

Recaldent™ (CPP-ACP)



Literature Overview

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Literature overview Recaldent™ (CPP-ACP)

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1. Dental caries in the cotton rat. VI. The effect of the amount of protein, fat and carbohydrate in the diet on the incidence and extent of carious lesions

Schweigert BS, Shaw JH, Zeppelin M and Elvehjem CA. J Nutr 31: 439-447 (1946)

Summary: The isocaloric substitution of 10 or 20 parts of lard for sucrose in a purified ration reduced the incidence and extent of carious lesions in the cotton rat in proportion to the amount of lard added.

When the casein content of the diet was increased from 24 to 50% at the expense of sucrose, some reduction in caries occurrence was observed.

When 50 parts of casein and 10 parts of lard were fed, the protective effect was additive. The number of cavities observed was comparable to that observed when the ration contained 20 parts of lard.

No carious lesions were noted when mineralized whole milk diets were fed. The incidence and extent of tooth decay were low when a ration approximating milk solids in composition was fed.

2. Effects of dietary composition on tooth decay in the albino rat

Shaw JH. J Nutr 41:13-23 (1950)

Summary: Weanling caries-susceptible albino rats were used as the experimental subjects in a series of investigations to determine whether the initiation and development of carious lesions would be influenced by dietary procedures already demonstrated to alter the incidence of tooth decay in caries-susceptible cotton rats. The isocaloric substitution of fat and protein for part of the sucrose in the purified ration resulted in substantial reductions in the incidence of tooth decay in comparison to that in the control rats. Administration of either mineralized fresh or evaporated milk as the sole source of nutrients resulted in extremely low degrees of dental decay. The addition of 10% of sucrose by weight to either mineralized fresh or evaporated milk did not result in any appreciable increase in tooth decay above the average for the animals on the comparable milk diets alone. Caloric restriction resulted in appreciably less tooth decay than in the control rats which were allowed to eat ad libitum. In each of the above results, the influence of dietary variation on the initiation and development of carious lesions in the caries-susceptible white rat is extremely comparable to the effect obtained with the same regimens in cotton rats. In contrast, the type of caging arrangement employed throughout the experimental period was not found to influence tooth decay in the white rat, unlike its strong influence in the cotton rat.

3. Protein factors and experimental rat caries

Bavetta LA and McClure FJ. Nutr 63: 107-117 (1957)

Summary: A cariogenic diet containing a roller-process skim milk powder remained highly cariogenic after supplementation with known vitamins and essential amino acids. Caries severity was significantly reduced, however, by the addition of 11% of casein in place of cornstarch to this diet.

A supplement of blood albumin proved as effective as L-lysine in the reduction of caries produced by a lysine-deficient skim milk powder diet. Diets containing 13% of casein developed a high incidence of severe caries but caries was very limited with 24% casein in the diet. This striking caries difference was accompanied by only a slight difference in rate of growth.

An autoclaved mixture of casein, lactose, and Hubbell, Mendel and Wakeman salts compared with unautoclaved casein was associated with an increased incidence of caries when the casein content of the diet was 13% but not when it was 24%.

An inhibitory effect of a lysine supplement on caries was observed using purified diets containing zein as a source of protein. The result supports prior evidence that the cariogenicity of diets containing heat-processed skim milk powders and deficient in lysine is due under some conditions to a critical deficiency of lysine.

The combined results of these studies suggest that the quantity of protein in the diet may be an important factor in the development of cariogenicity by experimental diets.

4. Calcium phosphate sequestering phosphopeptide from casein

Reeves RE and Latour NG. Science, Vol. 128, p. 472

Introduction: By the use of ion-exchange columns, a number of crude phosphopeptide fractions have been separated from pancreatic casein hydrolyzates. One of these fractions exhibits, to a remarkable degree, the property of sequestering calcium phosphate in the pH range from 7 to 10.5. Although numerous investigations have been made on phosphopeptides derived from casein by various enzymatic hydrolyses, no mention of this calcium phosphate sequestering property has been found in the literature.

The finding of a casein fragment with the property of solubilizing calcium phosphate, or preventing its precipitation at relatively high pH's, has obvious implications in regards to an understanding of the role of casein in calcium and phosphate transport and assimilation. Apart from its interest in this connection, the phosphopeptide is of practical use in preventing the formation of calcium phosphate precipitates in culture media containing relatively high concentrations of phosphate and calcium ion.

5. Effects of various sucrose-casein ratios in purified diets on the teeth and supporting structures of rats

Holloway PJ, Shaw JH and Sweeney EA. *Arch Oral Biol* Vol.3, pp.185-200, 1961

Purified diets of varying carbohydrate:casein ratios were fed to female rats of the Harvard caries-susceptible strain for a stabilizing period of 28 days and then throughout pregnancy and lactation. Offspring of females fed a low-protein: high-sucrose diet grew more slowly and their molar teeth erupted later than rats born to females fed a control diet with a normal protein content. The molars of the offspring of the protein-deficient females were significantly smaller than those of the controls. This change in size was due to a decrease in the distance between the outer borders of the dentine rather than to a reduction in enamel thickness. Many of the third molars of these rats had missing cusps. The offspring of the deficient females had a significantly higher susceptibility to caries than the offspring of the control females. Rats born to females fed a high-protein: low-sucrose diet had a tendency toward an increased susceptibility to a periodontal syndrome and were significantly less susceptible to dental caries than the offspring of females fed the control diet. There was also a slight tendency for a breakdown of the periodontal structures among the offspring of females fed the low-protein:high-sucrose diet, but the syndrome did not progress as rapidly as among the offspring of females on the high-protein:low-sucrose diet.

6. A review of the effect of milk on dental caries

Reynolds EC, Storey E. *Aust. J. Dairy Tech.* 34:175-179, 1979

Introduction: Milk contains all the essential nutrients required for the growth and development of bones and teeth. Whether milk is beneficial for teeth once they erupt however, is uncertain since it has been described as being both cariogenic (Gardner, Norwood and Eisenon, 1977; Shelton, Berkowitz and Forrester, 1977) and anticariogenic (Shaw, Ensfield and Wollman, 1959; Stephan, 1966). The reason for this apparent contradiction is due largely to the inherent problems of studying the effect of one item of food on dental health. Nevertheless, much circumstantial evidence is accumulating that, despite its content of potentially cariogenic lactose, milk contains factors which can contribute to the prevention of dental caries.

7. Effect of milk on caries incidence and bacterial composition of dental plaque in the rat

Reynolds EC and Johnson IH. *Arch Oral Biol* Vol. 26, p.p. 445 to 451, 1981

Summary: Supplementation of a cariogenic diet with pasteurized bovine milk substantially reduced the incidence of dental caries in both male and female Sprague-Dawley rats. The reduction in caries incidence was not associated with the consumption of less of the cariogenic diet and more milk, nor with the animals having a significantly altered bacterial composition of their dental plaque. The results lead to the hypothesis that the anticariogenic effect of milk is attributable to a direct chemical influence on the caries process in the oral environment of the rat.

8. Phosphoprotein inhibition of hydroxyapatite dissolution.

Reynolds EC, Riley PF, Storey E. *Calcif Tissue Int* 1982;34 Suppl 2:S52-6

Summary: A chromatography column containing hydroxyapatite beads was used to study the effect of different proteins on the rate of hydroxyapatite dissolution. The four phosphoproteins tested (phosvitin, alpha sI-casein, beta-casein and kappa-casein) markedly reduced the rate of hydroxyapatite dissolution. Three nonphosphorylated proteins had a relatively smaller effect. The effect of the protein in reducing the hydroxyapatite dissolution rate has been attributed to protein binding to the surface of hydroxyapatite. The reduction in dissolution rate, expressed as the change in nmol calcium released per min per nmol of phosphoprotein bound to hydroxyapatite, increased with increasing number of phosphoserine residues of the protein. The results are consistent with the proposition that phosphoproteins have a regulatory role in mineralization processes and could provide a mechanism by which dietary and salivary phosphoproteins exert an anticariogenic effect.

9. Effect of adsorbed protein on hydroxyapatite zeta potential and *Streptococcus mutans* adherence

Reynolds EC, Wong A. *Infect Immun* 1983 Mar;39(3):1285-90

The adherence of *Streptococcus mutans* PK1 to hydroxyapatite disks pretreated with various acidic and basic proteins in imidazole buffer was studied. Adsorption of a basic protein onto an hydroxyapatite disk enhanced or had no effect on bacterial adherence, whereas adsorption of an acidic protein reduced adherence. The effect of adsorbed protein on bacterial adherence was of both short and long range. The long-range effect of the acidic proteins in reducing the number of bacteria adhering to hydroxyapatite was related to protein adsorption causing an increase in surface net negative charge, as shown by zeta potential measurement. Basic protein produced a net positive surface charge which facilitated adherence. Within the acidic protein group, the acidic residue percentage of the adsorbed protein was negatively correlated with the number of bacteria adhering, whereas the nonpolar residue percentage was positively correlated with bacterial adherence. Within the basic protein group, the basic residue percentage was correlated with the number of cells adhering. These results indicate the involvement of short-range hydrophobic and ionic interactions in bacterial adherence to protein-coated hydroxyapatite.

10. Effect of casein and whey-protein solutions on caries experience and feeding patterns of the rat

Reynolds EC, del Rio A. *Arch Oral Biol* 1984;29(11):927-33

Summary: Casein (bovine milk phosphoprotein) at 2 per cent (w/v) in drinking water reduced the extent of fissure and smooth-surface caries of male Sprague-Dawley rats consuming a solid cariogenic diet. Whey protein (the non-phosphorylated protein group of bovine milk) also at 2 per cent (w/v) in the drinking water produced a smaller reduction and only of fissure caries. There was no significant difference in salivary-gland function (as determined by protein concentration), or in the amount or frequency of cariogenic diet consumed. The finding that a 2 per cent solution of whey protein reduced the extent of fissure caries in animals consuming a solid diet containing 26 per cent whey protein suggests that the anticariogenic action is mediated by the protein being in solution. These results suggest a topical anticariogenic action for dietary protein.

11. Effect of cheese, with and without sucrose, on dental caries and recovery of *Streptococcus mutans* in rats

Rosen S, Min DB, Harper DS, Herper WJ, Beck EX and Beck FM. *J Dent Res* 63(6):894-896, June, 1984

The objective of this study was to determinate the effect of aged and young cheddar cheese with and without added sucrose on dental caries and the associated recovery of implanted *Streptococcus mutans*. Very little caries was observed in rats consuming cheese without sucrose. There was an increase in caries in rats fed cheeses with 20% sucrose, but this increase was not significant. There was significantly greater caries activity in rats fed standard diets containing 20% or 5% sucrose (SLS or MIT 305) than in rats fed cheeses containing 20% sucrose. Rats fed cheese or powdered diets containing sucrose had significantly higher frequency of recovery and higher levels of *S.mutans* infection than did rats fed cheese containing no sucrose. This study confirms the low cariogenic potential and possible cariostatic activity of cheddar cheese in rats. Since cheddar cheese with sucrose did not significantly interfere with *S.mutans* implantation, the cariostatic mechanism is apparently unrelated to a direct antimicrobial effect on *S.mutans*.

12. Cariostatic evaluation of cheeses with diverse physical and compositional characteristics

Harper DS, Osborn JC, Hefferren JJ, Clayton R. *Caries Res* 20:123-130 (1986)

The caries-inhibitory potential of four cheeses with different permutations of texture, aging, levels of butterfat, protein, calcium, phosphate and lactose were evaluated in rat caries test using a controlled frequency feeding machine in a design similar to that of Edgar et al. [*Caries Res.* 14:384-389, 1982]. Test substrates were alternated with a cariogenic powdered diet and each fed 14 times daily for 28 days to albino rats superinfected with *Streptococcus mutans*. An additional group was fed an agar gel with 25% lactalbumin and 25% soybean oil to assess the caries inhibition provided by a moist, cheese-like substrate without butterfat or casein and low in calcium and phosphate. Food consumption, weight gains, plaque *S. mutans* levels and enamel caries lesions were recorded. Compared to the cheddar and gel control groups, alternate meals of processed cheese spread and mozzarella cheese substantially reduced caries incidence on buccal, but not sulcal tooth surfaces. The cream cheese group had more buccal caries than the gel group. *S.mutans* levels were lower in the mozzarella and cheddar cheese groups than in the cream cheese group. These results support previous reports of the cariostatic potential of cheese and suggest that a substantial portion of the protection may be related to textural influences and casein and/or calcium-phosphate content of the cheese. The amount of butterfat, aging or carbohydrate in the test substrates had no apparent effect upon the amount of caries inhibition observed in this model.

13. Role of *Streptococcus mutans* in human dental decay

Loesche WJ. *Microbiol. Rev.* Vol. 50, 1986, p. 353-380

These data provide convincing, albeit substantial, evidence that *S. mutans*, possibly *S. sobrinus*, and lactobacilli are human odontopathogens. As such, dental caries is a diagnosable and treatable infection (209). Aciduricity appears to be the most consistent attribute of *S. mutans* that can be associated with both its selection in stagnant areas and its cariogenicity. Other aciduric species such as *S. sobrinus* appear to be important primarily in smooth-surface decay and, as such, may be a cariogenic determinant when rampant decay occurs.

Colonization by *S. mutans* occurs after tooth eruption, and if the fissures become colonized in their depths, then decay may be inevitable. However, if this colonization is delayed until the fissure depths are occupied by other bacteria, there is the possibility that decay will not occur or its occurrence will be greatly reduced. This understanding of the ecology of *S. mutans* suggests that treatment strategies which interfere with the colonization of *S. mutans* may have a profound effect on the incidence of dental decay in human populations (182).

14. Modification of food cariogenicity in rats by mineral-rich concentrates from milk

Harper DS, Osborn JC, Clayton R, Hefferren JJ. *J Dent Res.* 1987 Jan;66(1):42-5

Dairy products, including milk, cheese, and casein, can reduce the caries-causing potential of cariogenic substrates as measured in various animal, plaque acidity, and in vitro systems. Although the mechanisms responsible for protection are not completely identified, substances containing Ca and P may contribute to the protective potential by reducing demineralization and/or promoting remineralization of enamel. Casein may reduce demineralization by forming a protective coat on the enamel surface. By means of a rat model, this study evaluated the ability of three casein-free milk mineral concentrates with various levels of whey protein, calcium, and phosphate to modify the cariogenicity of a powdered diet containing 20% sucrose. Analysis of these data indicates that there were no significant differences among groups for weight gain, total food consumption, or feeding frequency, as monitored by a computer-based infrared activity monitor. All three mineral concentrates significantly reduced buccal caries, and two of the three reduced sulcal caries by from 10 to 30%. The analysis further shows that casein-free milk mineral fractions can modify the cariogenicity of sucrose-containing foods in a rat model.

15. Effects of water-soluble components of cheese on experimental caries in humans

Silva MF, Burgess RC, Sandham HJ, Jenkins GN. *J Dent Res* 1987 Jan;66(1):38-41

The effect of water-soluble components of extra-old Cheddar cheese on experimental caries was tested by means of the seven-day intraoral cariogenicity test (ICT). Two bovine enamel blocks were placed in each buccal flange of the dental appliances of five volunteers. One side of each appliance (experimental) was dipped in a 25% water extract of the cheese for five min, while the other side (control) was dipped in de-ionized water. Immediately thereafter, the appliance was returned to the subject's mouth, and two 60-second rinses with 10% sucrose were performed. These procedures were repeated six times per day. The cheese-extract dippings reduced the cariogenicity of the sucrose by an average of 55.7% (p less than 0.01), as assessed by enamel microhardness. Neither the mean resting pH nor the mean minimum pH in response to sucrose was significantly different between the experimental and control sides. The concentration of calcium was significantly higher in plaque from the experimental side (32.44 micrograms/mg) as compared with the control side (19.36 micrograms/mg, p less than 0.01). The concentration of plaque phosphorus was higher on the experimental side (12.90 micrograms/mg) than on the control side (9.61 micrograms/mg); however, the difference was not statistically significant. These results show that cheese has one or more water-soluble components which reduce experimental caries in human subjects.

16. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model

Reynolds EC. *J Dent Res* 1987 Jun;66(6):1120-7

The ability of bovine milk phosphoprotein (casein) to be incorporated into plaque, prevent enamel sub-surface demineralization, and affect bacterial composition was determined using a modified intra-oral caries model. The intra-oral model consisted of a removable appliance containing a left and right pair of bovine enamel slabs placed to simulate an approximal area. Supragingival plaque was collected and impacted into the left and right inter-enamel spaces. The left side of the appliance was exposed to various sugar and salt solutions, while the right side was exposed to sugar and casein solutions. Sodium caseinate, the major fraction alpha s1-casein, and a tryptic digest of alpha s1-casein (TD-casein) were studied. Sodium caseinate at a level of 2% w/v in a 3% sucrose-3% glucose-salt solution (pH 7.0) prevented sub-surface enamel demineralization over a ten-day period as shown by microradiography and microhardness. Two exposures of a 2% w/v sodium caseinate, alpha s1-casein, or TD-casein solution (pH 7.0) per day prevented sub-surface enamel demineralization caused by six exposures of a 3% sucrose-3% glucose-salt solution per day over a ten-day period. Intact alpha s1-casein and tryptic peptides were shown immunochemically to be incorporated into the inter-enamel plaque. The incorporation of casein and its breakdown in plaque did not produce a significant change in the amount or composition of plaque bacteria. The ability of casein and tryptic peptides to prevent enamel demineralization was related to their incorporation into plaque, thereby increasing plaque calcium phosphate and acid-buffering capacity by the phosphoserine, histidine, glutamine, and aspartate residues and indirectly through catabolism by plaque bacteria.

17. Reduction of chocolate's cariogenicity by supplementation with sodium caseinate

Reynolds EC, Black CL. *Caries Res* 1987; 21:445-451

The cariogenicity of two chocolate confections was compared using a rat caries model. One chocolate confection contained 5.6% w/w casein while the other contained 16.6% w/w casein. The levels of fat and sucrose were the same in both confectionery. The casein enrichment was achieved by replacing the 18% w/w non-fat milk solids used normally in the manufacture of milk chocolate with soluble sodium caseinate. Forty Sprague-Dawley male rats infected with streptomycin-resistant *Streptococcus mutans* consumed the chocolate diets either ad libitum or in a Konig/Hofer programmed feeder. Animals consuming the casein-enriched chocolate either ad libitum or programme-fed had significantly ($p < 0.001$) lower smooth surface and fissure caries scores than the animals consuming the normal chocolate. There was no significant difference in the mean final body weight, salivary output, or salivary protein, calcium or phosphate concentrations between the animals on the two chocolate diets. There was also no significant difference in the number of total organisms, streptococci or streptomycin-resistant *S. mutans* recovered from the swabs of the molar teeth of animals on both diets. The work confirms an earlier report of a topical anticariogenic effect of soluble caseinate and shows that it is possible to reduce the cariogenicity of chocolate by caseinate supplementation.

18. Confectionery composition and rat caries

Reynolds EC, Black CL. *Caries Res* 1987; 21:538-545

Sucrose, milk chocolate, caramel or fudge were fed to rats infected with streptomycin-resistant *Streptococcus mutans* and receiving essential nutrients by gavage. The frequency of eating and drinking by the animals was monitored using a time-lapse video recorder. After 35 days, saliva was collected from the animals and caries was assessed. There was no significant difference between the caries experience of animals consuming fudge or sucrose. However, the caries experience of the animals consuming milk chocolate and caramel was significantly lower than that of the animals eating sucrose. The difference in caries activity was not attributed to a difference in salivary function as salivary protein, calcium and phosphate levels of all animals were normal. A significant difference in the frequency of eating and drinking periods of the animals from the four groups could not be demonstrated, indicating that the lower caries experience was not due to a less frequent sugar challenge. The caries reduction was attributed to a topical effect that was related to the difference in the composition of the confectionery. The levels of protein (casein) and fat of the test diets were inversely correlated with the animals' caries experience suggesting that one, or both of these components were responsible for the caries reduction.

