of the lesion down a concentration gradient. The CPP–supported metastable calcium phosphate solutions consume the acid generated during enamel lesion remineralisation by generating more calcium and phosphate ions, thus maintaining their high concentration gradients into the lesion (Reynolds, 1998; Reynolds et al, 2003; Shen et al, 2001; Reynolds, 1997). Enamel lesions, which have been remineralised with topical exposure to CPP-ACP, have been shown to be more resistant to subsequent acid challenge compared with normal remineralised enamel, as CPP-ACP is able to promote the remineralisation of enamel subsurface lesions with hydroxyapatite. In addition, the relatively low carbonate environment of the CPP-ACP treated subsurface lesion may also exhibit both improved crystallinity and lower microstrain than might be found in normal tooth enamel (Iijima et al, 2004). Ferrazzano and others (2007) observed the capability of CPPs to prevent demineralisation and promote remineralisation of early enamel lesions (Ferrazzano et al, 2007). In a systematic review with meta-analysis, Yengopal and Mickenautsch found the short-term remineralisation effect of CPP-ACP in clinical in situ trials, and long-term caries-preventing effect for CPP-ACP in the *in vivo* randomised control trial (Yengopal and Mickenautsch, 2009). CPPs could be a valid preventive system against demineralisation of early enamel lesions (Reynolds, 2008; Reynolds, 2009). CPPs contained in yogurt may have an inhibitory effect on demineralisation and promote the remineralisation of dental enamel (Ferrazzano et al, 2008).

CCP-ACP complex and glass ionomer cement

The CPP-ACP in the GIC increases the microtensile bond strength by the incorporation of the CPP-ACP nanoparticles into the crosslinked matrix of the GIC (Mazzaoui et al, 2003). CPP-ACP promotes the release of fluoride ions from the GIC by forming casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) nanocomplexes, which were released from the cement matrix (Reynolds, 1998). Inorganic phosphate is also released from the CPP-ACP-containing GIC. The CPP-ACP nanoparticles become physically encapsulated into the set GIC, as has been found with unreacted glass particles (Matsuya et al, 1984), and therefore released as the acid eroded the cement in the acidic buffer. The acid-catalysed release of the CPP-ACP nanoparticles from the GIC is consistent with the protection of the adjacent dentin observed during acid challenge.

CCP-ACP complex and resin bonding

The application of CPP-ACP may influence the subsequent resin adhesion to dentine (Adebayo et al, 2009). The use of conditioners prior to bonding with the self-etching primer adhesive system on treated enamel may significantly improve bond strength (Adebayo et al, 2007). The presence of CPP-ACP on the dentine surface, therefore, may

compromise bonding effectiveness of the etch-and-rinse adhesive system. However, it 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 (Vanthana et al, 2009). The enamel etching may not be inhibited by the use of a CPP-ACP paste with or without prior bleaching (Adebayo et al, 2009).

COMMERCIALLY AVAILABLE FORMS

The CPP-ACP complex was patented by the University of Melbourne, Australia, and the Victorian Dairy Industry Authority, Abbotsford, Australia. Bonlac Foods Limited (an Australian company owned by 2,300 dairy farmers in Victoria and Tasmania) has exclusive manufacturing and marketing rights for CPP-ACP and is the owner of the trademark (Recaldent). CPP-ACP has been incorporated into various products in order to exert a topical effect. These products include commercially available sugar-free chewing gum (Recaldent™; GC Corp, Japan and Trident White®; Cadbury Adams USA, Parsippany, New Jersey, USA), mints (Recaldent Mints™; Cadbury Japan Ltd, Japan), topical gels (Tooth Mousse™, Tooth Mousse Plus; GC Corp, Japan and MI Paste and MI Paste Plus; GC America, Alsip III) and experimentally tested sports drinks and glass ionomer cements (Reynolds et al, 2003; Shen et al, 2001; Cai et al, 2003; Ramalingam et al, 2005). CCP complex can be applied to teeth by the following means.

Chewing gum

Clinical trials of sugar-free chewing gum and mint have shown that they are non-cariogenic and can have an anticariogenic effect through the stimulation of saliva (Reynolds et al, 2003). Shen and coworkers (2001) evaluated the effect of incorporating CPP-ACP into sugar-free gum on enamel remineralisation. The subjects in the studies wore removable palatal acrylic appliances with six human enamel half-slabs inset containing subsurface demineralised lesions. The protocols of the three studies were identical except for the specific sweetener (sorbitol or xylitol), weight, and type (slab or pellet) of sugar-free gum, CPP-ACP dose, and number of treatments. The appliances were worn four times daily for the 20 minutes of gum chewing and for 20 minutes for the following 14 days. The enamel blocks were removed from the appliances for processing. Microradiographic analyses of the enamel lesions were evaluated using appropriate computerised imaging software. 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. These results indicated that the addition of CPP-ACP to sugar-free chewing gum significantly enhanced remineralisation of enamel subsurface lesions in a dose-related manner, independent of gum weight or type. Schirrmeister et al (2007) observed less enamel remineralisation for an equivalent chewing gum. This can be due to less direct contact with chewing gums, as they mounted the enamel slabs in buccal resin wings of mandibular appliances. In a recent study, 12 volunteers wore intra-oral appliances all day long, with specimens mounted lingually in the lower jaw and chewed three chewing gums containing urea (Itthagarun et al, 2005). One of the chewing gums contained no calcium phosphate, one dicalcium phosphate and another one CPP-ACP. Using the chewing gum without calcium phosphate, lesion depth decreased significantly less, and mineral gain was lower compared with the other two gums. It may be argued again that the lingual position of the specimens led to this significant difference. However, these authors did not find any significant difference between the chewing gums containing dicalcium phosphate and the CPP-ACP gums either. In the chewing gum studies, two randomised, double-blind cross over remineralisation trials were conducted with three pellet and three slab sugar-free gums containing different forms of calcium, including CPP-ACP (Reynolds et al, 2003). According to the results, the gum containing the CPP-ACP, although not containing the most calcium per piece of gum, produced the highest level of enamel remineralisation independent of gum-chewing frequency and duration. The CPP could be detected in plaque extracts three hours after subjects chewed the CPP-ACP-containing gum. These results highlight the importance of CPP in delivering ACP to the tooth surface. Iijima and others showed that sugar-free gum containing CPP-ACP produced approximately twice the level of remineralisation as the control sugar-free gum (Iijima et al, 2004). The remineralised enamel was more resistant to decalcification than native enamel. The eight- and 16-hour acid challenge of the lesions remineralised with the control gum resulted in