19. The effects of cheese snacks on caries in desalivated rats

Krobicka A, Bowen WH, Pearson S, Young DA. *J Dent Res* 1987 Jun;66(6):1116-9

Rats that had had their submandibular/sublingual glands removed surgically, and their parotid ducts tied, developed fewer and less severe caries lesions on coronal and root surfaces when fed cheese snacks in addition to a cariogenic diet than when fed additional cariogenic snacks or no additional snacks. The effects of cheese snacks were particularly dramatic on root-surface caries. These observations may be relevant for elderly humans who are most likely to develop root surface caries. Populations of *Streptococcus mutans* did not differ among the groups. *Actinomyces viscosus* was not detected at the end of the experiment in any of the groups. The results of this study demonstrate that cheese exerts a protective effect against coronal and root-surface caries in rats with a severely limited salivary function.

20. Protein dissimilation by human salivary-sediment bacteria

Reynolds EC, Riley PF. *J Dent Res* 1989 Feb;68(2):124-9

Proteins of known composition and structural characteristics were incubated (1.0 mg/mL) with re-suspended salivary sediment (2.5% v/v) in a lactate-salt medium with an initial pH of 5.2 for two hr at 37 degrees C. Hydrolysis of the proteins was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Hydrogen ion, amines, and ammonia were measured by use of a combined pH electrode, high-performance liquid chromatography, and glutamate dehydrogenase, respectively. Of the proteins studied, the caseins alpha s1, beta, and kappa and the histones H1 and H3 were extensively hydrolyzed by the salivary-sediment bacteria. The hydrolysis of these proteins was attributed to their relative lack of tertiary (folded) structure. The only amine detected was the polyamine putrescine arising from the catabolism of arginine following the hydrolysis of the arginine-rich histone H3. None of the other proteins extensively hydrolyzed by salivary sediment, although containing arginyl and lysyl residues, served as substrates for putrescine or cadaverine production. Pre-hydrolysis of the arginine-rich histone H3 and poly-L-arginine with trypsin resulted in a marked increase in putrescine produced, suggesting that the salivary-sediment proteolytic activity was not "trypsin-like". Incubation of salivary-sediment bacteria with the caseins and the histone H3 resulted in an increase in ammonium ion concentration and an associated decrease in hydrogen ion concentration. The increase in ammonium ion concentration not attributed to arginine hydrolysis was correlated with the content of glutaminyl plus asparaginyl residues of the proteins.

21. Cariogenicity of a confection supplemented with sodium caseinate at a palatable level

Reynolds EC, Black CL. *Caries Res* 1989;23:368-370

Summary: Recently Reynolds (1987) showed that tryptic peptides of caseinate were incorporated into plaque, increased the level of plaque calcium and inorganic phosphate and prevented subsurface enamel demineralization in a human intra-oral caries model. It was concluded that the tryptic peptides associated with the anticariogenic effect were the calcium phosphate sequestering phosphopeptides of α_s -caseinate and β -caseinate (Reynolds, 1987). These phosphopeptides are approximately 10% by weight of caseinate and are tasteless (Swaisgood, 1982). It could be possible therefore to supplement confectionery with the phosphopeptides at a level of one tenth that of caseinate (e.g. 2.0%) without affecting palatability or consistency but still achieving the reduction in cariogenicity obtained with caseinate at 20.0%. The phosphopeptides can be easily purified from a tryptic digest of caseinate by selective precipitation (Reynolds, unpublished) and their anticariogenic potential is presently being investigated.

22. A 24-month clinical study of the incidence and progression of dental caries in relation to consumption of chewing gum containing xylitol in school preventive programs

Kandelman D, Gagnon G. *J Dent Res*. 1990 Nov;69(11):1771-5

The effect of chewing gum containing xylitol on the incidence and progression of dental caries was tested in a sample of 274 children, aged eight and nine years, of low socio-economic status and high caries rate. They were divided into two experimental groups (15% and 65% xylitol chewing gum distributed three times a day at school) and one control group (without chewing gum). The three groups were exposed to the same basic preventive program. Children who chewed gum had a significantly lower net progression of decay (progressions-reversals) over a 24-month period than did the controls. Results for the two groups chewing gum were similar. Chewing xylitol gum had a beneficial effect on the caries process for all types of tooth surfaces, and especially for bucco-lingual surfaces. The two experimental groups had a DMF(S) increment of 2.24 surfaces, compared with 6.06 surfaces for the control group. For this indicator, there was no difference between the two experimental groups. Results for the plaque index were in agreement with those of the DMF(S) increment and the net progression of decay.

23. Efficient solution-phase synthesis of multiple O-phosphoseryl-containing peptides related to casein and statherin

Perich JW, Kelly DP, Reynolds EC. Int J Pept Protein Res 1992 Aug;40(2):81-8

The multiple Ser(P)-containing peptides, H-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-NHMe.TFA, H-Asp-Ser(P)-Ser(P)-Glu-Glu-NHMe.TFA and H-Glu-Ser(P)-Ser(P)-Glu-Glu-NHMe.TFA were prepared by the use of Boc-Ser(PO₃Ph₂)-OH in the Boc mode of solution phase peptide synthesis followed by platinum-mediated hydrogenolytic de-protection of the Ser(PO₃Ph₂)-containing peptides. The protected peptides were assembled using the mixed anhydride coupling methods with 40% TFA/CH₂Cl₂ used for removal of the Boc group from intermediate Boc-protected peptides.

24. The use of synthetic phosphopeptides for epitope mapping of the α_{s1} -casein phosphopeptide segment 59-79

Perich JW and Reynolds EC. Bioorg Med Chem Lett, Vol.2, No.9, pp. 1153-1154, 1992

Through the use of synthetic Ser- and Ser(P)-containing peptides, both sequences –Ser(P)-Ser(P)-Ser(P)- and Ile-Val-Pro-Asn-Ser(P)-Val-Glu-Glu of the tryptic phosphopeptide segment 59-79 of α_{s1} -casein were shown to be recognized by anti- α_{s1} -casein polyclonal antibodies in a competitive ELISA. Since replacement of Ser(P) with Ser in the later peptide resulted in complete loss of antibody recognition, this indicates that the phosphorylated seryl residue is a critical residue for antibody recognition.

25. An in situ model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization

Featherstone JD, Zero DT. *J Dent Res* 1992 Apr;71 Spec No:804-10

In situ models to assess the ability of oral care products or food components to enhance remineralization and/or inhibit demineralization of tooth enamel or roots must be very carefully designed to minimize the confounding effects of the many variables involved. Controlling these variables as closely as possible is essential if meaningful answers are to be obtained from the models. We have developed an in situ model which combines the experience of several groups. Detailed screening of subjects is essential. Selection criteria should include good general health, good dental health, mandibular partial denture, at least eight natural teeth, no active caries lesions, known fluoride history, normal salivary function, and no medications that affect salivary function. Each subject carries a sound enamel slab and an enamel slab with a pre-formed caries-like lesion (demineralized in vitro) in his/her denture on each side of the mouth for test periods of two or four weeks. The demineralization challenge is controlled by extra-oral immersion of the appliances in sucrose daily. Daily product exposure or daily food component exposure is used as desired. Compliance indicators and a diet diary are included. Whole saliva flow rate (unstimulated), plaque acidogenicity, and salivary fluoride are monitored during the test periods. At the end of the test period, the test slabs are assessed for mineral change, after being sectioned, by means of cross-sectional microhardness or microradiography. The mineral loss or gain (ΔM , microns \times vol%), compared with adjacent control sections retained in the lab, is calculated as change in ΔZ (microns \times vol%), namely, $\Delta M = \Delta Z_{TEST} - \Delta Z_{CONTROL}$.

26. The analysis of multiple phosphoserine-containing casein peptides using capillary zone electrophoresis

Adamson N, Riley PF, Reynolds EC. *J Chromatogr* 1993 Sep 3;646(2):391-6

Multiple phosphoserine-containing sequences of peptides and proteins stabilize amorphous calcium phosphate at neutral and alkaline pH and have been implicated in the nucleation/regulation of biomineralization. In an approach to analyze these peptides using capillary zone electrophoresis (CZE) we have attempted to relate the absolute electrophoretic mobility of various casein phosphopeptides to their physicochemical properties. Multiple phosphoserine-containing peptides were selectively precipitated from enzymic digests of sodium caseinate and further purified using RP-HPLC and anion-exchange fast protein liquid chromatography. Purified fractions were then analyzed by CZE. Absolute electrophoretic mobilities of 13 peptides were determined by measurement of migration times relative to that of a neutral marker, mesityl oxide. A linear relationship ($r^2 = 0.993$) was obtained between absolute electrophoretic mobility and $q/M(r)^{2/3}$ where q is the net negative charge of the peptide calculated using relevant pKa values and $M(r)$ is the molecular mass. $M(r)^{2/3}$ is a measure of the surface area of a sphere that has a volume proportional to the $M(r)$ of the peptide and relates to the frictional drag exerted on the peptide during electrophoretic migration. As absolute electrophoretic mobility is influenced by charge and size CZE can be used to monitor peptide phosphorylation, dephosphorylation, deamidation and truncation. This technique therefore would be suitable for quantitative analysis of peptide substrates in kinase and phosphatase studies. In conclusion CZE is a rapid and efficient technique for the resolution of multiple phosphoserine-containing peptides from enzymic digests of casein.

27. A selective precipitation purification procedure for multiple phosphoseryl-containing peptides and methods for their identification

Reynolds EC, Riley PF, Adamson NJ. *Anal Biochem* 1994 Mar; 217(2):277-84

Multiple phosphoseryl-containing sequences of proteins stabilize amorphous calcium phosphate and have been implicated in the regulation of biomineralization, protein structure, and enzyme activity. To facilitate studies on the identification and characterization of multiple phosphoseryl-containing sequences of proteins we have developed a simple and efficient purification procedure involving precipitation of Ca^{2+} /ethanol-induced aggregates of the multiple phosphoseryl-containing peptides from enzymic digests. The multiple phosphoseryl-containing peptides of a tryptic digest of casein were selectively precipitated using Ca^{2+} (20 mol/mol protein) and 50% (v/v) ethanol at pH 3.5, 4.6, and 8.0. The individual peptides of the precipitates were purified using anion-exchange fast-performance liquid chromatography and reversed-phase HPLC and then identified by solid-phase sequence analysis and amino acid composition analysis after vapor-phase hydrolysis. Prior to sequence analysis the phosphopeptides were covalently coupled to arylamine membranes and the phosphoseryl residues converted to S-ethylcysteinyl residues by calcium-ion-catalyzed beta-elimination in the presence of ethanethiol. The modified peptides were sequenced using an Applied Biosystems Inc. automated protein sequencer fitted with a membrane cartridge. Only peptides containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)- were precipitated by Ca^{2+} /ethanol at pH 3.5. The pH 4.6 precipitate contained all the cluster peptides plus two diphosphorylated peptides containing -Ser(P)-Glu-Ser(P)- and -Ser(P)-Thr-Ser(P)-. At pH 8.0, a monophosphorylated peptide containing -Ser(P)-Glu-Glu- was also present in the precipitate with the diphosphorylated and cluster peptides. The recoveries of the peptides in the pH 8.0 selective precipitate ranged from 83 to 95% of that present in the hydrolysate.

28. High performance capillary electrophoresis of casein phosphopeptides containing 2-5 phosphoseryl residues: Relationship between absolute electrophoretic mobility and peptide charge and size

Adamson NJ, Reynolds EC. *Electrophoresis* 1995, 16, 525-528

Multiple phosphoseryl-containing peptides from casein containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- stabilize amorphous calcium phosphate at neutral and alkaline pH and have been shown to be anticarcinogenic in various *in vitro*, animal and human experiments. In an approach to obtain insight into the structure and function of these peptides, we previously developed a method for their analysis using high-performance capillary electrophoresis (HPCE). A linear relationship was obtained between absolute electrophoretic mobility and $q/M^{2/3}$. Di- and triphosphorylated peptide absolute electrophoretic mobility correlated with both $q/M^{2/3}$ and $\ln(q+1)/n^{0.43}$. However, for both the $q/M^{2/3}$ and $\ln(q+1)/n^{0.43}$ relationships with absolute electrophoretic mobility, the di- and triphosphorylated peptides formed a separate linear relationship to that of the cluster peptides. From these relationships, a di- or triphosphorylated peptide exhibited a greater absolute electrophoretic mobility than a corresponding cluster peptide with the same $q/M^{2/3}$ or $\ln(q+1)/n^{0.43}$ value. This implies that the cluster peptides have a reduced effective net negative charge due to an electrical double layer effect and/or a larger hydrodynamic volume or a more extended structure relative to the di- and triphosphorylated peptides which is associated with a greater frictional drag electrophoretic migration.

29. A 1H-NMR study of the casein phosphopeptide α_{s1} -casein (59-79)

Huq NL, Cros KJ, Reynolds EC. *Biochim Biophys Acta* 1247 (1995) 201-208

Complete sequence-specific resonance assignments have been determined for a calcium phosphate sequestering, phosphoserine-containing, tryptic peptide α_{s1} -casein(59-79) containing the phosphorylated motif –SSSEE–. Spectra have been recorded in the presence of excess Ca^{2+} and at three different values of sample pH to characterize the changes in peptide conformation as calcium binds to the phosphorylated residues. The secondary structure of the peptide was characterized by sequential ($i, i + 1$), medium-range ($i, i + 2/3/4$), and long-range ($i, i + 5$) NOE connectivities, ^1H chemical shifts, NH to ^1H coupling constants and the observation of slowly exchanging amide protons. Two structured regions have been identified: residues P73 to V76 implicated in β -turn conformations, and residues E61 to Σ 67 involved in a loop-type structure.

30. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat

Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, Perich JW. *J Dent Res* 1995 Jun;74(6):1272-9

Casein phosphopeptides (CPP) stabilize calcium phosphate through the formation of casein-phosphopeptide amorphous calcium-phosphate complexes (CPP-CP). The ability of CPP-CP to reduce caries activity was investigated by use of specific-pathogen-free rats inoculated with *Streptococcus sobrinus*. The animals consumed a defined cariogenic diet free of dairy products. Solutions (100 μL) of the CPP-CP (0.1, 0.2, 0.5, 1.0% w/v) were applied to the animals' molar teeth twice daily. Other groups of animals received solutions containing 500 ppm F, the non-phosphorylated peptides of a casein tryptic digest (0.5% w/v), or the calcium-phosphate complex of a synthetic octapeptide, Ac-Glu-Ser(P)-Ile-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-NHMe, corresponding to the common sequence in the CPP. The CPP-CP significantly reduced caries activity in a dose-response fashion, with 1.0% CPP-CP producing 55% and 46% reductions in smooth surface and fissure caries activity, respectively, being similar to that of 500 ppm F. The anticariogenic effects of CPP-CP and fluoride were additive, since animals receiving 0.5% CPP-CP plus 500 ppm F had significantly lower caries activity than those animals receiving either CPP-CP or fluoride alone. The tryptic digest of casein with the phosphopeptides selectively removed showed no anticariogenic activity. The synthetic octapeptide-calcium phosphate complex significantly reduced caries activity, confirming that this calcium-phosphate-stabilizing portion of the casein phosphopeptides is associated with anticariogenicity. The CPP-CP did not significantly affect the level of *S. sobrinus* in fissure plaque.

31. In situ caries models

Zero DT. Adv Dent Res 1995 Nov;9(3):214-30; discussion 231-4

By using in situ models, we have the potential to study both fundamental aspects of the caries process as well as more applied research problems such as the effect of food on dental caries and the role of fluoride in caries prevention in human subjects without actually causing caries in the natural dentition. The key experimental parameters that need to be considered in the development of an in situ model are the characteristics of the subject panel, the physical design of the model, the type of hard tissue substrate and the method of assessing mineral status, and the study design and clinical protocol. Each parameter must be carefully considered in relation to the objectives of the research, study design requirements, ethical considerations, impact on clinical relevance, and impact on the control of variation. The major source of variation associated with in situ models should be of biological and not experimental origin. The design and conduct of proper in situ model studies require a clear understanding of the caries process, sound analytical support, and a knowledge of how to work with research subjects to achieve a high level of compliance. Given the complex nature of caries, a combination of hard tissue substrates—including sound, surface-softened lesions and subsurface lesions—may be necessary to model all aspects of caries progression and prevention successfully. Internal validation of in situ models using fluoride dose-response controls is considered to be necessary for studies evaluating the efficacy of new fluoride dentifrice formulations.

32. Characterization of tryptic casein phosphopeptides prepared under industrially relevant conditions

NJ Adamson, EC Reynolds. Biotech Bioeng 1995 45:196-204

Anticariogenic casein phosphopeptides (ACPP) contain the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- and have commercial potential as toothpaste, mouthwash, and food additives for the prevention of dental caries. In an approach to develop a commercial-scale process for the production of ACPP we have comprehensively characterized casein phosphopeptides (CPP) produced under industrially relevant conditions. Sodium caseinate (10% w/v) was hydrolyzed by Novo trypsin (commercial grade) at 50°C for 2 h and CPP were purified from the acid clarified hydrolysate by a single-step selective precipitation procedure involving Ca^{2+} (20 mol/mol casein) and ethanol (50% v/v) at pH 4.6 or 8.0. The individual peptides of the CPP preparations were purified by reversed-phase high-performance liquid chromatography (HPLC) and then identified by amino acid composition and sequence analyses. The yield of the pH 8.0 precipitate (13.85 ± 0.48 wt % of the caseinate) was slightly higher than that of the pH 4.6 precipitate (11.04 ± 0.30 wt % of the caseinate). However, the pH 4.6 precipitate contained predominantly (86.4 mol %) ACPP cluster peptides with small amounts of the diphosphorylated peptides (13.6 mol %), α_{s1} (43-58) and α_{s2} (126-136). In the pH 8.0 precipitate the cluster peptides represented a smaller proportion of the total peptides (61.9 mol %) due to increased recoveries of the diphosphorylated peptides (24.4 mol %) as well as the additional recovery of the monophosphorylated peptide β (33-48) (13.7 mol %) indicating increased cross-linking by Ca^{2+} at the higher pH. The recovery of the ACPP from the original caseinate was similar for both the pH 4.6 and 8.0 precipitates. Slight chymotryptic activity was detected in the industrial-grade enzyme, resulting in minor truncation of some peptides. Also some deamidation and methionine oxidation of one peptide, α_{s1} (59-79), were detected. In conclusion, ACPP can be produced under industrially relevant conditions with only minor modifications such as slight truncation, deamidation, and methionine oxidation. However, in order to prepare casein phosphopeptides predominantly containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu-, the single-step selective precipitation with Ca^{2+} /ethanol should be performed at pH 4.6 rather than pH 8.0.

33. Role of models in assessing new agents for caries prevention-non-fluoride systems

Roberts AJ. Adv Dent Res. 1995 Nov;9(3):304-11; discussion 312-4

While fluoride is an effective anti-caries agent, the search for more effective alternative therapies continues. A wide range of non-fluoride anti-caries agents has been postulated, and this paper reviews some of the pre-clinical models that have been utilized in their evaluation and some of the pitfalls that must be avoided. Using data on the potential anti-caries efficacy of phosphopeptides obtained from casein, the caution that must be applied in extrapolating laboratory data to predict clinical performance is discussed. Evaluation strategies that focus on only one potential mode of action (e.g., inhibition of demineralization) may overestimate the true clinical efficacy which may arise from a combination of two or more effects (e.g., inhibition of demineralization and stimulation of remineralization). Although laboratory and in situ data predict anti-caries efficacy for sodium trimetaphosphate in combination with fluoride, this was not found in three-year clinical trials. A possible reason for this, the lack of suitable calibration methods, is discussed. Finally, some comments on the appropriateness of laboratory evaluation strategies are made.

34. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci

Schupbach P, Neeser JR, Golliard M, Rouvet M and Guggenheim B. J Dent Res, Vol 75, 1779-1788 (1996)

The protective effects of milk and milk products against dental caries have been demonstrated in many animal studies. We have shown that this effect was mediated by micellar casein or caseinopeptide derivatives. A reduction in the *Streptococcus sobrinus* population in the oral microbiota of animals fed diets supplemented with these milk components was consistently observed. A possible explanation for these findings is that milk components are incorporated into the salivary pellicle, thereby reducing the adherence of *S. sobrinus*. This hypothesis was tested in vitro by the incubation of bovine enamel discs with unstimulated saliva. The resulting pellicle was washed and incubated with caseinoglycomacropeptide (CGMP) and/or caseinophosphopeptide (CPP) labeled with 17- and 12-nm gold particles. All samples were prepared for electron microscopy by high-pressure freezing followed by freeze-substitution. It was demonstrated by high-resolution scanning electron microscopy with back-scattered electron imaging, as well as by transmission electron microscopy, that both peptides were incorporated into the pellicle in exchange for albumin, confirming previous findings. This protein was identified with a mouse anti-human serum albumin followed by goat anti-mouse IgG labeled with 25-nm gold particles. Incorporation of CGMP and/or CPP into salivary pellicles reduced the adherence of both *S. sobrinus* and *S. mutans* significantly. It is suggested that the calcium and phosphate-rich micellar casein or caseinopeptides are incorporated into the pellicle. The resulting ecological shifts, together with the increased remineralization potential of this biofilm, may explain its modified cariogenic potential.

35. Ability of a β -casein phosphopeptide to modulate the precipitation of calcium phosphate by forming amorphous dicalcium phosphate nano-clusters

Holt C, Wahlgren NM and Drakenberg T. Biochem. J (1996) 314 (1035-1039)

The ability of casein in the form of colloidal-sized casein micelles to modulate the phase separation of calcium phosphate during milk secretion is adapted to produce nanometre-sized particles of calcium phosphate stabilized by a casein phosphopeptide (nanoclusters). The nanoclusters were prepared from an undersaturated solution of salts and the peptide by raising the pH homogeneously from about 5.5 to 6.7 with urea plus urease. Chemical analysis and IR spectroscopy showed that they comprise an amorphous dicalcium phosphate bound to the phosphopeptide. Multinuclear NMR spectroscopy of the cluster solutions showed that the small ions and free peptide in the solution were in a state of dynamic exchange with the nanoclusters. The peptide is linked to the calcium phosphate through its sequence of phosphorylated residues, but, in a proportion of adsorbed conformational states, the termini retain the conformational freedom of the unbound peptide. The ability of casein to form nanoclusters in milk suggests a more general mechanism for avoiding pathological calcification and regulating calcium flow in tissues and biological fluids exposed to or containing high concentrations of calcium.

36. Dairy products and dental health

Reynolds EC. Proceedings of the Nutrition Society of Australia (1995) 19

Dental caries (tooth decay) is initiated via the demineralization of tooth hard tissue by organic acids from the fermentation of dietary sugar by dental plaque odontopathogenic bacteria. Even though in most developed countries the prevalence of dental caries has decreased through the use of fluorides, the disease remains a major public health problem. Except for some reports associating nursing bottle caries with milk consumption, dairy products have been recognized for over 40 years as exhibiting an anticaries effect. Using laboratory, animal and human in situ caries models it has been shown that casein phosphopeptide amorphous calcium phosphate complexes (CPP-ACP) exhibit an anticariogenic activity. The casein phosphopeptides (CPP) are produced from a tryptic digest of the milk protein casein by aggregation with calcium phosphate and purification by ultrafiltration. The 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 phosphoserine residues the CPP bind to clusters of amorphous calcium phosphate (ACP) in metastable solution, preventing their growth to the critical size required for nucleation and precipitation. The proposed mechanism of anticariogenicity for the CPP-ACP is that they localize ACP in dental plaque which buffers the free calcium and phosphate ion activities thereby helping to maintain a state of supersaturation with respect to tooth enamel. This depresses demineralization and enhances remineralisation. The CPP-ACP, unlike fluoride, can be added to sugar-containing foods and therefore have commercial potential as an anti-cariogenic additive to foods and toothpastes.

37. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions

Reynolds EC. *J Dent Res* 1997 Sep;76(9):1587-95

Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP), localize ACP in dental plaque, and are anticariogenic in animal and in situ human caries model. In this vitro study, CPP-stabilized calcium phosphate solutions were shown to remineralize subsurface lesions in human third-molar enamel. Solutions were used to examine the effect of CPP-calcium phosphate concentration on remineralization. Other solutions were used to examine the effect of increasing pH, which decreased the concentrations of free calcium and phosphate ions and increased the level of CPP-bound ACP. Although most of the remineralizing solutions were supersaturated with respect to the amorphous and crystalline calcium phosphate phases, the solutions were stabilized by the CPP such that spontaneous precipitation of calcium phosphate did not occur. After a ten-day remineralization period, enamel lesions were sectioned, subjected to microradiography, and the mineral content determined by microdensitometry. All solutions deposited mineral into the bodies of the lesions, with the 1.0% CPP-calcium phosphate (pH 7.0) solution replacing 63.9 +/- 20.1% of mineral lost at an averaged rate of $3.9 \pm 0.8 \times 10^{-8}$ mol hydroxyapatite/m²/s. The remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions. Remineralization was not significantly correlated with either the CPP-bound ACP or the degrees of saturation for hydroxyapatite, octacalcium phosphate, or ACP. However, remineralization was significantly correlated with the degree of saturation for dicalcium phosphate dihydrate (CaHPO₄·2H₂O), but this was attributed to the significant correlation of remineralization with the activity gradients from the solution into the lesion of some calcium phosphate ions and ion pairs, in particular the neutral ion pair CaHPO₄(0). The CPP, by stabilizing calcium phosphate in solution, maintain high-concentration gradients of calcium and phosphate ions and ion pairs into the subsurface lesion and thus effect high rates of enamel remineralization.

38. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review

Reynolds EC. *Spec Care Dentist* 1998 Jan-Feb; 18(1):8-16

Using laboratory, animal, and human in situ caries models, investigators have shown that casein phosphopeptide amorphous calcium phosphate complexes (CPP-ACP) exhibit an anticariogenic activity. The casein phosphopeptides (CPP) are produced from a tryptic digest of the milk protein casein by aggregation with calcium phosphate and purification by ultrafiltration. The CPP have a remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque. Through their multiple phosphoserine residues, the CPP bind to forming clusters of amorphous calcium phosphate (ACP) in metastable solution, preventing their growth to the critical size required for nucleation and precipitation. The proposed mechanism of anticariogenicity for the CPP-ACP is that they localize ACP in dental plaque, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. The CPP-ACP, unlike fluoride, can be added to sugar-containing foods and therefore have commercial potential as an additive to foods as well as to toothpastes and mouthwashes for the control of dental caries.

39. Anticariogenic casein phosphopeptides

Reynolds EC. *Prot Peptide Lett*, Vol. 6, No 5, pp. 295-303, 1999

Proteins and peptides containing clusters of phosphoserine residues have been shown to stabilize amorphous calcium phosphate in solution, and have been implicated in the regulation of biomineralisation processes. Casein phosphopeptides (CPP) containing the cluster sequence –Ser(P)-Ser(P)-Ser(P)-Glu-Glu- have been demonstrated to significantly reduce the level of dental caries (tooth decay) in animal and human experiments and also to repair early stages of decay. Through their multiple phosphoserine residues the peptides bind to forming nanoclusters of amorphous calcium phosphate (ACP) in metastable solution, preventing their growth to the critical size required for nucleation and phase transformation. Conformational and binding studies have shown that all the phosphoserine residues are important in interaction with ACP. The CPP localize ACP at the tooth surface providing a reservoir of calcium and phosphate ions thereby helping to maintain a state of supersaturation with respect to tooth enamel. The CPP-ACP, unlike fluoride, can be added to sugar-containing foods as well as oral care products for the control of dental caries.

40. Advances in enamel remineralization: anticariogenic casein phosphopeptide amorphous calcium phosphate

E.C. Reynolds, C.L. Black, F. Cai, K.J. Cross, D. Eakins, N.L. Huq, M.V. Morgan, J.W. Perich, P.F. Riley, P. Shen, G. Talbo, F. Webber. *J Clin Dent* 10: 86-88, 1999

Casein phosphopeptides (CPP) are multiphosphorylated peptides from an enzymatic digest of the bovine milk protein casein. These peptides have a remarkable ability to stabilize calcium phosphate in solution as amorphous calcium phosphate (ACP). Through their multiple phosphoserine residues the CPP bind to forming nanoclusters of ACP in metastable solution, preventing their growth to the critical size required for nucleation and phase transformations. The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanoclusters have been shown to localize at the tooth surface and prevent caries in laboratory, animal and human *in situ* caries models. The CPP-ACP have also been shown to remineralize enamel subsurface lesions *in vitro* and *in situ* when delivered in a sugar-free chewing gum. The proposed anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. The CPP-ACP have potential as an additive to foods and oral care products for the control of dental caries.

41. Calcium phosphates in demineralization/remineralization processes

Zapanta LeGeros R. *J Clin Dent* 10: 65-73, 1999

Enamel and dentin caries occurs as a result of a shift in the equilibrium between demineralization and remineralization processes, with the demineralization process predominating. Caries lesion can be arrested by timely remineralization strategies. This paper reviews the calcium phosphates and other calcium compounds (e.g., calcium fluoride, CaF_2) associated with the demineralization/remineralization processes. The caries process is initiated by the dissolution of the tooth mineral (calcium carbonatehydroxyapatite) by organic acids (lactic and acetic acid) produced by plaque bacteria acting on dietary carbohydrates or by lowered pH from ingested food and drink. The dissolution increases the concentration of calcium, phosphate/acid phosphate, magnesium, carbonate/bicarbonate ions in the microenvironment of the caries lesion, leading to the formations and transformations of different types of calcium phosphates. In the presence of low levels of fluoride (F^-) ions, (F,OH)-apatite, FHA, forms directly or indirectly by transformation of the other calcium phosphates; at higher concentrations, CaF_2 -like materials form which can dissolve to form FHA and provide a supply of F^- in solution. The progress of the caries lesion is decelerated and remineralization accelerated, in F-containing enamel. The demineralization/remineralization processes consist of the dissolution of F-poor, Mg- and CO_3 -rich dental apatites and the precipitation of F-rich, Mg- and CO_3 -poor dental apatites which are more resistant to subsequent acid challenges. The simultaneous presence of calcium (Ca), phosphate (P) and fluoride (F) ions in solution compared to the combined presence of (Ca+P), (Ca+F) or (P+F) or F alone, is most effective in inhibiting the dissolution of synthetic or enamel apatite.

42. The role of phosphopeptides in caries prevention

Reynolds EC. *Dental Perspectives* 1999 3:6-7

Conclusion: The anticariogenicity of the CPP-ACP has been demonstrated in the rat and human *in situ* caries models. The CPP-ACP has also been shown to remineralize enamel subsurface lesions *in vitro* and *in situ* when delivered in a sugar-free gum. The proposed anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization.

The CPP-ACP therefore have exciting potential for use in oral care products and foods to help prevent dental caries and to help repair early stages of the disease, a development which will add significantly to dentists' armoury of tools in preventive dentistry.

43. Molecular modeling of a multiphosphorylated sequence motif bound to hydroxyapatite surfaces

Huq NL, Cross KJ and Reynolds EC. *J.Mol.Model.* 2000, 6, 35-47

Proteins and peptides containing the multiphosphorylated motif –Ser(P)-Ser(P)-Ser(P)-Glu-Glu- stabilize amorphous calcium phosphate (ACP) in body fluids and bind with high affinity to crystalline calcium phosphate phases such as hydroxyapatite (HA) regulating crystal growth. Binding of this motif to hydroxyapatite surfaces was investigated in this study using molecular modeling techniques. Using a three-step computational procedure, we have determined the relative binding energies of the motif –Ser(P)-Ser(P)-Ser(P)-Glu-Glu to different crystalline surfaces of HA. This analysis revealed preferences of the motif for (100) and (010) surfaces of the crystal and preferences for particular orientations on a given surface. These preferences are principally governed by electrostatic interactions between the crystal lattice and the peptide with the most stable conformers adopting structures where alternate residues exhibit backbone angles characteristic of a β -strand and values of an α -helix or a distorted α -helix, allowing maximal interaction between the acidic side groups and surface calciums. The results of this study are consistent with experimentally-derived data on the interaction of multiphosphorylated proteins/peptides with HA and have implications for the role of these proteins/peptides in calcium phosphate stabilisation and biomineralisation processes.

44. The use of casein phosphopeptides in oral care products for the prevention and treatment of early enamel caries

Reynolds EC. *Aust J Dairy Technol* 2000 55:1-6

(not published)

45. Binding characteristics of *Streptococcus mutans* for calcium and casein phosphopeptide

Rose RK. *Caries Res* 2000 Sep-ct;34(5):427-31

Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP) and may be used to localize ACP in dental plaque, maintaining a state of supersaturation with respect to tooth enamel, reducing demineralization and enhancing remineralization [Reynolds, J Dent Res 1997;76:1587-1595]. The aim of this paper is to investigate these effects by measuring the affinity and capacity of *Streptococcus mutans* for CPP-ACP. Using the equilibrium dialysis system described by Rose and Hogg [Biochim Biophys Acta 1995;1245:94-98], assessment of calcium binding by a plaque streptococcus at a fixed CPP-ACP concentration gives a series of CPP-ACP-influenced dissociation constants for calcium. These data can then be used to derive a true dissociation constant for CPP-ACP itself. The results demonstrate that CPP-ACP binds with about twice the affinity of the bacterial cells for calcium up to a value of 0.16 g/g wet weight cells. Application of CPP-ACP to plaque may cause a transient rise in plaque fluid free calcium which may assist remineralization. Subsequently, CPP-ACP will form a source of readily available calcium to inhibit demineralization. Hence, CPP-ACP binds well to plaque, providing a large calcium reservoir, which is likely to restrict mineral loss during a cariogenic episode and provide a potential source of calcium for subsequent remineralization. Overall, once in place, CPP-ACP will restrict the caries process.

46. Remineralisation of fluorotic enamel lesions by casein phosphopeptide - amorphous calcium fluorophosphate (CPP-ACFP) solution

Roberts MJ, Messer LB, Reynolds EC. *IADR, ANZ division, Abstract 54, 2000*

Epidemiological studies show an increasing prevalence and severity of fluorosis amongst children in Australia especially for Thylstrup-Fejerskov (TF) indices of 2 and 3 (Riordan PJ. *Caries Res* 1993; 27:71-7). *In situ* and *in vitro* studies have shown CPP-ACFP able to remineralise enamel carious sub-surface lesions (Reynolds EC. *J Dent Res* 1997; 76:1587-95). Histological studies indicate fluorotic lesions are similar to enamel carious lesions. To determine whether CPP-ACFP could remineralise fluorotic lesions and improve the appearance, premolar teeth (n=7) with fluorosis (TF=3) were sectioned into buccal and lingual samples and randomly assigned to two groups, with and without surface conditioning (5.25% w/v NaOCl for 20 minutes) prior to remineralisation with CPP-ACFP (5% w/v, pH 7) for 10 days. The relative whiteness of the lesions before and after remineralisation was quantitated by light reflectance (L value, lightness). Fluorotic lesions were sectioned, subjected to microradiography, and the remineralisation determined by microdensitometry. CPP-ACFP treatment significantly reduced whiteness of fluorotic lesions (from 81.3 \pm 2.7 to 76.0 \pm 4.3; $p < 0.05$). The reduction in whiteness increased with surface conditioning (from 85.6 \pm 3.0 to 72.6 \pm 5.9; $p > 0.05$). Literature-based values for non-fluorosed enamel are 69.0 \pm 2.9 (Den Besten PK, Giambro N. *Ped Dent* 1995; 17:340-5). CPP-ACFP replaced 44.0 \pm 9.7% mineral within the fluorosed lesions. The percentage of mineral deposited increased with surface conditioning (80.0 \pm 7.8%). We conclude that treatment with CPP-ACFP significantly reduced the whiteness and partially remineralised fluorotic lesions. Surface conditioning improved the effectiveness of the treatment with CPP-ACFP. (Study supported by Research Committee School of Dental Science, The University of Melbourne).

47. Experimental study of phosphopeptide in promoting tooth remineralization

Li Zhang, Zongtong Li, Yingjie Dong. *ChinJ Dent Res* 2000 May;3(1):27-30

Objective: To explore a new method for effective and safe prevention and treatment of caries.

Methods: Following the theory of tooth remineralization, we searched for the mechanism and clinical application of tooth remineralization with phosphopeptide.

Results: Phosphopeptide, with calcium and phosphorus, had a high affinity for hydroxyapatite and could enhance tooth remineralization.

Conclusion: Phosphopeptide was effective in the prevention and treatment of tooth decay. Clinically, it could be safely applied.

48. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques

Rose RK. *Arch Oral Biol* 2000 Jul;45(7):569-75

Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP) and may be used to localize ACP in dental plaque, maintaining a state of supersaturation with respect to tooth enamel, reducing demineralization and enhancing remineralization. The aim here was to investigate these effects by measuring the effect of CPP-ACP on calcium diffusion in plaque. Using Dibdin's effusion system, calcium diffusion was measured in streptococcal model plaques. This demonstrated that by providing a large number of possible binding sites for calcium, 0.1% CPP-ACP reduces the calcium diffusion coefficient by about 65% at pH 7 and 35% at pH 5. Hence, CPP-ACP binds well to plaque, providing a large calcium reservoir within the plaque and slowing diffusion of free calcium. This is likely to restrict mineral loss during a cariogenic episode and provide a potential source of calcium for subsequent remineralization. Overall, once in place, CPP-ACP will restrict the caries process.

49. Enamel remineralization by chewing gum containing casein phosphopeptide-amorphous calcium phosphate

EC Reynolds, P Shen, F Cai, A Nowicki, J Vincent. IADR, General session, Chiba 2001 - Abstract 0489

The ability of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in sugar-free chewing gum to remineralize enamel subsurface lesions in a human *in situ* model was studied. Thirty subjects in randomized, cross-over, double-blind studies wore removable palatal appliances with six, human-enamel, half-slabs inset containing subsurface demineralized lesions. The other half of each enamel slab was used as the control demineralized lesion. The studies involved a dose response of the CPP-ACP in sorbitol- and xylitol-based sugar-free gum and also included a nil-treatment (no gum chewing) control. The intra-oral appliances were inserted immediately before gum chewing for 20 min and were retained for a further 20 min immediately after gum chewing. This was performed four times per day. Each treatment was for 14 days duration and at the completion of each treatment the enamel slabs were removed, paired with their respective demineralized control, embedded, sectioned and subjected to microradiography and densitometric image analysis. The nil treatment resulted in 3.60% enamel remineralization. The use of either the sorbitol- or xylitol-based gum more than doubled the amount of remineralization with the average level of enamel remineralization for both gum types being 9.05%. Addition of CPP-ACP to either the sorbitol- or xylitol-based gum resulted in a dose-related increase in enamel remineralization with 0.19 mg, 10 mg, 18.8 mg and 56.4 mg of CPP-ACP producing an increase in enamel remineralization of 9%, 62%, 101% and 151%, respectively, relative to the control gum. Supported by Warner-Lambert, a division of Pfizer, Inc.

50. Structural studies of the β -casein phosphopeptide bound to amorphous calcium phosphate

KJ Cross, NL Huq, D Eakins, EC Reynolds. IADR, General session, Chiba 2001 - Abstract 0490

Tryptic phosphopeptides from milk caseins (CN), complexed with amorphous calcium phosphate (ACP) exhibit anticariogenic activity based on laboratory, animal and human *in situ* caries models. To understand the mechanisms of the anticariogenicity of the CPP-ACP complex, we have been studying the structure-function relationships of the predominant peptides α 1-CN (59-79), β -CN (1-25), when bound to calcium ions, bound to calcium phosphate in solution and bound to hydroxyapatite crystals. The purpose of this study was to examine the solution structure of β -CN (1-25) complexed with calcium phosphate using Nuclear Magnetic Resonance (NMR) Spectroscopy. Powder diffraction X-ray crystallography confirmed that the calcium phosphate phase stabilized by β -CN (1-25) was non-crystalline. The translational diffusion experiments indicate that the β -CN(1-25)-ACP particles observed by NMR are less than 10 nm in diameter. Furthermore, nOe transfer from water solvent to solute revealed that specific protons are solvent exposed in the β -CN(1-25)-ACP. In conclusion, β -CN(1-25) adopts a preferred conformation when complexed with ACP, and this conformation has similar features to that observed in β -CN(1-25) in the presence of calcium ions. Supported by the National Health and Medical Research Council of Australia (Grant No. 991486).

51. Ultrastructural studies of the casein phosphopeptide-amorphous calcium phosphate nanoclusters

KJ Cross, NL Huq, D Eakins, EC Reynolds. IADR, General session, Chiba 2001 - Abstract 0491

Tryptic phosphopeptides derived from milk caseins are known to associate with amorphous calcium phosphate (ACP) forming stable complexes that behave as calcium phosphate delivery vehicles and are effective in the remineralization of early enamel lesions. The aim of this study was to examine the structures of these complexes using a range of physico-chemical techniques, including NMR spectroscopy, powder diffraction X-ray crystallography, and light scattering. The colloidal solutions of CPP-ACP exhibited a complex rheological behaviour. At low concentrations (1-10%, w/v) they behaved as free-flowing solutions whereas at higher concentrations (20-40% w/v) they transformed into thixotropic gels. Microfiltration studies suggest that the complexes can reach sizes over 0.2µm. NMR studies suggest that the inner core of these complexes are less than 10 nm. Powder diffraction patterns have confirmed the amorphous nature of these complexes. In conclusion, the CPP-ACP complexes consist of small, nano-sized sub-unit particles that are cross-linked to form much larger particles (>200nm) or a gel network. Supported by the National Health and Medical Research Council of Australia (Grant No. 991486).

52. Cation-dependent structural features of beta-casein-(1-25)

Cross KJ, Huq NL, Bicknell W, Reynolds

EC. *Biochem J* 2001 May 15;356(Pt 1):277-86

Complete sequence-specific, proton-resonance assignments have been determined for the calcium phosphate-stabilizing tryptic peptide beta-casein-(1-25) containing the phosphorylated sequence motif Ser(P)(17)-Ser(P)-Ser(P)-Glu-Glu(21). Spectra of the peptide have been recorded, in separate experiments, in the presence of excess ammonium ions, sodium ions and calcium ions, and of the dephosphorylated peptide in the presence of excess sodium ions. We observed significant changes to chemical shifts for backbone and side-chain resonances that were dependent upon the nature of the cation present. Medium-range nuclear Overhauser effect (nOe) enhancements, characteristic of small structured regions in the peptide, were observed and also found to be cation dependent. The secondary structure of the peptide was characterized by sequential and medium-range ($i, i+2/3/4$, which denotes an interaction between residue i and residue $i+2, i+3$ or $i+4$ in the peptide) nOe connectivities, and ^1H chemical shifts. Four structured regions were identified in the calcium-bound peptide: residues Arg(1) to Glu(4) were involved in a loop-type structure, and residues Val(8) to Glu(11), Ser(P)(17) to Glu(20) and Glu(21) to Thr(24) were implicated in beta-turn conformations. Comparison of the patterns of medium-range nOe connectivities in beta-casein-(1-25) with those in alpha(S1)-casein-(59-79) suggest that the two peptides have distinctly different conformations in the presence of calcium ions, despite having a high degree of sequential and functional similarity.

53. MALDI-PSD-MS analysis of the phosphorylation sites of caseinomacro-peptide

Talbo GH, Suckau D, Malkoski M, Reynolds EC. Peptides. 2001 Jul;22(7):1093-8

Caseinomacropeptide (CMP) is a 64 amino acid polypeptide corresponding to kappa-casein 106-169. CMP naturally exists in several forms due to extensive posttranslational modifications including glycosylation and phosphorylation. The aglycosylated, phosphorylated form of CMP has been shown to exhibit antibacterial activity. The aim of this study was to use matrix assisted laser desorption/ionization post source decay mass spectrometry (MALDI-PSD-MS) to identify the phosphorylation sites in the CMP sequence. CMP was isolated from a chymosin digest of casein by HPLC and then digested with endoproteinase Glu-C to generate peptides suitable for MALDI-PSD-MS analysis. This analysis showed that CMP is fully phosphorylated at Ser(149) and only partially phosphorylated at Ser(127.) Dehydroalanyl residues corresponding to the phosphoserines of CMP were detected upon MALDI-PSD-MS analysis suggesting that the phosphoryl bond in phosphoserine is very labile during PSD analysis such that the phosphoryl group may be lost before backbone fragmentation.

54. Health aspects of dairy products – Dairy products in relation to caries prevention and oral health.

Reynolds EC. Encyclopedia of Dairy Sciences 2001.

55. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate

Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. *J Dent Res* 2001 Dec;80(12):2066-70

Casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) exhibit anticariogenic potential in laboratory, animal, and human in situ experiments. The aim of this study was to determine the ability of CPP-ACP in sugar-free chewing gum to remineralize enamel subsurface lesions in a human in situ model. Thirty subjects in randomized, cross-over, double-blind studies wore removable palatal appliances with six human-enamel half-slabs inset containing sub-surface demineralized lesions. The appliances were inserted immediately before gum-chewing for 20 min and then retained for another 20 min. This was performed four times per day for 14 days. At the completion of each treatment, the enamel half-slabs were paired with their respective demineralized control half-slabs, embedded, sectioned, and subjected to microradiography and densitometric image analysis, for measurement of the level of remineralization. The addition of CPP-ACP to either sorbitol- or xylitol-based gum resulted in a dose-related increase in enamel remineralization, with 0.19, 10.0, 18.8, and 56.4 mg of CPP-ACP producing an increase in enamel remineralization of 9, 63, 102, and 152%, respectively, relative to the control gum, independent of gum weight or type.

56. Remineralization of early enamel caries by anticariogenic casein phosphopeptide – amorphous calcium phosphate nanocomplexes

Reynolds EC. *Dental Practice* 2001 November / December

Casein phosphopeptides (CPP) are multi-phosphorylated peptides from an enzymatic digest of the bovine milk protein casein. These peptides have a remarkable ability to stabilize calcium phosphate in solution as amorphous calcium phosphate (ACP). Through their multiple phosphoserine residues the CPP bind to forming nanoclusters of ACP in metastable solution, preventing their growth to the critical size required for nucleation and phase transformations. The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes have been shown to localize at the tooth surface and prevent caries in laboratory, animal and human in situ caries models. The CPP-ACP have also been shown to remineralize enamel subsurface lesions in situ when delivered in a sugar-free chewing gum. The proposed anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. The CPP-ACP have potential as an additive to foods and oral care products for the control of dental caries.

57. Abstract 2810 - An in vitro investigation of the effects of casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) on erosion of human dental enamel by a sports drink

Ramalingam L, Messer LB and Reynolds EC. IADR, General Session, San Diego 2002 - Abstract 2810

Objectives: There have been numerous studies that have attempted to reduce the erosive potential of acidic beverages. Previous studies have demonstrated the erosivity of Powerade™ and the purpose of this study was to determine the lowest concentration of casein phosphopeptide - stabilised amorphous calcium phosphate (CPP-ACP) required to be added to Powerade™ to eliminate erosion of human enamel *in vitro*.

Methods: Surface changes of 30 human enamel specimens were examined following immersion in Powerade™ (P) containing 0.063%, 0.09%, 0.125%, 0.25% CPP-ACP for 30 minutes at 37°; under constant agitation in a water bath; P and double deionized water (DDW) were positive and negative controls respectively. Enamel surface characteristics were examined qualitatively (stereomicroscopy, scanning electron microscopy) and quantitatively (surface profilometry).

Results: The pH values of test solutions were higher than that of P (2.70), and the pH of each solution increased slightly with increasing concentrations of CPP-ACP (pH 3.07, 3.27, 3.40, 3.90). The titratable acidity decreased on addition of CPP-ACP (P=1.83, 1.61, 1.63, 1.47, 1.36). All enamel specimens immersed in P with or without CPP-ACP exhibited red superficial staining. The mean profile depths were: P: 3.87µm (SD 0.56), P+0.063% CPP-ACP: 1.80µm (SD 0.31), P+0.09% CPP-ACP: 0.43µm (SD 0.06), P+0.125% CPP-ACP: 0.34µm (SD 0.17), P+0.25% CPP-ACP: 0.19µm (SD 0.06), DDW: 0.25µm (SD 0.07). P alone and P+0.063% CPP-ACP displayed an erosive step between test and control areas of enamel. The mean erosive depths of DDW, P with 0.09%, 0.125% and 0.25% CPP-ACP did not differ significantly ($p>0.05$). Type 1 etch patterns predominated with scattered areas of Type 3 in P alone and P+0.063% CPP-ACP. Superficial surface irregularities were displayed for P+0.09% and 0.125% CPP-ACP.

Conclusion: The erosive potential of Powerade™ was eliminated or reduced on addition of low concentrations of CPP-ACP (0.09%, 0.125% and 0.25%) *in vitro*.

58. A clinical trial of the anticaries efficacy of casein derivatives complexed with calcium phosphate in patients with salivary gland dysfunction

Hay KD^a and W. Murray Thomson. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;93:271-5

Objective: The purpose of this study was to compare the caries preventive efficacy of a mouthrinse solution containing casein derivatives coupled with calcium phosphate (CD-CP) with that of a 0.05% sodium fluoride mouthrinse among individuals with dry mouth. **Study**

Design: A randomized control trial design was used. Participants included individuals who had had radiotherapy for head and neck cancer (n = 82) and others with Sjögren's syndrome (n = 56). Baseline data collection was followed by reexamination 12 months later. Posterior bite-wing radiographs were taken on both occasions.

Results: A total of 124 participants, 61 (49.2%) in the sodium fluoride group and 63 (50.8%) in the CD-CP group, completed the 12-month examination. The baseline characteristics of the 2 groups did not differ. Coronal caries incidence was higher in the sodium fluoride group than in the CD-CP group (34.4% and 27%, respectively), but the difference was not statistically significant. Similarly, the small difference in coronal caries increment between the 2 groups was not statistically significant (0.4 and 0.3 surfaces, respectively). There was insufficient root surface caries experience between the 2 groups observed for differences to be examined. Proportionately more of the CD-CP group lost 1 or more teeth, and the mean number of tooth loss was higher. The participants with the highest incidence and increment were those with Sjögren's syndrome in the CD-CP group. Some of that difference was accounted for by differences in baseline caries status.

Conclusion: It appears that CD-CP preparations hold promise as caries preventive agents for individuals with dry mouth, although confirmation of this study's findings in other settings is warranted before a definitive conclusion can be reached.

59. Incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass-ionomer cement

Mazzaoui SA, Burrow MF, Tyas MJ, Dashper SG, Eakins D, Reynolds EC. *J Dent Res* 2003 Nov;82(11):914-8.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes have been shown to prevent demineralization and promote remineralization of enamel subsurface lesions in animal and in situ caries models. The aim of this study was to determine the effect of incorporating CPP-ACP into a self-cured glass-ionomer cement (GIC). Incorporation of 1.56% w/w CPP-ACP into the GIC significantly increased microtensile bond strength (33%) and compressive strength (23%) and significantly enhanced the release of calcium, phosphate, and fluoride ions at neutral and acidic pH. MALDI mass spectrometry also showed casein phosphopeptides from the CPP-ACP nanocomplexes to be released. The release of CPP-ACP and fluoride from the CPP-ACP-containing GIC was associated with enhanced protection of the adjacent dentin during acid challenge in vitro.

60. Abstract 1007- Caries prevention potential of a tooth-coating material containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)

Sato T, Yamanaka K and Yoshii E. *IADR, General Session, Göteborg 2003 - Abstract 1007*

Objectives: The purpose of this study was to evaluate the caries prevention potential of a newly developed tooth coating material *in vitro*.

Methods: A newly developed tooth coating material: Tooth Mousse (TM, GC Corp), a CPP-ACP free placebo (PP) and fluoride (900ppm F) added placebo (FP) were prepared. Bovine enamel specimens were prepared and assigned to 6 test treatments using 10 specimens per treatment group. They were immersed in 10 wt. % diluted solution of each material for 10 minutes, followed by 10 minutes immersion in demineralization solution (pH=4.75, Ca: 0.75mM, P: 0.45mM) twice a day for 4 days. Except those treatments, the specimens were immersed in ion exchanged water (IEW) or were stayed in air of 100% RH at 37 degree Celsius. The extent of demineralization of the enamel specimens was determined by Knoop hardness measurements. The hardness reduction (Δ KHN) was chosen as the primary efficacy viable. Buffer capacity of TM was also measured by pH monitoring. Ten vol. % diluted solution of TM and IEW was added to suspension of *Streptococcus mutans* (pH=4.5) and the pH of suspension was monitored for 2 hours.

Results: In IEW immersing group, Δ KHN of TM, FP and PP were 22.8, 51.6 and 59.6 respectively and in air staying group, Δ KHN of TM, FP and PP were 9.3, 24.2 and 30.6 respectively. Compared with FP and PP, TM showed less Knoop hardness reduction ($p<0.05$). Two hours after the addition of samples, the pH of TM and IEW showed 6.0 and 4.0 respectively.

Conclusions: The results show that TM is effective in preventing the enamel demineralization in vitro and has strong buffer capacity to acid produced by *S. mutans*.

61. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum

Reynolds EC, Cai F, Shen P, Walker GD. *J Dent Res* 2003 Mar;82(3):206-11

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes incorporated into sugar-free chewing gum have been shown to remineralize enamel subsurface lesions in situ. The aim of this study was to compare the ability of CPP-ACP, with that of other forms of calcium, to be retained in supragingival plaque and remineralize enamel subsurface lesions in situ when delivered in a mouthrinse or sugar-free gum in randomized, double-blind trials. In the mouthrinse study, only the CPP-ACP-containing mouthrinse significantly increased plaque calcium and inorganic phosphate levels, and the CPP were immunolocalized to the surfaces of bacterial cells as well as the intercellular matrix. In the chewing gum studies, the gum containing the CPP-ACP, although not containing the most calcium per piece of gum, produced the highest level of enamel remineralization independent of gum-chewing frequency and duration. The CPP could be detected in plaque extracts 3 hrs after subjects chewed the CPP-ACP-containing gum. The results showed that CPP-ACP were superior to other forms of calcium in remineralizing enamel subsurface lesions.

62. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate

Cai F, Shen P, Morgan MV, Reynolds EC. *Aust Dent J* 2003 Dec;48(4):240-3

Background: The anticariogenic potential of casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) has been demonstrated using laboratory, animal and human in situ caries models. The aim of this study was to determine the effect of CPP-ACP incorporation into a sugar-free lozenge (pressed mint tablet) on enamel remineralization in a human in situ model.

Methods: The study utilized a double-blind, randomized, cross-over design with four treatments: (i) a lozenge containing 56.4mg (3 per cent w/w) CPP-ACP; (ii) a lozenge containing 18.8mg (1 per cent w/w) CPP-ACP; (iii) a lozenge not containing CPP-ACP; and (iv) a no lozenge nil-treatment control. Ten subjects wore removable palatal appliances with four, human-enamel, half-slab insets containing subsurface lesions. Lozenges were consumed, without chewing, four times per day for 14 days duration. After each treatment period the enamel slabs were removed, paired with their respective demineralized control, embedded, sectioned and subjected to microradiography and computer-assisted densitometric image analysis to determine the level of remineralization.

Results: The incorporation of CPP-ACP into the lozenge significantly increased enamel subsurface lesion remineralization with 18.8 and 56.4mg of CPP-ACP increasing remineralization by 78 and 176 per cent respectively, relative to the control sugarfree lozenge.

Conclusion: This study demonstrates that lozenges are a suitable vehicle for the delivery of CPP-ACP to promote enamel remineralization.

63. Erosion of human dental enamel by sports drinks

Ramalingam L, Messer LB, Reynolds EC. *Accepted for publication Paed Dent*

(not published)

64. Dairy components in oral health

Reynolds EC. *Aust. J. Dairy Technol.* 58, 79-81

Dental caries (tooth decay) is the localized destruction of tooth tissue initiated by specific dental plaque bacteria that ferment dietary sugar to organic acids. Even though in most developed countries the prevalence of dental caries has decreased through the use of fluorides, the disease remains a major public health problem. A substantial volume of literature now exists demonstrating an anticariogenic effect of dairy products (milk, milk concentrates, powders and cheeses) in laboratory, animal and human *in situ* caries models. This anticariogenic effect has been attributed to the multiphosphoryl-containing sequences of casein and their ability to stabilize calcium phosphate. These sequences can be released enzymically as casein phosphopeptides (CPP), which stabilise amorphous calcium phosphate (ACP) in solution by the formation of CPP-ACP nanocomplexes. The CPP-ACP localise at the tooth surface and prevent demineralisation and promote remineralisation of enamel subsurface lesions. CPP-ACP is now being used commercially as an ingredient (RecaldentTM) in oral care products.

65. Molecular modeling of the multiphosphorylated casein phosphopeptide α_{S1} -casein (59-79) based on NMR constraints

Huq NL, Cross KJ, Reynolds EC. *Journal of Dairy Research* (2004) 71 28-32

Introduction: Casein micelles contain stabilized amorphous calcium phosphate that is bioavailable to the neonate (Holt & Sawyer, 1988). Tryptic phosphopeptides formed from casein digestion associate with amorphous calcium phosphate forming stable nanocomplexes that have been described as calcium phosphate delivery vehicles (Holt et al. 1996; Reynolds et al. 1999, 2003; Farrell et al. 2002). The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) technology has been commercialized by the food and oral care industries as agents for the prevention and repair of early stages of dental caries (tooth decay) (Reynolds, 2000) (<http://www.recaldent.com>).

66. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)

Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. *Caries Res* 2004;38:551-556

The aim of this clinical study was to investigate the acid resistance of enamel lesions remineralized in situ by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP: RecaldentTM). The study utilized a double-blind, randomized, crossover design with two treatments: (i) sugar-free containing 18.8 mg of CPP-ACP, and (ii) sugar-free gum not containing CPP-ACP as control. Subjects wore removable palatal appliances with inserts of human enamel containing demineralized subsurface lesions and chewed the gum for 20 min 4 times per day for 14 days. After each treatment the enamel slabs were removed and half of each lesion challenged with acid in vitro for 8 or 16 h. The level of remineralization was determined using microradiography. The gum containing CPP-ACP produced approximately twice the level of remineralization as the control sugar-free gum. The 8- and 16-hour acid challenge of the lesions remineralized with the control gum resulted in 65.4 and 88.0% reductions, respectively, of deposited mineral, while for the CPP-ACP remineralized lesions the corresponding reductions were 30.5 and 41.8%. The acid challenge after in situ remineralization for both control and CPP-ACP-treated lesions resulted in demineralization underneath the remineralized zone, indicating that the remineralized mineral was more resistant to subsequent acid challenge. The results show that sugar-free gum containing CPP-ACP is superior to an equivalent gum not containing CPP-ACP in remineralization of enamel subsurface lesions in situ with mineral that is more resistant to subsequent acid challenge.

67. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle- α_{S1} -casein (59-79) by stabilized amorphous calcium fluoride phosphate nanocomplexes

Cross KJ, Huq NL, Stanton DP, Sum M, Reynolds EC. *Biomaterials*

The repair of early tooth enamel lesions has been recently demonstrated by tryptic phosphopeptides derived from milk caseins that associate with amorphous calcium phosphate (ACP) forming stable complexes. These casein phosphopeptides (CPP), containing the cluster sequence-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-, form calcium phosphate delivery vehicles that retard enamel demineralization and promote remineralization. Recently, we have shown that these peptides also stabilize calcium fluoride phosphate as soluble complexes. These complexes designated CPP-ACFP, have the potential to provide superior clinical efficacy in preventing dental caries and treating and repairing early stages of disease. In an approach to determine the ultrastructure of the casein phosphopeptide-amorphous calcium fluoride phosphate complexes, we have studied the structure of the predominant peptide α_{S1} -CN(59-79) bound to ACFP using nuclear magnetic resonance (NMR) spectroscopy and X-ray diffraction. The α_{S1} -CN(59-79) peptide stabilized calcium fluoride phosphate as amorphous nanocomplexes with a hydrodynamic radius of 2.12 ± 0.26 nm. The nanocomplexes exhibited stoichiometry of one peptide to 15 calcium, nine phosphate and three fluoride ions. Sequence-specific resonance assignments were characterized by sequential ($i, i + 1$), medium-range ($i, i + 2$) NOEs and ^1H chemical shifts. The spectral data were compared with that of the peptide α_{S1} -CN(59-79) bound to calcium ions, revealing that the structurally significant secondary NH and α -chemical shifts were similar.

68. Abstract 2045 - Fluoride effect on acid resistance capacity of CPP-ACP containing material

Kariya S, Sato T, Sakaguchi Y, Yoshii E. *IADR, 82nd General Session, Honolulu 2004 - Abstract 2045*

Objectives: The purpose of this *in vitro* study was to evaluate the effect one acid resistance and neutralizing capacity when fluoride is added to a CPP-ACP containing material.

Methods: A CPP-ACP containing material (Tooth Mousse: TM, GC Corp.) and a second material containing 900ppm fluoride (TMP) were prepared. Bovine enamel specimens were cut out and early caries lesions were formed in acetic acid buffer solution (pH4.8, Ca:1.5mM, P:0.9mM) for 3 days. Thirty wt. % dilution by remineralizing solution (pH7, HEPES: 20mM, Ca 1.5mM, P:0.9mM) was prepared for each material. Specimens were immersed in each diluted material at 37 degree Celcius for 10 days. The acid resistance of the specimen was determined by measuring the amount of calcium loss after the immersion in lactic acid buffer solution (pH4.5) for 3 hours. Neutralizing capacity of each sample was also measured by pH monitoring during acid addition. Ten μL of 1N hydrochloric acid solution was continuously added to 10mL of 10wt.% diluted solution of TM and TMP.

Results: The amount of calcium loss of TMP and TM was $8.86 \mu\text{g}/\text{mm}^2$ (SD:1.6) and $21.7 \mu\text{g}/\text{mm}^2$ (SD:1.9) respectively and TMP showed lower levels of calcium loss compared with TM ($p < 0.05$). After the addition of 100 μL hydrochloric acid solution, the pH of TM and TMP were reduced from 7.7 to 5.9 and 5.4 respectively. The neutralizing capacity of TM was a little superior to TMP. Fluoride has the capacity to improve the crystalline tooth structure, generation of fluoroapatite and accelerate remineralization. These effects would assist the improvement of acid resistance of TMP.

69. Abstract 2046 – Remineralization power by xylitol chewing gums

Murata Y, Matsuda Y, Tanaka T, Ikeda T, Komatsu H, Sano H, Miyake R, Morita M. IADR, 82nd General Session, Honolulu 2004 - Abstract 2046

It is considered that chewing gums containing some additives promote remineralization of enamel.

Objectives: To investigate the effect of xylitol chewing gums on remineralization of enamel in vitro.

Methods: Xylitol chewing gum containing *Gloiopeltis furcata* extract and calcium hydrogenphosphate (GF), xylitol chewing gum containing casein phosphopeptide-amorphous calcium phosphate (CP), and experimental chewing gum only containing xylitol (CO) were used. Twenty-four demineralized enamel slabs, which were prepared by immersing in 0.01 M acetate buffer (pH4.0) for 2 days at 50°C, were partially covered with wax for preserving demineralized surface. For remineralization process, enamel slabs with the wax were immersed for 14 days at 37°C in a remineralizing solution (1.0 mM CaCl₂, 0.6mM KH₂PO₄, 0.1 m NaCl:pH 7.3 with KOH) containing extracts from each chewing gum. The remineralized enamel slabs without the wax were dehydrated and embedded in polyester resin. Approximately 100µm-thick enamel sections were prepared. Remineralization power was analyzed quantitatively by contacting microradiography for mineral change (vol%µm) between demineralized and remineralized surface, which was standardized with NIH image. Paired t-test was conducted to analyze the remineralization ability of each xylitol chewing gum. One-way ANOVA and Tukey's test were then conducted between each independent variable.

Results: Paired t-test demonstrated that the remineralization ability for each GF (X=1682.3, SD=667.4) and CP (X=780.0, SD=705.2) was significant (p <0.05), while we failed to find the same ability in CO (X=393.3, SD=703.2, ns). Tukey's test showed that the remineralization power for GF was significantly higher than that of CP and CO (p<0.05).

Conclusion: The remineralization power of chewing gums differs depending on the additives the chewing gum contained.

70. Abstract 2049 – Effects of cheese and milk containing CPP-ACP on enamel remineralization

Minami K, Inaba D, Yonemitsu M, Koganei M, Oda M. IADR, 82nd General Session, Honolulu 2004 - Abstract 2049

Objectives: It has been known that a sort of food such as cheese can enhance remineralization. In this study, effects of processed cheese and milk containing CPP-ACP on enamel remineralization were examined in vitro.

Methods: The enamel blocks prepared from bovine incisors were demineralized by immersion in a 0.1 M lactic gel containing 6wt% carboxymethylcellulose at 37°C for 2 w. Each 6 demineralized enamel samples were exposed to one of the following solutions for 60 min: A: untreated (baseline), B: 10% soln. of processed cheese (Maiji Dairies Co., Japan), C: 10% soln. of cheese containing 0.5% CPP-ACP, D: 30% soln. of cheese, E: 30% soln. of cheese containing 0.5% CPP-ACP, F: milk (Meiji Dairies Co., Japan) G: milk containing 0.2% CPP-ACP, H: milk containing 0.5% CPP-ACP. Subsequently, the samples were immersed in the mineral solution (1.5 mM Ca, 0.9 mM phosphate, 20 mM Hepes, pH 7) for 23hr. These treatments were repeated for 7 d and, finally, the samples were microradiographed to measure the lesion depth (Id, µm).

Results: The groups B, C, E, G and H showed significantly lower Id values by 35-46% compared to the group A (105±16µm; p<0.05). The group E (57±7µm) had significantly lower Id values compared to the group D (84±12µm). So did the group G (64±10µm) and H (63±12µm) with respect to the group F (91±6µm) indicating improved mineral recovery.

Conclusions: In conclusion, it was suggested that addition of CPP-ACP into processed cheese or milk would be effective for enhancement of remineralization in enamel lesions.

71. Abstract 2997 – Molecular of anticariogenic casein phosphopeptide aS2-CN(2-20) NMR spectroscopy derived constraints

Deangelis EF, Huq NL, Cross KJ, Reynolds EC. IADR, 82nd General Session, Honolulu 2004 - Abstract 2997

Objective: The repair of early enamel lesions has been demonstrated by tryptic phosphopeptides derived from milk caseins that associate with amorphous calcium phosphate (ACP) forming stable complexes. These casein phosphopeptides (CPP), form calcium phosphate delivery vehicles that retard enamel demineralization and promote remineralization.

Method: The aim of this project was to characterize, using molecular modeling techniques, the calcium phosphate delivery vehicle aS2-CN(2-20). The in silico peptide aS2-CN(2-20) was constrained using distance constraints obtained from nuclear magnetic resonance (NMR) spectroscopy data. All calculations were performed with the molecular package SYBYL 6.8 on a Silicon Graphics Octane workstation. After energy minimization, dynamics and simulated annealing, fifty conformers were produced that were stereochemically correct with low nOe violations indicating good consistency with the nmr data.

Results: The RMSD values, together with the changes in the conformation along the backbone as revealed by the Ramachandran plots and orientation of the side-chains indicate the presence of turns and loops with a high degree of flexibility between these secondary structural elements. These results correlate well with the ability of the CPP to bind to both amorphous and crystalline phases of calcium phosphate.

Conclusion: In conclusion molecular modeling is an essential tool in the investigation of the anticariogenic mechanism of the CPP because it enables the visualization of the peptide conformational preferences and allows the identification of structural features that are important in its role as a calcium phosphate delivery vehicle.

72. Determination of demineralization of tooth substrate by use of an ultrasonic device.

Takamizawa T, Yamaguchi K, Inage H, Miyazaki M, Ando S, Hinoura K. Japan J Conserv Dent 2004 Jun 47 Spring Issue 24 – Abstract B-4

Objective: It has been proven that a tooth structure is maintaining its dynamic balance by repeating demineralization and remineralization. Accordingly, it is considered as important to determine the condition of demineralization on the tooth structure in order to prevent a caries formation to be caused as a result of the demineralization. Therefore we focused on the ultrasonic pulse method and tried to identify the condition of demineralization with time, by measuring the changes on the tooth structure, which means, the changes of the sonic speed, in order to establish an in-vitro model of demineralization or remineralization.

Method:

1) Fabrication of the specimens for the measurement

The extracted bovine lower anterior teeth without crack or deficiently mineralization, at the age from 2 to 3 years old were used for this trial. The specimens were prepared by cutting the enamel and dentin of labial surface of the above bovine teeth in a block (4x4x1mm), using low-speed hard tissue precision cutting machine (Isomet 1000, Buehler). All the surfaces of the block were polished accordingly to the degree of a water-resistant SiC paper #2,000. This block was used as the measuring specimen.

2) Storage in the demineralizing solution

The specimens were stored in the 0.1M lactic acid buffer solution for 10 and 30 minutes, and stored in the artificial saliva (pH 7.0). Other specimens were stored in the 10-times diluted solution of CPP-ACP Paste (Tooth Mousse, GC) and placebo paste (no CPP-ACP contained) for 10 minutes prior to the storage of the specimens in the demineralizing solution. After that, they were also stored in the 0.1M lactic acid buffer solution and then in the artificial saliva at 37°C. The specimens were stored in the demineralizing solution twice a day.

3) Measuring of the propagation time of ultrasonic waves

Pulser/Receiver (MODEL 5900, Panametrics) as an ultrasonic transmitter/receiver, Oscilloscope (Wave Runner LT584, Lecroy) and a specimen stage were used as the measurement system. The specimen was settled on the stage and contacted by the transducer. Each propagation time of ultrasonic waves, which occurred in longitudinal wave, was measured by ultrasonic transmission method. Next, the thickness of each specimen and its longitudinal wave sonic speed was measured. Above measurement was conducted before the storage in the solution every day for one week, and every 7 days until 28 days after starting the storage in the solution. Each measurement was conducted at $23 \pm 1^\circ\text{C}$, $50 \pm 5\%\text{RH}$ in the temperature-controlled room. The number of specimens used was 6 pieces for each condition.

Results: The ultra-sonic speed of enamel and dentin specimens was found to decrease with time for a specimen stored in the demineralizing solution compared to a control specimen stored in the artificial saliva. This tendency to decrease of the sonic speed meant the decrease in the inorganic component of the tooth structure; therefore, it could be considered that the inorganic component of the tooth structure dissolved by the demineralizing solution. On the other hand, no decrease in the sonic speed was found for both of enamel and dentin specimens stored in the 10-times diluted solution of CPP-ACP paste and even an increase of the sonic speed was found especially for the enamel. It could be considered that the inorganic component contained in high concentrations in CPP-ACP acted to prevent demineralization or to enhance remineralization of the tooth structure.

Conclusion: From the result of this study, it was suggested that the condition of demineralization of the tooth structure could possibly be measured non-destructively by using ultrasonic pulse method.

73. Clinical effectiveness of a CPP-ACP crème for tooth hypersensitivity treatment.

Poitevin A, Peumans M, De Munck J, Van Landuyt K, Coutinho E, Braem M, Van Meerbeek B. EADR Istanbul, 25-28 August 2004, Abstract 0136

Tooth hypersensitivity is a widespread oral discomfort that remains difficult to treat.

Objectives: To evaluate the clinical effectiveness of a newly developed tooth-coating crème (GC Tooth Mousse), containing Casein PhosphoPeptide - Amorphous Calcium Phosphate (CPP-ACP) for the treatment of tooth hypersensitivity.

Methods: 11 private practitioners treated 61 patients (37 female, 24 male, 19-67 yr age range) for tooth hypersensitivity using GC Tooth Mousse. Medical and dental history, oral hygiene and dietary habits were recorded before treatment. At baseline, sensitivity of the test teeth was scored by the dentist on a 0-10 visual analogue scale (VAS) after air-blast (A), instrumentation with a probe (P) and an ultrasonic scaler (U). The patient was instructed to apply GC Tooth Mousse during 21 days (brush teeth at evening, apply GC Tooth Mousse in tray or with swab, leave for 3 min, spread remaining crème throughout the mouth for 1-2 min, do not eat or drink for 30 min). Sensitivity of the test teeth was daily evaluated by the patient using VAS. After 3 weeks, patients sensitivity was re-scored by the dentist (A, P, and U).

Results: Before treatment, the test teeth showed a VAS sensitivity of 6.0 ± 2.7 (A), 3.3 ± 2.9 (P) and 5.9 ± 2.9 (U). A significant reduction (Wilcoxon signed rank statistics, $p < 0.0001$) in tooth sensitivity was noticed by the dentist after 21 days to 3.2 ± 2.8 (A), 2.0 ± 2.4 (P) and 4.1 ± 3.3 (U). The change in sensitivity was more apparent after the air stimulus than the tactile stimuli. Regarding the daily evaluation of tooth sensitivity by the patient, the sensitivity also decreased from day 1 (5.6 ± 2.4) to day 21 (2.3 ± 2.3). Half of the patients reported a general reduction in sensitivity (44%) and wanted to repeat the treatment if sensitivity re-occurred (43%).

Conclusion: Out of this clinical study, GC Tooth Mousse appears to be effective in reducing tooth hypersensitivity.

74. Enamel remineralization by a mouthrinse containing casein phosphopeptide-amorphous calcium phosphate and fluoride in an *in situ* model.

Shen P, Cai F, Walker G, Reynolds C, Reynolds EC. Australian Dental Journal ADRF Special Research Supplement 2004;49:4

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) have been demonstrated to have anticariogenic activity in laboratory, animal and human *in situ* experiments. The aim of the current study was to compare the enamel remineralization ability of mouthrinse containing CPP-ACP with that of a mouthrinse containing fluoride in an intra-oral enamel remineralization model. The study utilized a double blind, randomized, four-way crossover design with four treatments: (i) 0.4% CPP-ACP, (ii) 0.4% CPP-ACP and 220 ppm F⁻, (iii) 220ppm F⁻, and (iv) mouthrinse containing neither CPP-ACP nor fluoride (placebo). Approval for the study was obtained from The University of Melbourne Human Research Ethics Committee.

Sound relatively planar buccal and lingual surfaces free of cracks, stains and fluorosis were selected from extracted human third molars and polished wet to a mirror finish using Softex™ (3M) discs. Each polished surface was then sawn from the tooth as an approximately 8 x 4 mm slab and lesions were created in the enamel windows using a lactate pH 4.8, carbopol demineralization buffer. Nine healthy subjects wore removable palatal appliances with four half-slab insets of human enamel containing demineralized subsurface lesions. The other half of each enamel slab was stored in a humidified container and was used as the control demineralized lesion. All subjects were instructed to insert the intra-oral appliance immediately before mouthrinsing for 60 seconds and to wear the appliance for a further 20 minutes. This was performed four times per day at the following times: at 10 am, 11.30 am, 2 am and 3.30 pm. Each treatment was for 14 days duration and at the completion of each treatment the enamel half-slabs were removed, paired with their respective demineralized control, embedded, sectioned and subjected to microradiography and computer-assisted densitometric image analysis to determine the level of mineral content. The mouthrinse containing 0.4% CPP-ACP and 220 ppm F⁻ produced 19% enamel subsurface lesion remineralization compared with 2% remineralization produced by the placebo rinse, 8% remineralization by the 220 ppm F⁻ rinse, and 14% remineralization by the 0.4% CPP-ACP rinse. All these values were significantly different to each other ($p < 0.01$). The results support the role of fluoride in promoting remineralization and demonstrate an important facilitation of the effect of fluoride by CPP-ACP.

75. Investigation of the binding of casein phosphopeptides to the major enamel pellicle proteins.

Ung M, Huq NL, Cross KJ, Reynolds EC. *Australian Dental Journal ADRF Special Research Supplement 2004*;49:4

Tryptic phosphopeptides derived from milk caseins are known to associate with amorphous calcium phosphate (ACP) forming stable complexes that behave as calcium phosphate delivery vehicles and are effective in the remineralization of early enamel lesions. The major casein phosphopeptides (CPP) are α_{s1} -CN(59-79) and β -CN(1-25) and its deamidated forms with smaller amounts of α_{s2} -CN(1-21) and α_{s2} -CN(46-70). The sequences are shown below, using the three letter codes for the amino acid residues, where Ser (P) denotes an O-phosphoserine residue.

All peptides contain the sequence motif –Ser(P)-Ser(P)-Ser(P)-Glu-Glu-. To date the potential anti-cariogenicity of the CPP-ACP complex has been demonstrated in a rat caries model, in situ human caries models, in vitro remineralization/demineralization models and in short-term mouthwash trials (Reynolds, 1991; Reynolds, et al., 1995; Shen et al., 2001; Cai et al., 2003). The anticariogenic mechanism of the casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complexes has not been fully described. It has been proposed that CPP localize ACP in dental plaque, thus acting as a reservoir of free calcium and phosphate, depressing enamel demineralization and enhancing enamel remineralization of early lesions. However, the interactions between CPP and salivary proteins has not been previously investigated. Oral cavity surfaces are constantly exposed to free flowing saliva. Upon contact with saliva, tooth enamel is immediately covered by a thin layer of salivary proteins that forms the acquired enamel pellicle. The aim of this study was to investigate the binding of the CPP to salivary proteins using an enzyme-linked immunosorbent assay (ELISA). This interaction may represent a crucial phase in the anticariogenic activity of CPP. Therefore, understanding the exact mechanism of CPP activity may enable its use in a greater number of therapeutic applications and lead to further improvement of its specific activity and efficacy.

The casein phosphopeptides α_{s1} -CN (59-79) and β -CN (1-25) were selectively precipitated from a tryptic digest of casein using Ca^{2+} and ethanol and further purified by anion exchange FPLC and reversed phase HPLC. The purity was checked by MALDI-TOF mass spectrometry.

Whole unstimulated saliva was collected from adults. Parotid saliva was collected using a Lashley cup after stimulation with lemon drops. The profiles of intact untreated saliva were examined by SDS-PAGE, HPLC and MALDI-TOF mass spectrometry. Similarly the profiles of the salivary proteins bound to HA were also examined by SDS-PAGE and MALDI-TOF mass spectrometry for comparison with previously published reports.