65.4% and 88.0% reductions, respectively, of deposited mineral, while for the CPP-ACP-remineralised lesions the corresponding reductions were 30.5% and 41.8%. (Iijima et al, 2004). The 54 mg CPP-ACP sugar-free gum significantly slowed progression and enhanced regression of approximal caries relative to a control sugar-free gum in a 24-month clinical trial on 2,720 adolescent patients (Morgan et al, 2008). Chewing with gum containing citric acid and CPP-ACP resulted in significantly higher remineralisation (13%) than chewing with either gum containing no CPP-ACP or citric acid (9.4%) or gum containing citric acid alone (2.6%). The acid challenge of the remineralised lesions showed that the level of mineral after acid challenge was significantly greater for the lesions exposed to the gum containing CPP-ACP (Cai et al, 2007). Cai and others (2009) compared the remineralisation efficacy of sugar-free chewing gums and observed that chewing with gum containing CPP (Trident Xtra Care) resulted in significantly higher remineralisation (20%) than chewing with gum containing calcium carbonate with added citric acid (Orbit Professional-12.43%) or gum without added calcium (Orbit-9.27% or Extra-9.32%) (Cai et al, 2009). Manton and colleagues (2009) also observed significantly greater remineralisation with the gum containing CPP (Trident White-18.4%) than gum without CPP (Orbit -8.9% and Orbit Professional -10.5% (Manton et al, 2008).

Mouth rinse

The fact that the remineralising effect is not confined to chewing gum as a vehicle for Recaldent™ was demonstrated by Cai et al (2003) with rinsing solutions, which also produced as much as 176% higher remineralisation than the controls (Shen et al, 2004). The ability of CPP-ACP to be retained in supragingival plaque and demineralise enamel subsurface lesions in situ when delivered in a mouth rinse was studied using four mouth rinses (Reynolds et al, 2003) Two of the mouth rinses contained CPP-ACP (2% w/v and 6% w/v, respectively), the third mouth rinse contained an unstabilised slurry of 60mM $CaCl_2$ and 40mM sodium phosphate. The fourth mouth rinse was de-ionized water and acted as the control. Only the CPP-ACP containing mouth rinses significantly increased plaque calcium and inorganic phosphate levels, and the CPP were immunolocalised to the surfaces of bacterial cells, as well as to the intercellular plaque matrix.

A human *in situ* caries model study evaluated the ability of a 1.0% CPP-ACP solution as a mouth rinse, twice daily, to prevent enamel demineralisation. It resulted in a 144% increase in calcium level and a 160% increase in inorganic phosphate level in the interenamel plaque recovered from the removable intraoral appliance used in the study. Moreover, CPP-ACP produced a 51 ± 19% reduction in enamel mineral loss caused by frequent sugar-solution exposure (Reynolds, 1998).

Lozenges

Microradiographs and densitometry have shown the use of lozenges with different CPP-ACP concentrations increases remineralisation in subsurface caries lesions, which is dose-dependent. Two percent CPP-ACP solutions have also shown their effectiveness in reducing subsurface caries lesions, obtaining higher remineralisation with longer application times (Cai et al, 2003).

Topical gels

Lennon and colleagues applied a tooth cream containing 5% casein/calcium phosphate to bovine enamel specimens for 120 seconds twice daily. They found no significant difference with respect to erosive loss after seven and 14 days of erosive cycling (Lennon et al, 2006). Higher remineralisation was observed in the specimens treated with Tooth Mousse GC after demineralization than the untreated specimens (Rahiotis and Vougiouklakis, 2007). In another *in vitro* study, the demineralisation of dentine was measured non-destructively using an ultrasonic pulse-echo method (Oshiro et al, 2007; Yamaguchi et al, 2006; Yamaguchi et al, 2007). The twice-daily application of 10-fold diluted CPP-ACP paste resulted in maintenance of a sonic velocity normal for sound dentin, in contrast to the reduced velocity in specimens treated with placebo paste, thus indicating that the CPP-ACP prevented dentine demineralisation.

Clinical cases of root caries lesions have been described which have been stabilised by fluoescence laser using a CPP-ACP paste (Tooth Mousse GC) (Vlacic et al, 2007). Enamel microabrasion together with prolonged use of a CPP-ACP based paste is useful for treating white spot enamel lesions (Ardu et al, 2007). The micro-abrasion can be performed with a 2 to 3 mm layer of abrasive paste containing