An ELISA was developed using polyclonal rabbit anti-bovine casein antibodies to detect the CPP. Flat bottomed, 96-well polyvinyl microtitre plates were coated with 50 μ L of clarified whole saliva (10 μ g/mL) in PBS and incubated at 4°C overnight. The coating solution was removed and the wells blocked with 200 μ L of 1% (w/v) BSA in PBST for one hour at RT. The plate was washed four times with PBST, followed by the serial dilution of CPP (5mg/mL) in PBS and incubation for three hours at RT. The plate was washed and 50 μ L of polyclonal rabbit anti-bovine casein antibodies (1:640) in 0.5% (w/v) BSA was added and incubated overnight at RT. Six washes were performed followed by the addition of 100 μ L of horse radish peroxidase-conjugated goat anti-rabbit antibody (1:2000) in 0.5% BSA and incubation for three hours at RT. The plate was washed six times and 100 μ L of substrate solution was added to each well. After 15 minutes of development the optical density was measured at 415nm.

Since the salivary statherin is also multi-phosphorylated and was postulated to be delivered from the ancestral milk casein, binding of the anti-bovine casein antibodies to whole and parotid saliva was tested and confirmed to be negligible. The ELISA revealed that both α_{s1} -CN (59-79) and β -CN (1-25) bind to whole and parotid saliva. Superimposition of the binding curves suggested that β (1-25) casein had a greater degree of binding to whole saliva.

Previous studies have shown that CPP-ACP is incorporated into dental plaque, and it has been suggested that the hydrophobic residues in CPPs may be involved as attachment anchors at hydrophobic sites in plaque and oral tissues. However, this is the first time the direct binding of the anticariogenic CPP to salivary proteins has been demonstrated.

76. Additional aids to the remineralisation of tooth structure.

Reynolds EC, Walsh LJ. *Textbook: Preservation and Restoration of Teeth - 2nd edition 2005, p111-118*

For details ref. Textbook: Preservation and Restoration of Teeth - 2nd edition 2005, p111-118

77. Acid Resistance of Enamel Subsurface Lesions Remineralized by a Sugar-Free Chewing Gum Containing Casein Phosphopeptide-Amorphous Calcium Phosphate

Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Caries Res 2004;38:551-556

The aim of this clinical study was to investigate the acid resistance of enamel lesions remineralized in situ by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP: Recaldent™). The study utilized a doubleblind, randomized, crossover design with two treatments: (i) sugar-free gum containing 18.8 mg of CPPACP, and (ii) sugar-free gum not containing CPP-ACP as control. Subjects wore removable palatal appliances with insets of human enamel containing demineralized subsurface lesions and chewed the gum for 20 min 4 times per day for 14 days. After each treatment the enamel slabs were removed and half of each lesion challenged with acid in vitro for 8 or 16 h. The level of remineralization was determined using microradiography. The gum containing CPP-ACP produced approximately twice the level of remineralization as the control sugar-free gum. The 8- and 16-hour acid challenge of the lesions remineralized with the control gum resulted in 65.4 and 88.0% reductions, respectively, of deposited mineral, while for the CPP-ACP-remineralized lesions the corresponding reductions were 30.5 and 41.8%. The acid challenge after in situ remineralization for both control and CPP-ACP-treated lesions resulted in demineralization underneath the remineralized zone, indicating that the remineralized mineral was more resistant to subsequent acid challenge. The results show that sugar-free gum containing CPP-ACP is superior to an equivalent gum not containing CPP-ACP in remineralization of enamel subsurface lesions in situ with mineral that is more resistant to subsequent acid challenge.

78. La reminéralisation des lésions carieuses (2) synergies thérapeutiques / The remineralisation of caries lesions: joint therapies

Lasfargues JJ, Martin JM, Miller C. Realites Cliniques Vol. 15 n°3, 2004 pp.261-275

Despite the widespread diffusion of topical fluorides, 20% of those patients who do not, in principle, belong to a risk group still present with carious lesions, and the patients who have the greatest risk of developing caries may not always benefit from optimal fluoridation. In addition, if carious lesions must be intercepted at an early stage, in the patient at high risk the caries are already present. These findings justify focusing on therapeutic agents which are capable of reacting jointly with fluorides, and it is proper, at the clinical level, to develop dual strategies of remineralization and restoration. In this article, the various therapeutic possibilities for remineralization that may be associated with fluorides are discussed, and joint therapies with these are proposed. The roles of chlorhexidine, xylitol, and caseine phosphopeptides on remineralization are discussed. The treatment with ozone is debated and illustrated in a case of remineralization of cervical lesions in a patient under neuroleptic medication. The capacity of the glass ionomer cement restorations to remineralize the dental tissues internally and externally is also discussed. It is shown that even if the prophylactic measures using these agents and materials show undeniable clinical importance, none of them can substitute for optimal topical fluoride application. By way of conclusion, a clinical case is presented which allows us to stress the importance of the complementary use of prophylactic and restorative measures.

79. Using ultrasound transmission velocity to analyze demineralization of tooth substrate

Lasfargues JJ, Martin JM, Miller C. *Realités Cliniques* Vol. 15 n°3, 2004 pp.261-275

Miyazaki M, Yamaguchi K, Takamizawa T, Inage H, Hinou K. Abstract 94 – 52nd ORCA Congress, July 2005, Indianapolis, USA / *Caries Res* 2005; 39:319

The purpose of this study was to investigate the use of an ultrasonic pulse method to analyze demineralization of dental hard tissues, and to determine the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste (Tooth Mousse, GC Corp.) on demineralization by measuring changes in the ultrasound transmission velocity. The specimens were blocks of bovine enamel and dentin. The specimens were stored in 0.1 M lactic acid buffer for 10 min, and then in artificial saliva (pH 7.0). Other specimens were stored in a 10 x diluted solution of CPP-ACP paste and a placebo paste without CPP-ACP for 10 min, followed by 10 min immersion in a demineralization solution (pH 4.75, Ca 0.75 mM, P 0.45 mM) twice a day before storage in artificial saliva. The propagation time of longitudinal ultrasonic waves was measured by a pulser-receiver with a transducer. Six specimens were used for each condition, and one-way ANOVAs followed by the Tukey HSD test ($\alpha = 0.05$) were done. The ultrasound transmission velocity in specimens was found to decrease with time for specimens stored in the demineralization solution. On the other hand, no decrease was found for specimens stored in the CPP-ACP solution and a significant increase was found for enamel specimens. From the results of this study, it is suggested that the condition of demineralization of the tooth structure could possibly be measured non-destructively by using ultrasonic pulse method. It is also possible that CPP-ACP acted to prevent demineralization or to enhance remineralization of the tooth tissues. Supported by a grant to promote multi-disciplinary research project.

80. Fluoride uptake and distribution in enamel and dentin after application of different fluoride solutions

Holler BE, Friedl KH, Jung H, Hiller KA, Schmalz G. *Clin Oral Invest* (2002) 6 :137-144

The aim of this in vitro study was to examine the fluoride accumulation in enamel and dentin after short- and long-term application of four fluoride solutions, including a casein-based fluoride preparation. Cubical enamel and dentin specimens were cut out from extracted, caries-free, human third molars. The buccal surface of each specimen was moistened for 5 min or 24h with 10µl of the control or one of the four test solutions Olaflur, Oleaflur, sodium fluoride, or experimental fluoride containing hydrolyzed casein. The specimens were ground in 20-µm steps and the fluoride content was determined in each enamel and dentin layer. After application of the fluoride solutions, significantly more fluoride was associated with the superficial layer up to 20 µm. The values were 3-4 times higher in enamel and 4-8 times higher in dentin after 5-min application time and 10-24 times higher than the initial fluoride content in both hard tooth tissues after 24-h application time. Focusing on the experimental solution, the fluoride levels in enamel and dentin were somewhere in the order of the values of sodium and amine fluoride solutions. However, a tendency towards higher values could be observed after application of the experimental solution.

81. Preventing acid induced enamel demineralization using CPP-ACP containing paste

Sakaguchi Y, Kato S, Sato T, Kariya S, Chen Li. IADR, 83rd General Session, Baltimore 2005 - Abstract 2055

Objectives: The objective of this in vitro study is to evaluate, by use of Quantitative Light-Induced Fluorescence (QLF), the caries preventive potential of MI Paste (MIP, GC Corp.; also known as GC Tooth Mousse™, which contains 10% Casein Phosphopeptide – Amorphous Calcium Phosphate (CPP-ACP).

Methods: Bovine enamel specimens were prepared, treated with 0.3 grams of MIP and then challenged with 10 ml of a demineralization solution (0.1M Lactic acid buffer, pH 5.0) at 37 degree Celcius RH 100% for 72 hours. The enamel surface of each specimen was scanned, using QLF (Inspektor pro, Inspektor Dental Care) before and after the procedure. The collected images were then analyzed to determine the degree of demineralization. A placebo paste without CPP-ACP, a CPP-ACP free paste containing 900 ppm Fluoride and water were included as controls. The sections of enamel samples were also subjected to X-ray CT (TOSCANER-30000μhd, Toshiba) analysis.

Results: QLF analysis determined that the mean change in fluorescence radiation (ΔF , %) of the MIP, the placebo paste, fluoride paste and water controls were -1.55; -9.03; -6.98; and -16.50 respectively. Compared to the placebo and fluoride paste, MIP showed a significant protective affect against acid induced demineralization ($p < 0.05$). X-Ray CT further confirmed this result.

Conclusion: This QLF study confirmed the inhibitory effect of CPP-ACP on acid induced surface demineralization and demonstrated a functional role for MIP™ as a caries preventative agent for use in minimum intervention dentistry.

82. Casein phosphopeptide-amorphous calcium phosphate paste: root surface caries formation

Hicks J, Flaitz C. IADR, 83rd General Session, Baltimore 2005 - Abstract 3275

Objective: This in vitro study evaluated the effect of a casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) on artificial caries formation in human root surfaces using polarized light microscopy.

Methods: 12 human teeth with sound root surfaces were sectioned from into 3 portions. Each portion a single tooth was assigned to one of the three treatment groups: 1) CPP-ACP (MI Paste, GC America, n=12); 2) 0.05% sodium fluoride rinse (NaF, ACT McNeil-PPC, n=12); and 3) No Treatment Control (n=12). CPP-ACP and NaF root segments were treated with the appropriate assigned agent for 60 seconds, followed by air-water rinsing for 60 seconds, and then exposed to synthetic saliva for 24 hours. CPP-ACP and NaF treatment, rinsing and synthetic saliva exposure were repeated on a daily basis over a 14-day period. No treatment control root segments were exposed to synthetic saliva only for a 14-day period. Artificial root surface caries were created over a 10-day period (alternating 16 hours demineralization followed by 8 hours remineralization). Longitudinal sections (3 sections/root segment) were obtained and lesion depths were determined using polarized light microscopy (n=36 lesions/group, ANOVA, DMR).

Results: Mean root surface lesion depths were: 1) No Treatment Control Group: 310 +/- 27μm; 2) NaF Rinse Group: 216 +/- 21μm; 3) CPP-ACP Group: 144 +/- 19μm. Mean lesion depth reductions compared with the No Treatment Control Group were: 30% for NaF Rinse ($P < .05$, ANOVA, DMR) and 54% for CPP-ACP ($P < .05$, ANOVA, DMR). Mean lesion depth reduction for CPP-ACP was 33% compared with NaF Rinse ($P < .05$, ANOVA, DMR).

Conclusions: Casein phosphopeptide-amorphous calcium phosphate paste markedly enhanced the resistance of root surfaces to artificial caries formation, when compared with fluoride rinsing (0.05% NaF). Bioavailable calcium and phosphate in CPP-ACP has been demonstrated to bind to hydroxyapatite, and this may be an important factor in reducing susceptibility of root surfaces to a cariogenic challenge.

83. Nuove strategie nella prevenzione della carie dentaria: studio sperimentale sui caseino-fosfopeptidi

Ferrazzano GF, Troianiello S, Ingenito A. Prevenzione odontostomatologica 2005; 4:15-21

84. Minimal intervention dentistry: part 1. Strategies for addressing the new caries challenge in older patients

Chalmers JM. JCDA, July 2006, Vol.72,N°5

The aging of the population combined with increased retention of natural teeth into old age means that clinicians now face a new caries challenge in older dentate patients. An increase in the onset of dental caries is evident among patients who may not have had high levels of caries in the past and who may have undergone extensive restorative procedures during their lifetimes. Minimal intervention dentistry (MID), a modern evidence-based approach to caries management in dentate patients, uses the medical model, whereby disease is controlled by the "oral physician" and an affiliated dental team. The main components of a geriatric approach to MID are assessment of the risk of disease, with a focus on early detection and prevention; external and internal remineralization; use of a range of restorations, dental materials and equipment; and surgical intervention only when required and only after disease has been controlled. This first in a series of 2 articles describes and illustrates oral disease management in geriatric MID, which involves the assessment and management of a diverse range of primary and modifying factors; integrated with an evaluation of the plaque-biofilm interface and the resultant dynamic oral disease process.

85. Promoting remineralization: using casein phosphopeptide - stabilized amorphous calcium (fluoride) phosphate. A chemical approach

Manton DJ. *EAPD, 8th Congress of European Academy of Paediatric Dentistry; Amsterdam, June 2006 – Abstract 14*

Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP, Recaldent™) is a casein derived peptide, with added calcium and phosphate, which acts as a calcium and phosphate reservoir when incorporated into dental plaque and on the tooth surface. The CPP-ACP nano-complexes release calcium and phosphate ions via a pH or concentration gradient mechanism to maintain a supersaturated environment with respect to hydroxyapatite, therefore reducing demineralization and enhancing remineralization. The ability of CPP-ACP to remineralise white spot lesions of enamel (WSL) both in vitro and in situ has been widely reported. Recently, CPP-ACP in sugar-free gums has been shown to significantly slow the progression and promote the regression of caries in a randomized, controlled clinical trial. The addition of low concentrations (<1.0%) of CPP-ACP to acidic sports drink has been shown to reduce erosive potential without significantly altering taste. The inclusion of fluoride into CPP-ACP (as CPP-ACFP) forms a novel material with fluoride incorporated into the nano-complex. Advantages of increased remineralization are indicated for CPP-ACFP over CPP-ACP due to the availability of the fluoride ion with calcium and phosphate ions at the enamel surface, increasing fluorapatite formation. Remineralization with CPP-ACFP of mild fluorotic lesions of enamel in vitro resulted in remineralization of $44.0 \pm 9.7\%$ of the hypomineralised fluorotic lesion, significantly increasing after enamel pre-conditioning with NaOCl, with a concomitant improvement in aesthetic appearance. The addition of CPP-ACP to Glass Ionomer Cement (GIC) restorative material resulted in the inhibition of demineralization of adjacent dentine, with increase in microtensile bond and compressive strength, and significant enhancement of calcium, phosphate and fluoride ions at neutral and acid pH; however setting time was increased. Clinically, CPP-ACP can be delivered to the tooth surface in several vehicles: chewing gum, lozenge, topical crème, mouthrinse, toothpaste and added to GIC restorative material

86. Acid resistance of remineralized enamel by a sugar-free chewing gum

Iijima Y, Nishimura M, Iijima S. *IADR, 84th General Session, Brisbane 2006 - Abstract 184*

Objectives: This study examined the effect of a sugar free chewing gum (Tablet type) containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP; Recaldent, CASRN: 691364-49-5) on an acid resistance of remineralized enamel without fluoride conditions in a human in situ model.

Methods: Twenty subjects in randomized, cross-over, double-blind studies wore removable mandibular appliances with a demineralized human enamel sample. The appliances were inserted in oral environment immediately before gum-chewing for 20 min and then retained for another 20 min. This was performed four times per day for 14 days. Intra-oral experiment using another gum was performed after 1 week of washout time. Half of the enamel samples were covered with nail varnish after intra-oral exposure for remineralization. The enamel samples were acid challenged for 3 days in vitro, sectioned and subjected to microradiography to determine the level of mineral vol%. Results: During the second acid challenge, the mineral loss level of CPP-ACP tablet gum group showed less mean mineral loss (-2142) than that of placebo gum (-2804). The difference between the two groups was statistically significant and the probability was 0.036. The reason why there was a less mineral loss during the second acid attack for 3 days in case of CPP-ACP gum, it seems that this gum remineralizes subsurface enamel with mineral of higher crystallinity than saliva. The formation of higher crystallinity makes the mineral less soluble in acid.

Conclusion: Remineralized enamel by CPP-ACP tablet gum was much higher acid resistant than placebo gum.

87. Remineralisation of white spot lesions in situ by tooth mousse

Manton DJ, Shen P, Cai F, Cochrane NJ, Reynolds C, Messer LB, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 185

Objectives: To investigate the potential of a commercially available dental crème containing casein phosphopeptide – amorphous calcium phosphate (CPP-ACP), ["GC Tooth Mousse" (10% w/v CPP-ACP) (GC Corp, Japan)] to remineralize sub-surface white spot lesions of enamel (WSL) in a double blind, randomized, 2-way cross-over in situ study.

Methods: Enamel specimens were sectioned from either buccal or lingual surfaces of extracted sound human third molars and WSL windows were created using the Carbopol method. Specimens were divided into test and control half slabs. Healthy volunteers (n=6) wore mid-palatal appliances containing four enamel test slabs with WSL. A slurry (1g crème, 4ml H₂O) of Tooth Mousse (TM_{test}) or placebo Tooth Mousse (TM_{placebo}) was placed intra-orally (60s) after appliance insertion. The appliance was worn (40min) and the process repeated 4 times per day for 10d. Cross-over occurred after a 7d washout. Mineral content of each remineralized half-slab invested in resin with its control half slab was determined by microradiography after sectioning and lapping to $85 \pm 5\mu\text{m}$. The % mineral profiles of each WSL and adjacent sound enamel were compared and differences between sound and lesion values calculated (ΔZ_d , ΔZ_r). Proportional change in mineralization (%R) was calculated according to the formula: $\%R = ((\Delta Z_d - \Delta Z_r) / \Delta Z_d) \times 100$ and data analysed (ANOVA, Tukey's post-hoc, $p < 0.05$).

whereas TM_{test} produced %R of $24.22 \pm 3.31\%$.

Results: TM_{placebo} resulted in a %R of $3.72 \pm 2.10\%$,

Conclusion: A 20% dilution of Tooth Mousse crème containing CPP-ACP produced 551% more WSL mineralisation than a placebo crème in an in situ model. This study was supported by the Cooperative Research Centre for Oral Health Science, School of Dental Science, The University of Melbourne.

88. Remineralization by a mouthrinse containing CPP-ACP at pH 5.5

Shen P, Cai F, Walker GD, Reynolds C, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 189

Objectives: To investigate the efficacy of mouthrinse containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) at pH 5.5 in remineralization of enamel subsurface lesions in an human in situ model.

Method: This study utilized a double blind, cross-over design with two treatments: (i) a mouthrinse containing 0.5% CPP-ACP at pH 7.0 and (ii) a mouthrinse containing 0.5% CPP-ACP at pH 5.5. Subjects wore removable palatal appliances with four human-enamel half-slabs inset containing subsurface demineralized lesions. The subjects were instructed to rinse with 5 ml of mouthrinse for 60 seconds 4 times a day (at 10:00am; 11:30am; 2:00pm and 3:30pm) for 10 consecutive days. The subjects did not wear the appliances during eating, drinking and oral hygiene procedures. There was a one-week washout period after which the subjects crossed over to the other mouthrinse. After each treatment period the enamel slabs were removed, paired with their respective demineralized control, embedded, sectioned and subjected to microradiography and computer-assisted densitometric image analysis to determine the level of remineralization.

Results: Use of the mouthrinse containing 0.5% CPP-ACP at pH 5.5 produced $14.2 \pm 1.9\%$ remineralization of the enamel subsurface lesions in the 10 day period whereas the pH 7.0 rinse resulted in a $10.3 \pm 2.3\%$ enamel subsurface remineralization.