Table 1 Various clinical studies on CCP-APC complex

Author, year	Population	Intervention	Controls	Outcome	Conclusion
Shen et al, 2001 [Australia]	30 adults (age, 23–40 years)	*In situ*: gum with different concentrations of CPP-ACP (0, 0.19, 10, 18.8 and 56.4 mg)	Crossover washout 1 week	% subsurface remineralisation	Dose related increase in remineralisation with CPP-ACP and sorbitol or xylitol-based sugar-free gum
Cai et al, 2003 [Australia]	10 adults (age 34 ± 6.6 years)	*In situ:* 3 lozenge types with CPP-ACP (0%, 1% and 3% weight per weight ratio)	No lozenge	% subsurface remineralisation	Lozenges may be suitable for delivery of CPP-ACP to promote enamel remineralisation (dose related)
Reynolds et al, 2003 [Australia]	30 adults (age, 22–44 years)	Mouth rinse: 2% CPP-ACP, 6% CPP-ACP, calcium and phosphate mixture deionized water; sugar-free gum; *in situ;* $CaCO_3$, $CaHPO_4/CaCO_3$ or CPP-ACP	Crossover washout 4 weeks for mouthrinse; not needed for gum	Mouth rinse; plaque calcium and inorganic phosphate levels; gum: % subsurface remineralization	Importance of CPP in delivering ACP to tooth surface and stabilising ACP
Iljlma et al, 2004 [Australia]	10 adults (age, 32.3±7.9 years)	*In situ:* sugar-free gum containing 18.8 mg CPP-ACP	Control; sugar-free gum lacking CPP-ACP; crossover washout 1 week	% subsurface remineralisation	Sugar-free gum and CPP-ACP effective in remineralisation
Walker et al, 2006 [Australia]	10 adults	*In situ:* subjects drank 200 ml control milk or test milk with 2 or 5 gm CPP-ACP/L	Crossover washout 1 week	Subsurface remineralisation	More remineralising ability for milk and CPP-ACP
Cai et al, 2007 [Australia]	10 subjects (age, 23–46 years)	*In situ:* 3 sugar-free gums: 20 mg citric acid, 18.8 mg CPP-AC, 20 mg citric acid; no added ingredient	Crossover washout 1 week	% subsurface remineralisation	Significantly greater ($P < 0.05$) mineral level after acid challenge with ACP-CPP
Anderson et al, 2007 [Sweden]	26 adolescents, 60 teeth, (age 14.6 years)	*In vivo:* CPP-ACP paste daily for 3 months, then fluoride paste daily for 3 months	Daily 0.05% NaF mouth rinse and fluoride paste for 6 months	Blind assessment of clinical and laser fluorescence of white spots at 1, 3, 6 and 12 months	Both treatments reversed white spots; better visual outcome for test
Schirrmeister et al, 2007 [Germany]	15 subjects (age, 27.5 ± 2.5 years)	*In situ:* 4 sugar-free gums: without zinc citrate; with zinc citrate and dicalcium phosphate, calcium gluconate, calcium lactate: with CPP-ACP; no calcium	chewed test gums 14 days each: control subjects wore appliances without chewing gum	Lesion depth reduction and mineral change	No additional remineralising benefit even with gum and CPP-ACP; mandibular buccal appliances may have resulted in less direct contact of slabs with gum
Hay and Thompson, 2002 [New Zealand]	124 subjects with salivary gland dysfunction (age, 53 ± 14 years)	*In vivo:* Self-administered topical CD-CP mouth rinse 3 times daily	Self-administered topical 0.05% NaF mouth rinse 3 times daily	Coronal caries increment (bitewing radiographs at baseline and 12 months)	CD-CP may be useful for caries prevention in dry mouth syndrome

Table 1 Various clinical studies on CCP-APC complex

Author, year	Population	Intervention	Controls	Outcome	Conclusion
Ithagarun et al, 2005 [Hong Kong]	12 adults (age, 20–47 years)	*In situ;* gum with 30 mg urea and no calcium phosphate, 25 mg dicalcium phosphate dihydrate or 47 mg CPP-ACP	Crossoverwash-out 5 days	Mean % change in lesion depth	Caries preventive potential of urea-containing gum and dicalcium phosphate or CPP-ACP
Kowalczyk et al, 2006 [Poland]	13 patients with dentine hypersensitivity (age, 23–48 years)	GC Tooth Mousse applied on surfaces for 3 minutes	None	Pain intensity at baseline; testing soon after applying GC Tooth Mousse, and at 15 minutes, 1 week and 4 weeks after application	Insufficient effectiveness and short-term therapeutic effect in soothing pain
Hay and Morton, 2003 [New Zealand]	38 adults with severe xerostomia (age, older than 25 years)	*In vivo:* CD-CP for 14 days	Patients mouth moistening strategies (sipping water, chewing gum, artificial saliva)	Questionnaire about benefit	Potential benefits of CD-CP mouth rinse in oral moistening and dental caries prevention in xerostomia
Morgan et al, 2008 [Australia]	2,720 students, (age 11.5–13.5 years)	Sugar-free gum containing 54 mg CPP- ACP	Control; sugar-free gum lacking CPP-ACP, 24 month study	Standardised digital bitewing radiographs	CPP-ACP significantly slowed progression and enhanced regression of enamel caries on approximal surfaces
Rao et al, 2009 [India]	150 school children	Toothpastes containing 2% w/w CPP or containing 1,190 mg/kg fluoride as 0.76% sodium monofluorophosphate (SMFP)	Control; placebo toothpaste without CPP or fluoride	Oral hygiene and caries experience were assessed at baseline, and 12 and 24 months.	Toothpaste containing 2% CPP seemed to have an efficacy similar to paste containing 1,190 mg/kg SMFP in the prevention of caries
Bailey et al, 2009 [Australia]	408 white spot lesions, 45 post orthodontic patients (age, 12–18 years)	Cream containing CCP-ACP complex twice daily after fluoridated tooth paste	Control: placebo paste	Lesion regression in 12 weeks	More regression of lesions
Zhou et al, 2009 [China]	White spot lesions in 10 orthodotic patients (age 17.7 years)	10% CCP-ACP paste twice daily	None	Intraoral image analysis for 2 months	More regression of lesions

silicon carbide microparticles in water-soluble paste and 6.6% hydrochloric acid with slight pressure for 60 to 120 seconds. The paste containing CCP-ACP is then applied onto the treated enamel surface and left undisturbed for 15 minutes and finally removed by aspiration, but not water sprayed. The patient is instructed to apply the paste after brushing, twice a day, after breakfast and just before bedtime. The best results were achieved when salivation was prevented for a few minutes. Studies on patients with post-orthodontic demineralised white spots, showed that daily application of a CPP-ACP cream for three months, followed by brushing with a fluoride toothpaste for three months achieved complete removal of the spots after monitoring for 12 months (Anderson et al, 2007). In an *in vitro*