Conclusion: A 0.5% CPP-ACP mouthrinse at pH 5.5 produced significantly greater (38%) remineralization in situ than a 0.5% CPP-ACP rinse at pH 7.0.

89. Remineralisation by chewing gum containing CPP-ACP and citric acid

Cai F, Shen P, Walker GD, Yuan Y, Manton DJ, Reynolds C, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 190

Objectives: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) have been shown to remineralize enamel subsurface lesions in situ. The aim of this study was to investigate the effects of CPP-ACP in a fruit-flavoured sugar-free chewing gum containing citric acid on enamel remineralization, and the acid resistance of the remineralized enamel, using an in situ remineralization model. **Method:** The study utilized a double blind, randomized, crossover design with three treatments: (i) sugar-free gum (2 pellets) containing 18.8 mg CPP-ACP and 20 mg citric acid, (ii) sugar-free gum containing 20 mg citric acid alone, and (iii) sugar-free gum not containing CPP-ACP or citric acid. Ten subjects were instructed to wear removable palatal appliances, with 4 half-slab insets of human enamel containing demineralized subsurface lesions, and to chew gum (2 pellets) for 20 min (4times/d, 14d). At the completion of each treatment the enamel half-slabs were removed and half of the remineralized lesion treated with carbopol/lactic acid for 16hr. The enamel slabs (remineralized, acid challenged and control) were then embedded, sectioned and subjected to microradiography to determine the level of remineralization. **Results:** Chewing with the gum containing CPP-ACP and citric acid resulted in significantly higher remineralization ($13.1\% \pm 2.2\%$) than chewing with either the gum containing no CPP-ACP or citric acid ($9.3\% \pm 1.2\%$) or the gum containing citric acid alone ($2.6\% \pm 1.3\%$) ($p < 0.01$). The 16hr acid challenge of the remineralized lesions showed that the level of mineral after acid challenge was significantly greater for the gum containing CPP-ACP and citric acid when compared with the other two gums ($p < 0.01$). **Conclusion:** Sugar-free chewing gum containing CPP-ACP and citric acid significantly promoted remineralization of enamel subsurface lesions in situ.

90. Remineralization potential of CPP-ACP and its synergy with fluoride

Sakaguchi Y, Kato S, Sato T, Kariya S, Nagao S, Chen L. IADR, 84th General Session, Brisbane 2006 - Abstract 191

Objectives: To evaluate the remineralization potential of casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) in a tooth creme and its synergy with fluoride.

Methods: This cross-over in situ model involved five healthy adult subjects and four randomized treatments: tooth creme containing 10% CPP-ACP, (Tooth Mousse, GC Corporation; TM), creme containing 10% CPP-ACP plus 900ppm Fluoride (TMP), placebo creme without CPP-ACP and fluoride, and a professional paste containing 950 ppm fluoride. Enamel subsurface lesions ($2.5 \times 1 \text{ mm}^2$) on polished bovine tooth slabs ($10 \times 5 \times 1 \text{ mm}^3$) were created by exposure to a lactic acid gel (0.1 M, pH 5.1 with 3 mM CaCl_2 , 1.8 mM KH_2PO_4 and 5% HEC) at 37 degree Celsius for 5 days. The slabs were then mounted in a buccal flange on a removable maxillary appliance (4 lesions/appliance). Subjects were instructed to brush their teeth after lunch with a fluoride toothpaste (900 ppm), cover the lesions with the tooth creme, wear the appliance for 30 min, rinse the lesions with water, and keep the appliance in the mouth for four more hours. This was repeated every day for seven days. The appliance was kept in a humidified environment when outside the mouth. The lesions were subjected to analysis using an X-Ray CT scanner (TOSCANER-30000mhd, Toshiba) and Scion Image for Windows before and after intraoral treatments.

Results: The mean percentage remineralization for the placebo, fluoride paste, TM, TMP was 5.76 ± 10.13 , 12.14 ± 14.17 , 12.73 ± 11.80 , and 27.07 ± 14.57 respectively. TMP was superior to all other formulations ($p < 0.01$).

Conclusion: This study demonstrated significant subsurface enamel remineralization by CPP-ACP tooth creme in the mouth, with a synergistic effect of CPP-ACP and fluoride.

91. QLF and TMR analysis of CPP-ACFP remineralized enamel in vitro

Cochrane NJ, Cai F, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 192

Casein phosphopeptides (CPP) have been shown to stabilise amorphous calcium phosphate (CPP-ACP). CPP-ACP has been associated with anticariogenicity and remineralization in in vitro and in vivo models. CPP has also been shown to stabilise amorphous calcium fluoride phosphate (CPP-ACFP) which had an additive effect compared with the separate remineralization effects of fluoride or CPP-ACP.

Objectives: The aim of this study was to examine the remineralization potential of two concentrations of CPP-ACFP in terms of mineral and visual change. The mineral formed was subsequently analysed using wavelength dispersive microprobe spectrometry.

Methods: Enamel subsurface lesions were created using the Carbopol method and remineralized using CPP-ACFP solutions (0.5, 1.0%w/v) at pH 5.5 (2mL, 37°C, 10 days, daily solution changes, n=10 lesions per group). The lesions were then digitally photographed, analysed using quantitative light induced fluorescence (QLF) to determine percentage fluorescent loss integrated over area (%Q), embedded, sectioned, lapped and analysed using transverse microradiography (TMR) to determine percentage mineral change (%R). Elemental maps and quantitative line scans for calcium, phosphorous, fluoride, oxygen and chlorine were collected across the lesions using wavelength dispersive spectrometry on a JEOL 8900 SuperProbe Microprobe.

Results: The 0.5 and 1% CPP-ACFP solutions both produced statistically similar levels of remineralization as measured by TMR and QLF respectively, however there was more variation in the 0.5% lesions.

	0.5%	1%	P value
%R	56.3±17.7	58.7±5.9	0.85
%Q	83.4±16.0	93.1±6.6	0.36

All white spot lesions became more translucent after remineralization treatment. Microprobe analysis of remineralized enamel showed a single calcium phosphate phase with the ratio matching apatite. Fluoride was found evenly throughout the body of the lesions supporting fluorapatite formation.

Conclusion: CPP-ACFP solutions remineralized enamel subsurface lesions in vitro by the deposition of fluorapatite increasing mineral content and improving translucency.

92. Effect of CPP-ACP versus potassium nitrate on cervical dentinal hypersensitivity

Walsh LJ, Wang WS, Cakar A, Devani C, Tran KK, Hall A. IADR, 84th General Session, Brisbane 2006 - Abstract 947

The clinical problem of cervical dentinal hypersensitivity (CDH) can be managed by strategies that occlude patent dentine tubules exposed to the oral environment, or that reduce the excitability of pulpal nerves. Preliminary studies have shown that topical application of a CPP-ACP gel can cause blockage of dentine tubules. **Objectives:** This randomized clinical trial compared the therapeutic effect of a 10% CPP-ACP gel (GC Tooth Mousse) with a well established KNO₃ dentifrice (Colgate Sensitive) over 6 weeks. **Methods:** Patients (n=18 per group) screened from a general practice setting presenting with CDH used either CPP-ACP gel applied topically each night before retiring in conjunction with their conventional dentifrice twice daily, or the KNO₃ dentifrice twice daily. CDH responses to 4 types of stimuli were self-rated using a visual-analogue scale after 4wk and 6wk of treatment, and a composite score calculated. Data were analyzed in a blinded manner. **Results:** The two groups were well matched with no significance difference in baseline scores. In both the CPP-ACP and KNO₃ groups, when compared with their relevant baseline values, CDH scores were reduced significantly at 4wk (on average by 46.9% and 46.8%, respectively) and at 6wk (by 56.8 and 64.4%, respectively), when assessed using a repeated measures ANOVA (P<0.01 at 4wk and P<0.001 at 6wk). In each group, only 2 of the 18 subjects did not show a response, and each of these had relatively low baseline scores. The further reduction in CDH scores which occurred from weeks 4-6 in both groups was not statistically significant. At each of the 4wk and 6 wk time points, there were no significant differences between the two treatment groups. **Conclusions:** Despite differences in their apparent mechanisms of action, both CPP-ACP gel and the KNO₃ dentifrice give similar useful reductions in self-rated symptoms of CDH.

93. Plaque microcosm biofilm mineralization by CPP-ACP and calcium-phosphate-monofluorophosphate-urea mineralising solution

Wong L, Sissons CH. IADR, 84th General Session, Brisbane 2006 - Abstract 1269

Plaque mineralisation is a highly complex multi-factorial process influenced by microbial promoters and inhibitors, the oral environment, and plaque pH. Objective: To deposit caries-protective minerals in microcosm plaque biofilms using (i) casein phosphopeptide-amorphous calcium phosphate complex (CPP-ACP) which models salivary CaPi carriers, and examine the effect of pH and the presence of F, and (ii) CPMU (20mM Ca, 12mM phosphate, 5mM monofluorophosphate, 500mM urea, pH5.0) mineralising solution which deposits CaPi following a urea-induced pH rise. Methods: Plaque microcosms were grown for 11-21 days from plaque-enriched saliva in a multi-plaque 'artificial mouth' culture system (MAM), continuously supplied (3.6ml/hr/plaque) with nutritional saliva analogue (BMM) or chemically-defined artificial saliva (DMM), supplemented with urea (or glucose) to raise (lower) the resting pH. Sucrose (5%) was supplied periodically (1.5ml/6min, 8hrly). Microcosms were mineralised under continuous or periodic regimes. Plaque pH, Ca, Pi and F, were measured. Results: Mineralisation with CPP-ACP yielded Ca and Pi levels 20-50% that of CPMU, depending on application regime but under otherwise similar environmental conditions of growth and pH. A raised plaque pH range yielded greater CaPi deposition from CPP-ACP, and from CPMU. A sucrose-induced low pH during CPP-ACP exposure appeared to lower CaPi accumulation. When plaque biofilms were mineralised with CPP-ACP and CPMU together, there was a large increase in CaPi deposited. Deposition from CPP-ACP also increased considerably in the presence of a low concentration of fluoride (0.05 ppm) in DMM. Conclusions: CPP-ACP was effective in mineralising plaque microcosms but mineral deposition was less than with CPMU. Different mechanisms of mineralisation may be involved with more complex pH-dependent CPP-ACP accumulation, Ca and Pi release, and subsequent deposition as CaPi mineral. Plaque pH is a key regulator of plaque mineralisation with CPP-ACP as well as CPMU. Supported by Health Research Council of New Zealand and Wellington Medical Research Foundation.

94. Effect of CPP-ACP on hardness of enamel eroded by Cola-drink

Sukasaem H, Panich M, Poolthong S.. IADR, 84th General Session, Brisbane 2006 - Abstract 1673

Objective: This in vitro study evaluated the remineralization effect of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) from Toothmousse (GC Asia Dental, Japan) on hardness of enamel eroded by Cola-drink.

Methods: Ten extracted human premolars were bucco-lingually cut into 2 halves and embedded in resin providing 20 specimens with buccal and lingual experimental sites. The specimens were polished (1 micron diamond slurry, Impitech, South Africa) and baseline Vickers hardness was measured (100g force, 15s) to demonstrate the effect of mineral changes. The demineralization process was done by immersion of specimens in Cola-drink (Thai Namthip Co.Ltd, Thailand) and artificial saliva (Chulalongkorn University, Thailand) (5s each for 10 cycles) for 3 times with 2 immersions in artificial saliva for 6h among the 3 alternate immersions. All specimens were rinsed with deionized water and the hardness measurements were repeated. For remineralization process, the demineralized specimens were randomly divided into 4 groups (n=10) and 4 regimens of remineralization used CPP-ACP (group 1), artificial saliva (group 2), CPP-ACP and artificial saliva (Group 3) and deionized water as control. After remineralization process, hardness measurements were repeated. Statistical analysis used Oneway ANOVA followed by Bonferroni test ($\alpha = 0.05$).

Results: The hardness of the specimens at baseline, after demineralization and after remineralization of group 1 (246.7 ± 3.2 , 209.1 ± 13.6 , 247.3 ± 5.2), group 2 (245.2 ± 6.4 , 207.9 ± 7.8 , 228.1 ± 9.2), group 3 (248.3 ± 6.2 , 205.6 ± 6.5 , 242.6 ± 12.1) and control group (239.1 ± 17.9 , 207.0 ± 8.2 , 210.0 ± 8.5) were demonstrated with standard deviation respectively in bracket. After remineralization, all groups showed significantly higher hardness value than control ($p < 0.05$). The CPP-ACP and CPP-ACP with artificial saliva groups demonstrated significantly higher hardness value than the artificial saliva group ($p < 0.05$).

Conclusion: CPP-ACP from Toothmousse can increase hardness of enamel eroded by Cola-drink. The remineralization effect of CPP-ACP is significantly higher than that of artificial saliva in vitro.

95. An in vitro study of wear prevention in dentine

Narayana T, Ranjitkar S, Kaidonis JA, Townsens GC, Richards LC. IADR, 84th General Session, Brisbane 2006 - Abstract 2424

Tooth wear is a significant problem facing clinicians, and several approaches are being used to manage it. Objective: This study aims to quantitatively and qualitatively test the efficacy of Tooth Mousse® in managing dentine wear under highly controlled conditions.

Methods: Eight dentine specimens from the lingual halves of third molar teeth were worn against enamel antagonists under a load of 9.95kg in an electro-mechanical tooth wear machine with hydrochloric acid lubricant (HCl) (pH=3), and with regular Tooth Mousse® application. A further eight dentine specimens were worn with Tooth Mousse® as the sole lubricant. Dentine wear was quantified by measuring reduction in dentine volume using a Dr PICZA 3D Scanner (PIX-4) and a MATLAB software package (version 6, The Mathwork Inc, Natick MA, USA), and assessed qualitatively by examining epoxy resin replicas under a scanning electron microscope. These data were then compared using ANOVA with data from two control experiments conducted under a load of 9.95kg with deionised water and HCl (pH=3) as lubricants, but no Tooth Mousse® was applied.

Results: Dentine specimens worn with Tooth Mousse® as the sole lubricant exhibited minimal wear, and had very smooth and shiny wear facets. Those worn with HCl (pH=3) with regular Tooth Mousse® application showed less wear than both the control groups. Their wear facets were not obviously different from facets of control specimens worn with HCl (pH=3), but were smoother than those of specimens worn with deionised water as the lubricant. The control group worn with HCl (pH=3) exhibited less wear than that worn with deionised water, and also displayed smoother facets.

Conclusions: This study has shown that Tooth Mousse® is capable of reducing dentine wear, and has also highlighted the importance of lubricants in reducing wear. Further research is required to clarify its clinical usefulness of Tooth Mousse® in this context.

96. Enamel wear prevention under conditions simulating bruxism and acid regurgitation

Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC. IADR, 84th General Session, Brisbane 2006 - Abstract 2428

Tooth wear is a growing public health problem and there is a need to better understand its aetiology and management. Objective: Our aim was to investigate the effectiveness of frequent applications of Tooth Mousse (GC Corporation) in preventing enamel wear in vitro, under conditions simulating bruxism and acid regurgitation.

Methods: Sixteen human third molar teeth were sectioned longitudinally in a mesio-distal direction, and enamel halves of the same teeth were worn against each other in a purpose-built electromechanical tooth wear machine under a load of 10.0 kg with hydrochloric acid lubricant (pH = 1.2) for around 10,000 test cycles. In the experimental sample (n = 8), the machine was stopped every two minutes (160 cycles of wear) and the specimens washed and dried. Tooth Mousse was then applied for four minutes. The specimens were further washed and dried before the cycle was continued. The same protocol was followed for the control specimens (n = 8) but no Tooth Mousse was applied. Tooth wear was quantified by measuring reduction in enamel volume per 1,000 cycles using a Dr PICZA 3D Scanner (PIX-4) and MATLAB software package (version 6, The Mathwork Inc, Natick MA, USA). Wear rates were compared between the samples with an unpaired t-test. Qualitative assessment was also carried out using Scanning Electron Microscopy (SEM).

Results: The rate of enamel wear was significantly lower in the experimental sample (0.41 mm³ per 1,000 cycles) than in the control sample (1.01 mm³ per 1,000 cycles) (p<0.01). Enamel wear facets in the experimental sample were also found to be smoother than those in the control sample.

Conclusions: Frequent application of Tooth Mouse is effective in reducing enamel wear under conditions simulating bruxism and acid regurgitation, probably due to its lubrication properties. These findings open up new possibilities for the prevention of tooth wear.

97. CPP-ACP gum slows progression and enhances regression of dental caries

Morgan MV, Adams GG, Bailey DL, Tsao CE, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 2445

Objectives: To investigate the radiographic progression and regression of dental caries in adolescent subjects chewing a gum containing CPP-ACP over a two-year period.

Methods: 2720 subjects were randomly assigned to either a test or control group. All subjects received accepted preventive procedures, including fluoridated water, fluoridated dentifrice, and access to professional care. The test group received a sugar-free gum containing 54.4mg CPP-ACP while the control group received an identical gum without CPP-ACP. Subjects were instructed to chew their assigned gum for 10 minutes 3x/day, with 1 session supervised on school days, over the 2-year study period. Standardised digital radiographs were taken at the baseline and at the completion of the clinical trial using the Dexis digital X-ray system. The radiographs, scored by a single examiner, were assessed for approximal surface dental caries at both the enamel and dentine level. Analysis of caries progression or regression was undertaken using a transition matrix.

Results: There was a statistically significant difference in the distributions of the transition scores between the two groups (P value < 0.001). The CPP-ACP gum slowed progression of carious lesions compared with the control gum. For subjects chewing the CPP-ACP gum, 814 (4.41%) of approximal surfaces experienced caries progression compared to 932 (5.31%) approximal surfaces in the control group, a reduction of 16.9%. The CPP-ACP gum enhanced regression of carious lesions compared with the control gum. 56 (0.30%) of approximal surfaces experienced caries regression with the CPP-ACP gum compared to 36 (0.21%) approximal surfaces with the control gum. A greater percentage of approximal surfaces remained unchanged with the CPP-ACP gum than with the control gum.

Conclusion: A chewing gum containing 54.4mg CPP-ACP significantly slowed progression and enhanced regression of dental caries in a two-year clinical trial relative to a normal sugar-free gum. Study Sponsor: Cadbury Schweppes, Science and Technology.

98. Structure and ¹⁵N-dynamics of casein phosphopeptide-amorphous calcium phosphate nanocomplexes

Cross KJ, Attard TJ, Huq NL, Reynolds EC. IADR, 84th General Session, Brisbane 2006 - Abstract 2534

Tryptic phosphopeptides derived from milk caseins are known to associate with amorphous calcium phosphate (ACP) forming stable complexes. These complexes act as biological calcium phosphate delivery vehicles and are effective in the remineralization of early enamel lesions. **Aim:** To examine the structures of these complexes.

Methods: NMR spectroscopic techniques were used to characterize translational diffusion of the CPP-ACP nanocomplexes and to characterize interatomic distances and the nuclear relaxation behaviour of the peptides within the CPP-ACP nanocomplex.

Results: A molecular model of the CPP-ACP nanocomplex was developed.

Conclusion: The CPP-ACP nanocomplexes were shown to be particles with hydrodynamic radii ranging from 1.526 ± 0.044 nm at pH 6.0 increasing to 1.923 ± 0.082 nm at pH 9.0 in the case of casein phosphopeptide β -casein(1-25). Features of the model suggest a mechanism by which calcium ion transport through biological membranes and the formation of large CPP-ACP aggregates can be achieved. Supported by NH&MRC grant 209042

99. Improved plaque uptake and enamel remineralization by fluoride with CPP-ACP

Reynolds EC, Cochrane NJ, Shen P, Cai F, Walker GD, Morgan MV, Reynolds C. IADR, 84th General Session, Brisbane 2006 - Abstract 2538

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been shown to slow the progression of caries and to remineralize enamel subsurface lesions.