study, Tooth Mousse GC reduced demineralisation around orthodontic brackets, especially when the brackets were cemented with resin-reinforced glass ionomer cement (Sudjalim et al, 2007). Kumar and co-workers (2008) observed the effect of casein phosphopeptide-amorphous calcium phosphate on remineralisation of artificial caries-like lesions. They found that the CCP-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 a clinical trial on 150 school children, Rao et al (2009) reported that CPP can be effectively incorporated into calcium carbonate-based toothpaste and that toothpaste containing CPP is effective in preventing caries. Toothpaste containing 2% CPP seemed to have an efficacy similar to paste containing 1,190 mg/kg sodium monofluorophosphate (SMFP) in the prevention of caries. Bailey and others conducted a clinical trial in a post-orthodontic population using fluoride toothpastes and receiving supervised fluoride mouth rinses, and observed that more lesions regressed in participants using a remineralising cream containing casein phosphopeptide-amorphous calcium phosphate compared with a placebo over 12 weeks in 408 white-spot lesions (Bailey et al, 2009). Zhou and others quantify the changes in post-orthodontic demineralised enamel lesion surface areas after using casein phosphopeptide-amorphous calcium phosphate in ten orthodontic patients. The mean reduction in lesion size after treatment was 4.89% for one month, and 8.359% for two months. They concluded that CPP-ACP can effectively improve the long-standing post-orthodontic demineralised enamel white lesion (Zhou et al, 2009).

Glass ionomer cement

Studies have also been done to investigate the effect of adding the active complex to glass ionomer cements. Incorporation of 1.56% w/w CPP-ACP into the GIC was shown to increase compressive strength and microtensile bond strength, enhance the release of calcium, phosphate, and fluoride ions, and enhance protection of the adjacent dentin to acid demineralisation (Mazzaoui et al, 2003).

Sprays

Hay and co-workers observed good moistening and lubrication with the CD-CP mouth rinse, when used as an atomised spray in the mouth. CCP-ACP preparations hold promise as caries preventive agents for individuals with dry mouth (Hay and Thomson, 2002).

Energy drinks

Introducing CPP-ACP nanocomplexes to soft drinks and other frequently consumed acid products, especially for the adolescent and young adult population, could help to reduce the erosive action of these products (Ramalingam et al, 2005). The addition of 2·0-5·0 g CPP-ACP/l to milk substantially increases its ability to remineralise enamel subsurface lesions (Walker et al, 2006). The remineralising effect of CPP-ACP in milk was dose-dependent with milk containing 0.2% CPP-ACP and 0.3% CPP-ACP producing an increase in mineral content of 81% and 164%, respectively, relative to the control milk (Walker et al, 2009). Casein phosphopeptide-amorphous calcium phosphate (2%) is an important constituent of T.F.S.D.(Tooth Friendly Soft Drink) (Kolahi et al, 2009).

SOME RECOMMENDED PROFESSIONAL APPLICATIONS FOR CPP-ACP COMPLEX

These are white spot prevention/removal in orthodontics, bleaching, following professional tooth cleaning, after application of topical fluoride, and to provide a topical coating for patients suffering from erosion, caries, and conditions arising from xerostomia (GC Europe, Recaldent).

In view of its broad spectrum of action and virtually unlimited usability, CCP complex can be of use to all patients at any time – from infants through senior citizens. In gerostomatology the necks of the teeth are often a problem because of recession. If root caries is present, the cream together with toothpaste can promote remineralisation as part of preventive treatment. Professional tooth cleaning and root smoothing in periodontology can often result in hypersensitivity of the neck of the teeth, which can be controlled very quickly with the new paste. The CCP-ACP complex not only reduces the formation of caries, but it also brings about a marked reduction in hypersensitivity. In restorative dentistry, the sensitivity of prepared abutment

teeth can be reduced. Mineralisation of the dentinal tubule openings recloses the tubules and rapidly reduces the hypersensitivity at the neck of the teeth. The remineralisation of lesions in this respect is dose-dependent and is enhanced by fluorides. It is not only children who now benefit from this effective protection. As people are now retaining their own teeth longer and longer, adults and the elderly also require life-long protection for their dentition. During orthodontic treatments, the risk of caries is increased or there are enamel lesions left after treatment, which can be remineralised with the tooth protection cream (Moule et al, 2007; Kecik et al, 2008; Sudjalim et al, 2006).

Remineralisation could be used to prevent and cure the early natural enamel caries of fluorosed teeth (Luo et al, 2009) The use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) may enhance remineralisation and decrease postoperative sensitivity following tooth whitening and microabrasion procedures in hypomineralised teeth (Ng and Manton, 2007). Tooth Mousse may be applied concurrently with the bleach without reducing bleaching effectiveness (Manton, 2008). Topical applications of CPP-ACP could be effective in promoting enamel remineralisation after interdental stripping (Giulio, 2009).

It appears that CCP-ACP preparations hold promise as caries preventive agents for individuals with dry mouth (Hay and Thomson, 2002). Leaving aside considerations such as dental caries, adequate mouth moistening is a key contributor to the quality of life for sufferers of dry mouth. The acceptability of the CCP-ACP flavour means that it could be used to moisten the mouth throughout the day, and it would have a clear advantage over a fluoride-based preparation because it could be swallowed instead of spat out. The CCP-ACP formulation is nontoxic. However, clinicians should consider potential side effects from ingestion of casein derivative protein in people with immunoglobulin E allergies to milk proteins, (although CCP-ACP is digestible by people with lactose intolerance.) Initial clinical trials in patients with severe xerostomia have also revealed positive results in terms of caries prevention (Hay and Thomson, 2002) and mouth moistening (Hay and Thomson, 2002; Hay and Morton, 2003).

CPP-amorphous calcium phosphate (CPP-ACP) preparation may have a potential use as a transport medium for avulsed teeth. When highly diluted, the CPP-ACP preparation may help preserve L929 cell viability in the short term without inducing apoptosis (Cehreli et al, 2008).

CRITICAL DISCUSSION

Although the remineralisation potential of CCP-ACP complex has been observed, some points are still worth mentioning. There are fourteen studies in the literature on CCP-ACP complex [Iijima et al, 2004; Shen et al, 2001; Cai et al, 2003; Reynolds et al, 2003; Walker et al, 2006; Cai et al, 2007; Itthagarun et al, 2005; Schirrmeister et al, 2007; Morgan et al, 2008; Hay KD and Homson WM, 2002; Andersson et al, 2007; Rao et al, 2009; Bailey et al, 2009; Zhou et al, 2009). Six of them were conducted by the same group of investigators who patented the CPP-ACP complex. These six studies followed the same in situ protocol that resulted in significant findings in favour of this technology (Iijima et al, 2004; Shen et al, 2001; Cai et al, 2003; Reynolds et al, 2003; Walker et al, 2006; Cai et al, 2007).

Of the eight studies conducted by groups independent of those that patented the complex, one used a similar *in situ* model (Itthagarun et al, 2005). Although the findings point to significant caries preventive potential of CPP-ACP when added to urea-containing chewing gum, the study found no difference in outcome between CPP-ACP and dicalcium phosphate dehydrate. The second independent in situ study found no significant difference between chewing gums that contained or did not contain calcium with regard to both mineral change and depth of demineralized lesions (Schirrmeister et al, 2007). But they used only 15 subjects over 2 weeks, with different conditions in terms of placement of the slabs. Morgan et al (2008) conducted a large-scale clinical trial involving 2,720 subjects over 24 months. This large study obviates the concerns raised about the site of placement of enamel slabs in *in situ* models. This was the formal evidence that CPP-ACP could significantly slow progression and enhanced regression of enamel caries on approximal surfaces (Morgan et al, 2008). In the fourth independent study that was an *in vivo* trial, no difference was observed between a NaF mouthrinse and a CD-CP group (Hay KD and Homson WM, 2002). However, the number of teeth lost was higher in CD-CP group, while the material used in the Hay and Thomson study was the not same as CPP-ACP as in Tooth Mousse/ MI Paste. The results of the fifth independent study, which was conducted in Sweden, were conflicting (Andersson et al, 2007). They found that by using clinical scoring and laser fluorescence assessment, both CPP-ACP cream and 0.05% NaF mouth rinses could promote regres-

sion of white spot lesions after debonding of fixed orthodontic appliances. The visual evaluation suggested an aesthetically more favourable outcome of the CPP-ACP treatment. In the sixth independent study, i.e., a clinical trial on 150 school children, Rao and others reported that CPP can be effectively incorporated into calcium carbonate-based toothpaste and that toothpaste containing CPP is effective in preventing caries. Toothpaste containing 2% CPP seemed to have an efficacy similar to paste containing 1,190 mg/kg SMFP in the prevention of caries (Rao et al, 2009). Bailey and others conducted a clinical trial in a post-orthodontic population and observed regression of more lesions by a remineralising cream containing casein phosphopeptideamorphous calcium phosphate compared with a placebo (Bailey et al, 2009). Zhou and others concluded that CPP-ACP could effectively improve the long-standing postorthodontic demineralised enamel white lesion (Zhou et al, 2009).

In an *in vitro* study Pulido et al (2008) also did not observe any inhibitory effect of CCP-ACP complex (MI Paste) on the progression of artificial caries-like lesions in enamel (Pulido et al, 2008). The discussion of the Pulido study overlooks the fact that the laboratory model used did not include contact with human saliva, had no triggers for the release of calcium and other ions from CPP-ACP, and was inherently biased towards simple precipitation of fluoride from high concentration NaF products. None of the studies has tested for the potential formation of calculus resulting from the supersaturated calcium phosphate state in plaque.

CONCLUSION

Caries management by risk assessment (CAMBRA) focuses on treating and preventing the cause of the disease at an early stage, rather than waiting until it causes damage to tooth structure (Young et al, 2009). The calcium phosphate-based remineralisation technologies show promise as adjunctive treatments to fluoride therapy in the non-invasive management of early caries lesions. Remineralisation of early carious lesions by CCP-ACP complex may continue to emerge in importance as fluoride did in the past for caries prevention and reduction. Efforts have focused on reducing the risk of caries in patients, and have highlighted the importance of a "partnership" approach between patients and dentists in order to ensure ultimate success in the control of caries. There is accumulating evidence from controlled clinical studies which supports the effectiveness of casein derivatives, specifically CCP-ACP in caries prevention and lesion reversal. Further research is required to provide a scientifically supported recommendation for other clinical applications.

REFERENCES

1. Adebayo OA, Burrow MF, Tyas MJ. Effects of conditioners on microshear bond strength to enamel after carbamide peroxide bleaching and/or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatment. J Dent 2007;35:862-870.
2. Adebayo OA, Burrow MF, Tyas MJ. Dentine bonding after CPP-ACP paste treatment with and without conditioning. J Dent 2008; 36:1013-24.
3. Adebayo OA, Burrow MF, Tyas MJ. A SEM evaluation of conditioned and bonded enamel following carbamide peroxide bleaching and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatment. J Dent 2009;37:297-306.
4. Adebayo OA, Burrow MF, Tyas MJ. Resin-dentine interfacial morphology following CPP-ACP treatment. J Dent 2009;38:96-105.
5. Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr 2004;134:989-995.
6. Anderson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. Oral Health Prev Dent 2007;5:229-233.
7. Anderson EP, Smith JK, Elvehjem CA, Phillips PH. Dental caries in the cotton rat; the effect of fluidity of the ration. J Nutr 1948;35:371-377.
8. Ardu S, Castioni NV, Benbachir N, Krejci I. Minimally invasive treatment of white spot enamel lesions. Quint Int 2007;38:633-636.
9. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, Reynolds EC, Morgan MV. Regression of Post-orthodontic Lesions by a Remineralizing Cream. J Dent Res 2009;88:1148-1153.
10. Bibby BG, Averill HM. Caries control through phosphates. J Dent Res 1963;42:477-487.
11. Bibby BG, Huang CT, Zero D, Mundorff SA, Little MF. Dental caries in the cotton rat; the effect of fluidity of the ration. J Nutr 1980;35:371-377.
12. Bowen WH, Amsbaugh SM, Monell-Torrens S, Brunelle J, Kuzmiak-Jones H, Cole MF. A method to assess cariogenic potential of food-stuffs. J Am Dent Assoc 1980;100:677-681.
13. Bunting R. Bacteriological, chemical and nutritional studies of dental caries by the Michigan Research Group: A summary. J Dent Res 1934;17: 97-105.
14. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralisation of enamel subsurface lesions *in situ* by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aus Dent J 2003;48:240-243.
15. Caruana PC, Mulaify SA, Moazzez R, Bartlett D. The effect of casein and calcium containing paste on plaque pH following a subsequent carbohydrate challenge. J Dent 2009;37:522-526.