Objectives: The aim of the studies was to determine the ability of CPP-ACP to increase the incorporation of fluoride into supragingival plaque and to promote enamel remineralization in situ with acid resistant mineral.

Methods: Randomized, double-blind cross-over studies were designed involving three mouthrinses and five toothpastes as follows: Mouthrinses (i) 2% CPP-ACP, (ii) 2% CPP-ACP plus 450 ppm F and (iii) 450 ppm F; and Toothpastes: (i) placebo, (ii) 1100 ppm F, (iii) 2800 ppm F, (iv) 2% CPP-ACP and (v) 2% CPP-ACP plus 1100 ppm F. The mouthrinses (15 ml) were used for 60 s, three times per day for 5 d and supragingival plaque collected and analyzed for F content. The toothpastes (1 g) were added to 4 ml water to form a slurry and used for 60 s four times per day for 14 days in an in situ remineralization model.

Results: The addition of 2% CPP-ACP to the 450 ppm F rinse significantly increased the incorporation of fluoride ions into plaque where the plaque fluoride level (33.0 ± 17.6 nmol/mg dry wt) was over double that obtained with the fluoride-only rinse (14.4 ± 6.7 nmol/mg dry wt). Fluoride in the toothpaste slurry produced a dose-response related remineralization of subsurface enamel lesions. The toothpaste containing 2% CPP-ACP produced a level of remineralization ($13.5 \pm 1.5\%$) similar to the 2800 ppm F paste ($15.5 \pm 2.4\%$) and the paste containing 2% CPP-ACP plus 1100 ppm F was superior ($21.0 \pm 5.9\%$) to all other formulations in enamel lesion remineralization. Acid challenge of the remineralized lesions showed that the CPP-ACP/F mineralized lesions were relatively acid resistant.

Conclusion: CPP-ACP promotes the incorporation of fluoride into plaque and sub-surface enamel producing effects superior to fluoride alone.

100. Prevention of white spot lesions in orthodontic practice: a contemporary review

Sudjalim TR, Woods MG, Manton DJ. *Australian Dental Journal* 2006;51 :(4):284-289

The development of white spot demineralization associated with fixed appliance orthodontic treatment is a significant clinical problem. Both established and experimental methods for prevention of such lesions in day-to-day clinical practice are presented and discussed.

101. In situ remineralisation by sugar-free gums, one-containing CPP-ACP

Manton DJ, Cai F, Cochrane N, Walker GD, Shen P, Adams G, Messer LB, Reynolds C, Reynolds EC. 45th Annual Meeting of Australian/New Zealand Division of the IADR, September 2005 – Abstract 0020

Objectives: To compare the efficacy of a CPP-ACP containing sugar-free chewing gum TW (Trident™ White) with sugar-free gums not containing CPP-ACP: Or (Orbit™) and OrP (Orbit™ Professional) to remineralise enamel subsurface lesions (WSL) in an in situ model.

Methods: Specimens for WSL mineralization were sectioned from either buccal or lingual surfaces of extracted sound third molar teeth, and the WSL were created using the carbopol method. A randomized, double-blind cross-over study was conducted. Ten healthy adult subjects were recruited. Subjects chewed the gum for a 20 min period 4 times per day for 14 days. Enamel half slabs were paired with their control, embedded in resin and microradiographic images taken. The difference in mineralization profile in the control specimen between the sound and WSL enamel windows was designated ΔZ_c , with the difference in the test specimen designated ΔZ_t . Percentage remineralisation (%R) was calculated according to the formula $(1 - \Delta Z_t / \Delta Z_c) \times 100$.

Results: TW produced remineralisation (%R) of $18.4 \pm 0.9\%$, with Or $8.9 \pm 0.5\%$ and OrP $10.5 \pm 0.9\%$. Trident White™ therefore produced 107% more remineralisation than the Orbit™ gum and 75% more than the Orbit Professional™. All differences were statistically significant.

Conclusion: The addition of CPP-ACP to sugar-free chewing gum significantly increased the mineralization of WSL in situ when compared to two other gums not containing CPP-ACP. This study was supported by the Cooperative Research Centre for Oral Health Science, School of Dental Science, The University of Melbourne.

102. Incorporation of casein phosphopeptide-amorphous calcium phosphate into a temporary cement

Wong R, Palamara J, Wilson PR. IADR, 84th General Session, Brisbane 2006 - Abstract 0653

Objectives: The presence of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in a temporary cement may act as a surface modifying agent which alters the permeability of cut dentin beneath temporary crowns. This study was carried out to determine the viability of incorporating CPP-ACP into a zinc oxide non-eugenol temporary cement in terms of its effect on setting time, compressive strength and film thickness.

Methods: The base and catalyst pastes of Freegenol™ (GC Int Corp, Tokyo, Japan) were weighed to a ratio of 1:4 and mixed with 0.5%, 1.0%, 2.0%, 3.0%, 4.0% and 8.0% w/v of CPP-ACP powder. Test conditions were conducted using ISO recommendations for setting time, compressive strength and film thickness for Type 1-Class 1 zinc oxide non-eugenol cements. Control specimens contained no CPP-ACP. Five specimens were used in each test group.

Results: Addition of $\leq 3.0\%$ CPP-ACP reduced the setting time but $\geq 3.0\%$ CPP-ACP delayed it beyond the ISO requirements of 10 minutes. Increasing amounts of CPP-ACP tended to reduce the compressive strength of Freegenol™ from a mean of 5 MPa for the 0.5% CPP-ACP to 3 MPa for the 8.0% test group. These values were in compliance with ISO recommendations of 35 MPa as the maximum for compressive strength. Film thickness measurements for all test groups complied with the recommended maximum of 25µm.

Conclusion: The incorporation of $\geq 3.0\%$ w/v CPP-ACP into Freegenol™ would require the addition of an accelerator, due to its effect of delaying setting time beyond ISO recommendations. The addition of up to 8.0% CPP-ACP into Freegenol™ is otherwise viable in terms of its compressive strength and film thickness.

This study is supported by the NHMRC grant no: 359318 and the Cooperative Research Centre for Oral Health Science.

103. Incorporation of casein phosphopeptide-amorphous calcium phosphate into glass ionomer cement

Al-Zraikat H, Palamara J, Burrow M, Messer H. IADR, 84th General Session, Brisbane 2006 - Abstract 0654

Objectives: The incorporation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) into glass ionomer cement (GIC) fissure sealant may enhance the inhibition of demineralization and promote remineralization. The aim of this study is to investigate the effect of incorporating increasing concentrations of CPP-ACP into Fuji III fissure sealant (GC Int. Corp., Tokyo, Japan) on its physical properties.

Methods: Setting time (ST), Compressive Strength (CS), Diametral Tensile Strength (DTS), Film Thickness (FT) and Flow values (FV) of Fuji III containing 0, 1, 3, 5, 7 and 10% CPP-ACP were tested following the international Organization for Standardization (ISO) specifications 9917:1991 and 6876:1986. Results were analyzed using one-way ANOVA and the Kruskal-Wallis tests.

Results: The effect of incorporating up to 10% CPP-ACP into Fuji III on the values of ST, CS, DTS and FV remained within ISO recommendations. The values of CS and DTS decreased with a significant effect when 10% CPP-ACP was added; in addition to a prolonged ST. Fuji III containing 5% CPP-ACP failed the FT test.

Conclusion: There was no adverse effect following the incorporation of up to 10% CPP-ACP on the physical properties tested except on FT with 5% CPP-ACP.

This project is supported by The Cooperative Research Centre for Oral Health Science.

104. Effect of CPP-ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device

Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Moore BK. *Journal of Dentistry* (2006) 34. 230-236

Objective: The purpose of this study was to determine the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste on demineralization of bovine enamel by measuring changes in the ultrasound transmission velocity.

Methods: The enamel specimens were prepared by cutting bovine teeth into blocks. The specimens were stored in 0.1M lactic acid buffer solution (pH4.75, Ca 0.75mM, P 0.45mM) for 10 min twice a day, and then stored in the artificial saliva (pH 7.0). Other specimens were stored in a 10-times diluted solution of CPP-ACP paste and a placebo paste without CPP-ACP for 10 min, followed by 10 min immersion into a demineralization solution twice a day before storage in the artificial saliva. The propagation time of longitudinal ultrasonic waves was measured by a Pulser-Receiver (Model 5900, Panametrics) with a transducer (W112, Panametrics). Six specimens were used for each condition, and one-way ANOVAs followed by the Tukey HSD tests ($\alpha=0.05$) were done.

Results: The sonic velocity was found to decrease with time for specimens stored in the demineralization solution. On the other hand, a significant increase in sonic velocity was found for specimens stored in the CPP-ACP solution.

Conclusion: From the result of this study, it was suggested that the conditions of de- and remineralization of the enamel structure could be measured non-destructively by using an ultrasonic pulse method. It could be concluded that the inorganic components contained in high concentrations in CPP-ACP acted to enhance remineralization of the enamel structure.

105. Ultrasonic determination of the effect of casein phosphopeptide-amorphous calcium phosphate paste on the demineralization of bovine dentin

Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Hurokawa H. *Caries Res* 2007; 41:204-207

The purpose of this study was to investigate the demineralization of dentin by measuring changes in the velocity of the sonic longitudinal waves transmitted through this substrate. One group of samples was immersed in demineralization solution for 10 min twice a day and then stored in artificial saliva. Two additional groups of samples were treated with a solution of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste or a placebo paste without CPP-ACP before demineralization and a control group was stored in artificial saliva. The sonic velocity of the demineralized specimens was found to decrease significantly over time. No significant increase in sonic velocity was observed in specimens treated with CPP-ACP, suggesting that CPP-ACP acted to prevent demineralization.

106. Resin bonding using an all-etch or self-etch adhesive to enamel after carbamide peroxide and/or CPP-ACP treatment

Moule CA, Angelis F, Kim GH, Le S, Malipatil S, Foo MS, Burrow MF, Thomas D. *Australian Dental Journal* 2007; 52(2):133-137

Background: Limited evidence exists regarding the effect of carbamide peroxide and casein phosphopeptide amorphous calcium phosphate (CPP-ACP) on composite-enamel bonding. Microshear bond strengths, using either a total-etch or self-etching adhesive, to enamel treated with carbamide peroxide and/or CPP-ACP were investigated.

Materials & methods: Twenty-six extracted human third molars were sectioned into four parts, each being allocated into one of the four groups (n=26): bleach (Polanight, 16% carbamide peroxide), CPP-ACP (GC Tooth Mousse), bleach and then CPP-ACP, or untreated (control). The surfaces were bonded with a total-etch bonding system (Single Bond) or a self-etching primer system (Clearfil SE Bond) and tested using a microshear test.

Results: A significant difference in bond strength was found between bonding systems. SE Bond showed the highest bond strength to untreated enamel ($p < 0.05$). The microshear bond strength of SE Bond decreased when the enamel was treated with carbamide peroxide, CPP-ACP or both ($p < 0.05$). Only combined use of carbamide peroxide and CPP-ACP significantly affected microshear bond strength with Single Bond.

Conclusion: These findings suggest the shear bond strength of resin to enamel using a self-etching priming adhesive may be affected if the enamel is treated with a bleaching agent or CPP-ACP.

107. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study

Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. *Journal of Oral Science*, Vol.49, No.2, 115-120, 2007

Milk and milk products, such as cheese, have been shown to exhibit anticariogenic properties in human and animal models. CPP-ACP shows an anti-caries effect by suppressing demineralization, enhancing remineralization, or possibly a combination of both. The purpose of this study was to evaluate the effect of CPP-ACP paste on demineralization by observing the treated tooth surface using an FE-SEM. The specimens were prepared by cutting enamel and dentin of bovine teeth into blocks. A few specimens were stored in 0.1 M lactic acid buffer solution for 10 min and then in artificial saliva (negative control). The remaining specimens were stored in a 10 times-diluted solution of CPP-ACP paste or a placebo paste containing no CPP-ACP for 10 min, followed by 10 min immersion in a demineralizing solution (pH=4.75, Ca) twice a day before storage in artificial saliva. After treatment of the specimens for 3, 7, 21 and 28 days, they were fixed in 2.5% glutaraldehyde in cacodylate buffer solution, dehydrated in ascending grades of *test*-butyl alcohol, and then transferred to a critical-point dryer. The surfaces were coated with a thin film of Au in a vacuum evaporator, and were observed under field emission-scanning electron microscopy (FE-SEM). The SEM observations revealed different morphological features brought about by the various storage conditions. Demineralization of the enamel and dentin surfaces was more pronounced with the longer test period in the control and negative control specimens. On the other hand, enamel and dentin specimens treated with CPP-ACP paste revealed slight changes in their morphological features. From the morphological observations of the enamel and dentin surfaces, it could be considered that the CPP-ACP paste might prevent demineralization of the tooth structure.

108. Effect of a CPP-ACP agent on the demineralization and remineralization of dentine in vitro

Rahiotis C, Vougiouklakis G. *Journal of Dentistry* 35 (2007) 695-698

Objectives: The aim of this study was to determine in vitro the effect of a commercial paste based on CPP-ACP complex on the demineralization of sound human dentine and on remineralization potential of artificial caries-like lesions formed on dentine surfaces.

Methods: Forty dentine specimens were prepared with hard tissue microtome. The specimens were divided in four groups the A, B, C and D ($n=10$). The specimen surfaces were subjected to surface analysis by Fourier transformance micro multiple internal reflectance infrared spectroscopy (micro MIR-FTIR). Tooth mousse was applied on surface specimens of A group, while no agent were applied on the specimens of B group. Afterwards, groups A, B, C and D were immersed in demineralization solution for 7 days. Afterwards, the surfaces were subjected to micro MIR-FTIR analysis and the mineral to matrix ratio was used to assess the extent of dentin demineralization (DM). Tooth mousse was applied on specimens of group C, while no agent was applied on specimens of group D. The groups C and D immersed in artificial saliva for 7 days and were subjected to analysis by micro MIR-FTIR and the mineral to matrix ratio was used to assess the extent of dentin remineralization (RM).

Results: Group A showed significant lower %DM in comparison to group B. Group C resulted in a significant higher %RM compared to group D.

Conclusions: The presence of agent CPP-ACP on dentine surfaces provoked lower demineralization and higher remineralization in comparison with the dentine surfaces without agent.

109. Remineralization of enamel lesion by a novel cream with both CPP-ACP and fluoride

Kariya S, Sakaguchi Y, Sato T, Kato S, Chen L, Iijima Y. Poster session 136 – 54th Annual ORCA Congress 2007

Objective: The aim of this two-center, placebo-controlled, randomized trial is to evaluate the remineralization potential of a novel cream with both Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and Fluoride (GC MI Paste Plus / Tooth Mousse Plus, GC Corporation, Japan)

Methods: This was a two-center, randomized, crossover design study. Ethical approval was obtained from the University of Nagasaki Ethics Committee. Fifteen healthy adults (8 males and 7 females; mean age 25y) were recruited.

Extracted sound human first premolar teeth were obtained from the dental hospital of Nagasaki University. Enamel slabs with unpolished surfaces (2x3x4mm) were exposed to a lactic acid buffer gel (0.1M, pH4.5 with 3.0 mM Ca and 1.8mM P) at 37 degree Celsius for 5 days to create artificial enamel sub-surface lesions. After demineralization, one third of each enamel slab was covered by nail varnish as a negative control. The figure 1 is a photo of the device. When it is insert into the mouth, two enamel slabs are placed adjacent to the buccal surface of mandibular molars.

Experimental materials are shown in the table 1. Volunteers were divided randomly into 3 groups (Table 2). They were instructed to brush their teeth after lunch with fluoride free toothpaste, cover the lesions with the test materials, wear the appliance for 30 min, rinse the lesions with water, and keep the appliance in the mouth for a further four hours. This was repeated every day for 7 days. The appliance was stored in a humidified environment whilst not in the mouth.

The enamel slabs were removed from the appliance, cut into thin sections of approximately 200µm thickness. The microsections were analyzed using X-Ray µ-CT (TOSCANER-30000µhd, Toshiba IT & Control System Corp., Japan) and Scion Image for Windows (Scion Corporation).

Results: The mean percentage remineralization for the PP, FP and MIPP was 3.6±8.5, 14.3±7.1 and 20.2±7.6 respectively.

Discussion; In the current in situ study, MIPP and FP showed remineralization of enamel sub-surface lesions. Especially, MIPP showed higher remineralization potential than the FP in this test ($p<0.05$). These results indicate that the CPP-ACP and Fluoride (NaF) can coexist and remineralization potential of fluoride is further enhanced by CPP-ACP.

Conclusions: This in situ study confirmed the remineralization effect of CPP-ACP and demonstrated a synergistic effect of CPP-ACP and Fluoride.

110. Considering biomodification and remineralization techniques as adjuncts to vital tooth-bleaching regimens

Milnar FJ. *Compendium*, May 2007; 28(5):234-240

Although in-office and dentist-monitored vital night-guard bleaching have proved successful for whitening teeth, in some cases involving stark with spots, additional efforts may be required. This clinical case demonstrates the use of a combination in-office and take-home whitening regimen, followed by the in-office and home application of a paste containing casein phosphopeptide and amorphous calcium phosphate, both of which are believed to help replace lost minerals in teeth, make teeth stronger, and help protect them from decay and erosion. The patient's teeth successfully lightened from an initial shade of A2 to a uniform shade of B1. The white spots that were still visible after the bleaching process had disappeared into the tooth structure after the 3-week at-home use of the remineralizing paste.

However, at the 3-month recall appointment, some relapse in the appearance of the white spots was noted. Although more research is needed to assess the broader implications of the technique, the author concludes that this individualized result suggests that products with the potential to remineralize tooth structure might be useful adjuncts to traditional tooth-bleaching processes to achieve a uniform appearance when white spots are present.

111. Aesthetic management of severely fluorosed incisors in an adolescent female

Ng F, Manton DJ. *Australian Dental Journal* 2007;52(3):243-248

Background: Dental fluorosis is a condition of enamel hypomineralization due to the effects of excessive fluoride on ameloblasts during enamel formation. Delayed degradation of enamel matrix proteins or inhibited protein removal results in impaired and incomplete crystal growth, producing hypomineralized and porous enamel. Severely fluorosed teeth may undergo post-eruptive surface breakdown and post-eruptive dark brown to black staining.

Methods: A 13 year old girl presented with severely discoloured maxillary central incisors. Initial aesthetic management of these teeth was conservative, including in-office tooth whitening, microabrasion and take-home whitening.

Results: Dark brown to black staining of the teeth was reduced successfully without the need for gross mechanical preparation of the enamel. Further improvement of aesthetics was achieved with composite veneers.

Conclusions: Conservative treatment options such as tooth whitening and microabrasion can dramatically improve severely discoloured fluorosed teeth. This can provide a satisfactory interim outcome or minimize the removal of discoloured enamel and dentine prior to the provision of composite veneers. The use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) may enhance remineralization and decrease post-operative sensitivity following tooth whitening and microabrasion procedures in hypomineralized teeth.

112. Minimally invasive treatment of white spot enamel lesions

Ardu S, Castioni NV, Benbachir N, Krejci I. *Quintessence International* volume 38, number 8, September 2007

This article describes a technique used to treat superficial white spot lesions by a minimally invasive approach. The proposed technique is based on reactivation of enamel by elimination of its hypermineralized external layer through microabrasion, followed by daily home application of casein phosphopeptide-amorphous calcium phosphate complexes (CPP-ACP). The technique may allow elimination of white spot lesions without involving restorative procedures. Microabrasion followed by long-term daily home application of CPP-ACP may be considered an interesting alternative to the restorative approach for treatment of white spot lesions.