16. Cehreli SB, Gurpinar AO, Onur AM, Dagli FT. *In vitro* evaluation of casein phosphopeptide-amorphous calcium phosphate as a potential tooth transport medium: viability and apoptosis in L929 fibroblasts. Dent Traumatol 2008;24:314-319.
17. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res 2008;42:88-97.
18. Crisp S, Lewis BG, Wilson AD. Glass ionomer cements: chemistry of erosion. J Dent Res 1976;55:1032-1041.
19. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physiochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. J Biol Chem 2005;280:15362-15369.
20. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptides in oral health – chemistry and clinical applications. Curr Pharm Des 2007;13:793-800.
21. de Rooij JF, Nancollas GH. The formation and remineralization of artificial white spot lesions: A constant composition approach. J Dent Res 1984;63:864-867.
22. Elsayad I, Sakr A, Badr Y. Combining casein phosphopeptide-amorphous calcium phosphate with fluoride: synergistic remineralisation potential of artificially demineralized enamel or not? J Biomed Opt 2009;14:044039.
23. Ferrazzano GF, Cantile T, Ingenito A, Chianese L, Quarto M. New strategies in dental caries prevention: experimental study on casein phosphopeptides. Eur J Paediatr Dent 2007;8:183-187.
24. Ferrazzano GF, Cantile T, Quarto M, Ingenito A, Chianese L, Addeo F. Protective effect of yogurt extract on dental enamel demineralization *in vitro*. Aust Dent J 2008;53:314-319.
25. GC Europe Recaldent Tooth Mousse Product Manual. GC Corporation, Tokyo, Japan.
26. Giulio AB, Matteo Z, Serena IP, Silvia M, Luigi C. *In vitro* evaluation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) effect on stripped enamel surfaces. A SEM investigation. J Dent 2009;37:228-232.
27. Guggenheim B, Schmid R, Aeschlimann JM, Berrocal R, Neeser JR. Powdered milk micellar casein prevents oral colonization by *Streptococcus sobrinus* and dental caries in rats: a basis for the caries – protective effect of dairy products. Caries Res 1999;33:446-454.
28. Harper DS, Osborn JC, Clayton R, Hefferren JJ. Cariostatic evaluation of cheeses with diverse physical and compositional characteristics. Caries Res 1986; 20:123-130.
29. Hay KD, Morton RP. The efficacy of casein phosphoprotein-calcium phosphate complex (DC-CP) [Dentacal] as a mouth moistener in patients with severe xerostomia. N Z Dent J 2003;99:46-48.
30. Hay KD, Thomson WM. A clinical trial of the anticaries efficacy of casein derivatives complexed with calcium phosphate in patients with salivary gland dysfunction Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93:271-275.
31. Holler BE, Friedl K-H, Jung H, Hiller KA, Schmalz G. Fluoride uptake and distribution in enamel and dentin after application of different fluoride solutions. Clin Oral Invest 2002;6:137-144.
32. Holt C, van Kemenade MJJM. The interaction of phosphoproteins with calcium phosphate. In: Hukins DWL (ed). Calcified tissue. Boca Raton, FL: CRC Press, 1989:175-213.
33. Holt C, Wahlgren NM, Drakenberg T. Ability of a b-casein phosphopeptide to modulate the precipitation of calcium phosphate by forming amorphous dicalcium phosphate nanoclusters. Biochem J 1996;314:1035-1039.
34. Hubbell R, Bunting R. Calcium and phosphorous of saliva in relation to dental caries. J Nutr 1932;5:599-605.
35. Huq NL, Cross KJ, Reynolds EC. Molecular modelling of a multiphosphorylated sequence motif bound to hydroxyapatite surfaces. J Mol Mode 2000;6:35-47.
36. Huq NL, Cross KJ, Reynolds EC. Molecular modelling of the multiphosphorylated casein phosphopeptide alpha s1-casein (59-79). J Dairy Res 2004;71:1-5.
37. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptideamorphous calcium phosphate (CPP-ACP). Caries Res 2004;38:551-556.
38. Itthagarun A, King NM, Yiu C, Dawes C. The effect of chewing gums containing calcium phosphates on the remineralization of artificial caries like lesions in situ. Caries Res 2005;39:251-254.
39. Kecik D, Cehreli SB, Sar C, Unver B. Effect of acidulated phosphate fluoride and casein phosphopeptide-amorphous calcium phosphate application on shear bond strength of orthodontic brackets. Angle Orthod 2008;78:129-133.
40. Kolahi J, Fazilati M, Kadivar M. Towards tooth friendly soft drinks. Med Hypotheses 2009;73:524-525.
41. Krobicka A, Bowen WH, Pearson S, Youn DA. The effects of cheese snacks on caries in desalivated rats. J Dent Res 1987;66:1116-1119.
42. Kumar VL, Ittagarun A, King NM. The effect of casein phosphopeptide–amorphous calcium phosphate on remineralization of artificial caries-like lesions: an *in vitro* study. Aust Dent J 2008;53:34-40.
43. Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a casein/calcium phosphate-containing tooth cream and fluoride on enamel erosion *in vitro*. Caries Res 2006;40:154-157.
44. Luo LY, Wang Y, Li H, Zheng H, Gao SJ. Study on remineralization of human fluorosed teeth *in vitro*. Hua Xi Kou Qiang Yi Xue Za Zhi 2009;27:96-99.
45. Manton DJ, Bhide R, Hopcraft MS, Reynolds EC. Effect of ozone and Tooth Mousse on the efficacy of peroxide bleaching. Aust Dent J 2008;53: 128-132.
46. Matsuya S, Matsuya Y, Yamamoto Y, Yamane M. Erosion process of glass ionomer cement in organic acids. Dent Mat J 1984;3:210-219.
47. Mazzaoui SA, Burrow MF, Tyas MJ, Dashper SG, Eakins D, Reynolds EC. Incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass-ionomer cement. J Dent Res 2003;82:914-918.
48. Mellanby M. Diet and the teeth: an experimental study. Part II. A Diet and dental disease B Diet and dental structure in mammals other than the dog. Medical Research Council. London: His Majesty's Stationery Office Special report series No. 153 1930 162-278.
49. Mellanby M, Lee C, Pattison. The effect of diet on the environment and extension of caries in the teeth of children. Brit Med J 1924;2:354-355.
50. Meyers IA. Diagnosis and management of the worn dentition: risk management and pre-restorative strategies for the oral and dental environment. Ann R Australas Coll Dent Surg 2008;19:27-30.
51. Morgan MV, Adams GG, Bailey DL, Tsao CE, Fischman SL, Reynolds EC. 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.

52. Moule CA, Angelis F, Kim GH, Le S, Malipatil S, Foo MS, Burrow MF, Thomas D. Resin bonding using an all-etch or self-etch adhesive to enamel after carbamide peroxide and/or CPP-ACP treatment. Aus Dent J 2007;52: 133-137.
53. Navia JM, Lopez H. Rat caries assay of reference foods and sugar-containing snacks. J Dent Res 1983;62:893-898.
54. Neeser JR, Golliard M, Woltz A, Rouvet M, Dillmann ML, Guggenhiem B. *In vitro* modulation of oral bacterial adhesion to saliva-coated hydroxypatite beads by milk casein derivatives. Oral Microbiol Immunol 1994;9:193-201.
55. Neuhaus KW, Lussi A. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and its effect on dental hard tissues. Schweiz Monatsschr Zahnmed 2009;119:110-116.
56. Ng F, Manton DJ. Aesthetic management of severely fluorosed incisors in an adolescent female. Aus Dent J 2007;52:243-248.
57. Nyvad B, Fejerskov O. Experimentally induced changes in ultrastructure of pellicle on enamel *in vivo*. In: Ten Cate JM, Leach SA/(eds)., Bacterial adhesion and preventive dentistry. Oxford: IRL Press Ltd, 1984:143-151.
58. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. J Oral Sci 2007;49:115-120.
59. Panich M, Poolthong S. The effect of casein phosphopeptide–amorphous calcium phosphate and a cola soft drink on in vitro enamel hardness. J Am Dent Assoc 2009;140:455-460.
60. Pearce EIF, Bibby BG. Protein adsorption on bovine enamel. Arch Oral Biol 1966;11:329-336.
61. Perich JW, Reynolds EC. The use of synthetic phosphopeptides for epitope mapping of the αs1- casein phosphopeptide segment Bioorg Med Chem Lett 1992; 2:1153-1154.
62. Piekarz C, Ranjitkar S, Hunt D, McIntyre J. An *in vitro* assessment of the role of Tooth Mousse in preventing wine erosion. Aust Dent J 2008;53:22-25.
63. Pulido MT, Wefel JS, Hernandez MM, Denehy GE, Guzman-Armstrong S, Chalmers JM, Qian F. The inhibitory effect of MI paste, fluoride and a combination of both on the progression of artificial caries-like lesions in enamel. Oper Dent 2008;33:550-555.
64. Rahiotis C, Vougiouklakis G. Effect of a CPP-ACP agent on the demineralization and remineralization of dentine *in vitro*. J Dent 2007;35:695-698.
65. Rahiotis C, Vougiouklakis G, Eliades G. Characterization of oral films formed in the presence of a CPP-ACP agent: an *in situ* study. J Dent 2008;36:272-80.
66. Ramalingam L, Messer LB, Reynolds EC. Adding casein phosphopeptide-amorphous calcium phosphate to sports drinks to eliminate *in vitro* erosion. Pediatr Dent 2005; 27:61-67.
67. Ranjitkar S, Kaidonis JA, Richards LC, Townsend GC. The effect of CPP-ACP on enamel wear under severe erosive conditions. Arch Oral Biol 2009;54:527-532.
68. Ranjitkar S, Narayana T, Kaidonis JA, Hughes TE, Richards LC, Townsend GC. The effect of casein phosphopeptide-amorphous calcium phosphate on erosive dentine wear. Aust Dent J 2009;54:101-107.
69. Ranjitkar S, Rodriguez JM, Kaidonis JA, Richards LC, Townsend GC, Bartlett DW. The effect of casein phosphopeptide-amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion. J Dent 2009;37:250-254.
70. Rao SK, Bhat GS, Aradhya S, Devi A, Bhat M. Study of the efficacy of toothpaste containing casein phosphopeptide in the prevention of dental caries: a randomized controlled trial in 12- to 15-year-old high caries risk children in Bangalore, India. Caries Res 2009;43:430-435.
71. Rees J, Loyn T, Chadwick B. Pronamel and Tooth Mousse: an initial assessment of erosion prevention *in vitro*. J Dent 2007;35:355-357.
72. Rolla G, Rykke M. Evidence for presence of micelle-like protein globules in human saliva. Colloid Surface B Biointerfaces 1994;3:177-182.
73. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. J Dent Res 1987;66:1120-1127.
74. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587-1595.
75. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist 1998;18:8-16.
76. Reynolds EC. Anticariogenic casein phosphopeptides. Prot Peptide Lett 1999;6:295-303.
77. Reynolds EC. Dairy components in oral health. Aus J Dairy Tech 2003; 58:79-81.
78. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J 2008;53:268-273.
79. Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence. Adv Dent Res 2009; 21:25-29.
80. Reynolds EC, Black CL. Reduction of chocolate's cariogenicity by supplementation with sodium caseinate. Caries Res 1987a; 21:445-451.
81. Reynolds EC, Black CL. Confectionery composition and rat caries. Caries Res 1987b;21:538-545.
82. Reynolds EC, Black CL, Cross KJ, Eakins D, Huq NL, Morgan MV, Nowicki A, Perich JW, Riley PF, Shen P, Talbo G, Webber FW. Advances in enamel remineralization: anticariogenic casein phosphopeptide-amorphous calcium phosphate. J Clin Dent 1999;10:86-88.
83. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2008;87:344-8.
84. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouth rinse or sugar-free chewing gum. J Dent Res 2003;82:206-211.
85. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, Perich JW. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995;74:1272-1279.
86. Reynolds EC, del Rio A. Effect of casein and whey-protein solutions on caries experience and feeding patterns of the rat. Arch Oral Biol 1984;29:927-933.
87. Reynolds EC, Johnson IH. Effect of milk on caries incidence and bacterial composition of dental plaque in the rat. Arch Oral Biol 1981;26:445-451.
88. Reynolds EC, Riley PF, Adamson NJ. A selective precipitation purification procedure for multiple phosphoseryl-containing peptides and methods for their identification. Anal Biochem 1994;217:277-284.

89. Rolla G, Rykke M. Evidence for presence of micelle-like protein globules in human saliva. Colloid Surface B Biointerfaces 1994;3:177-182.
90. Rose RK. Binding characteristics of streptococcus mutans for calcium and casein phosphopeptide. Caries Res 2000a; 34:427-431.
91. Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol 2000b; 45:569-575.
92. Rosen S, Min DB, Herper DS, Herper WJ, Beck EX, Beck FM. Effect of cheese, with and without sucrose, on dental caries and recovery of S*treptococcus mutans* in rats. J Dent Res 1984;63:894-896.
93. Schirrmeister JF, Seger RK, Altenburger MJ, Lussi A, Hellwig E. Effects of various forms of calcium added to chewing gum on initial enamel carious lesions *in situ*. Caries Res 2007;41:108-114.
94. Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. J Dent Res 1996;75:1779-1788.
95. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80:2066-2070.
96. Shen P, Cai F, Walker G, Reynolds C, Reynolds EC. Enamel remineralization by a mouthrinse containing casein phosphopeptide-amorphous calcium phosphate and fluoride in an *in situ* model. Aus Dent J Special Research Suppl 2004;49:4.
97. Silva MfdeA, Burgess RC, Sandham HJ, Jenkins GN. Effects of water-soluble components of cheese on experimental caries in humans. J Dent Res 1987; 66: 38-41.
98. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. Aus Dent J 2006; 51:284-289.
99. Tantbirojn D, Huang A, Ericson MD. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36:74-79.
100. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36:74-79.
101. Ung M, Huq NL, Cross KJ, Reynolds EC. Investigation of the binding of casein phosphopeptides to the major enamel pellicle proteins Aus Dent J Special Research Suppl 2004;49:4.
102. Vanthana S, Michael B, Yasushi S, Junji T. Resin bonding to dentine after casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatments. J Adhes Sci Tech 2009;23:1149-1161.
103. Vitorino R, Lobo MJ, Duarte JR, Ferrer-Correia AJ, Domingues PM, Amado FM. The role of salivary peptides in dental caries. Biomed Chromatogr 2005;19:214-222.
104. Vlacic J, Meyers IA, Walsh LJ. Combined CPP-ACP and photoactivated disinfection (PAD) therapy in arresting root surface caries: a case report. Br Dent J 2007;203:457-459.
105. Walker G, Cai F, Shen P, Reynolds, Ward B, Christopher Fone C, Honda S, Koganei M, Oda M, Reynolds E. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. J Dairy Res 2006;73:74-78.
106. Walker GD, Cai F, Shen P, Bailey DL, Yuan Y, Cochrane NJ, Reynolds C, Reynolds EC. Consumption of milk with added casein phosphopeptide-amorphous calcium phosphate remineralizes enamel subsurface lesions in situ. Aust Dent J 2009;54:245-249.
107. Willershausen B, Schulz-Dobrick B, Gleissner C. *In vitro* evaluation of enamel remineralisation by a casein phosphopeptide-amorphous calcium phosphate paste. Oral Health Prev Dent 2009;7:13-21.
108. Wilson AD, McLean JW. Glass-ionomer cement Chicago, IL: Quintessence Publishing, 1988.
109. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Kurokawa H. Ultrasonic determination of the effect of casein phosphopeptide-amorphous calcium phosphate paste on the demineralization of bovine dentin. Caries Res 2007;41: 204-207.
110. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Moore BK. Effect of CPP-ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device. J Dent 2006;34:230-236.
111. Young DA, Kutsch VK, Whitehouse J. A clinician's guide to CAMBRA: a simple approach. Compend Contin Educ Dent 2009;30:92-98.
112. Zhou CH, Sun XH, Zhu XC. Quantification of remineralized effect of casein phosphopeptiode-amorphous calcium phosphate on post-orthodontic white spot lesion. Shanghai Kou Qiang Yi Xue 2009;18:449-454.
113. Zita AC, McDonald, Andrews AL. Dietary habits and the dental caries experience in 200 children. J Dent Res 1956;38:860-865.