Evidence that demands a verdict: latest developments in remineralization therapies

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"Remineralization is the natural repair process for non-cavitated lesions and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization."

In the last decade, there has been a veritable explosion of interest in technologies which may have value for remineralization of enamel and dentine. The purpose of this article is to lay the foundations for understanding the process of remineralization and from this, to then examine the bona fides of current products and therapies in this domain. The purpose is not to provide a detailed scientific critique of remineralization systems, as has been done in other recent publications,[1] but rather to demystify some of the current technologies and to flag some of the issues when assessing this fruit salad of apples, oranges and pears. The characteristics of an ideal remineralizing agent are summarized in Table 1, which provides a backdrop against which to contrast the available materials and technologies.

Enamel minerals

An appropriate entry point into this discussion is to recall that the mineral in dentine and enamel is not pure hydroxyapatite, but rather a mixture of compounds including a number of carbonated apatites, with greater diversity of composition in dentine than in enamel. These minerals occupy up to 98 weight per cent and up to 96 volume per cent, when constitutional water is included.[2]

Fluorapatite is less acid soluble than hydroxyapatite, which in turn is less soluble than carbonated apatites, and so forth. Approximately 14% of the human enamel mineral is very soluble in weak organic acids because of the presence of these more acid soluble minerals such as those with sodium or magnesium.[3,4] Because of this chemical inhomogeneity of enamel, the process of enamel remineralization is rather complex. While it is commonly thought that the ratio of components required for remineralization is 10 calcium ions to 6 phosphate ions to 2 fluoride or hydroxyl ions (or one carbonate ion), this is a simplistic approach and not in keeping with experimental evidence on the stoichiometry of the total mineral composition of teeth, which permits other ratios for calcium to other components.

Nevertheless, calcium availability remains the singular limiting factor in enamel remineralization. One of the most important properties of calcium phosphate/calcium fluoride materials is their solubility behavior, bearing in mind that the majority of calcium compounds are very insoluble. In fact, most reactions of these calcium compounds in the aqueous environment are driven by relative solubility/reactivity of both the reactant and the product.[5]

Remineralization

Remineralization is the natural repair process for non-cavitated lesions and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization. These remineralized crystals are less acid soluble than the original mineral.[6]

Table 1. Requirements of an ideal remineralization material

Diffuses into the subsurface, or delivers calcium and phosphate into the subsurface
Does not deliver an excess of calcium
Does not favour calculus formation
Works at an acidic pH
Works in xerostomic patients
Boosts the remineralizing properties of saliva
For novel materials, shows a benefit over fluoride

Based on Zero, 2006.[88]

The composition and the concentration of inorganic ions in saliva and in dental plaque significantly influence the degree of saturation of the water-rich fluid which is in immediate contact with enamel. Somewhat surprisingly, the ionic concentrations of most ions, other than sodium and chloride, are different between saliva and plaque fluid, and there is little or no relationship between levels of calcium, phosphate, or pH, between the two. This means that attempts to elevate salivary levels of calcium or phosphate may not necessarily give a benefit in terms of enamel remineralization.[7]

The role of saliva

The critical role played by salivary components in controlling the equilibrium between de- and remineralization is ably demonstrated when salivary output is compromised and patients suffer dramatic increases in risk for dental caries and/or dental erosion. The sites affected most are those at which salivary protective effects are restricted. Enhanced remineralization of white spot lesions by stimulated salivary flow (e.g. from chewing a sugar-free gum) illustrates the dynamic protective effects of saliva. Protective properties of saliva which increase on stimulation include salivary clearance, buffering power, and degree of saturation with respect to tooth mineral. These benefits are maximized when salivary output is stimulated.[8]

It has been noted in the dental literature that the design of experiments using dental caries or dental erosion models must take into account the static and dynamic effects of saliva.[9] In the context of remineralization, an important component of saliva are its proteins, such as the glycoproteins which adsorb onto tooth structure to form the protective pellicle layer, and the phosphoproteins which regulate calcium saturation of the saliva. Pellicle is known to reduce mineral loss from enamel under conditions of acid challenge, more so for enamel than for dentine.[10,11]

Table 2. Some key proteins which stabilize calcium and phosphate

Saliva
- statherin
- acidic proline-rich proteins
- histatins
Milk
- Alpha and beta caseins
Hard tissues
- Ameloblastin
- Enamelin
- Osteopontin
- Bone sialoprotein
- Dentine sialoprotein

Based on Huq et al. 2005.[89]

Moreover, the early pellicle glycoproteins, acidic proline-rich proteins and statherin are known to promote remineralization of the enamel by attracting calcium ions (Table 2). Acidic proline-rich proteins bind strongly to hydroxyapatite, inhibit crystal growth of calcium phosphate salts from solutions supersaturated with respect to hydroxyapatite, bind calcium ions, and interact with several oral bacteria on adsorption to hydroxyapatite. Statherins, as well as histatins, and cystatins also exhibit affinities to mineral surfaces, and inhibit calcium phosphate precipitation.[12-14]

Some experimental systems such as in situ studies which use enamel slabs embedded into appliances allow full expression of the impacts of saliva, whilst some laboratory bench models exclude the involvement of saliva, and create nonsensical interpretations from the standpoint of clinical practice. This is a problem across parts of the remineralization literature. Despite their advantages, *in vitro* testing protocols using ionic solutions have significant limitations, most particularly related to their inability to simulate the complex biological processes involved.[15,16]

For agents such as Recaldent™ which interact extensively with saliva, it is essential that they are tested in models where human saliva is used, rather than with artificial saliva solutions which have some of the ions of saliva but lack its repertoire of proteins. This issue affects even more recent studies reported in the literature, which as a consequence likely underestimate the true remineralizing actions of this agent.[17-19] It is therefore preferable that *in situ* models are used, with enamel or dentine slabs carried in the mouth and exposed to the normal oral environment. Such models can be used to study fundamental aspects of the demin-remin balance in human subjects without actually causing caries in the natural dentition of those subjects.[20]

It appears that protective effects of salivary components and therapeutic agents act in a cooperative manner. An example would be the similar role played by salivary statherins and by the casein phosphopeptides in Recaldent, both of which regulate the behaviour of calcium and phosphate, and stabilize calcium phosphate compounds.

A key salivary parameter to consider in terms of remineralization is the extent of variations in calcium concentration between resting saliva (where it is low) and stimulated saliva (where it is higher). While phosphate levels in resting saliva do not vary markedly, large fluctuations in calcium concentrations occur in the one individual.[21] A lower calcium concentration results in a lower thermodynamic driving force for hydroxyapatite precipitation at normal oral pH, a higher driving force for hydroxyapatite dissolution at low pH, and a higher critical pH.

Salivary calcium concentrations are lower in children than adults, whereas their phosphate concentrations are not significantly different. The critical pH is significantly higher for children than for adults in both resting and stimulated saliva. Therefore, when compared to adults, children have a greater thermodynamic driving force for demineralization at low oral pH, and a lower force for remineralization at

normal oral pH. This is one contributor to the increased risk of demineralization in children compared with adults.[22]

Differences in calcium concentration have important implications for the critical pH and for the possibility of remineralization, since the latter will not occur when the degree of saturation of saliva with respect to tooth mineral is low.[23] In other words, remineralization may be enhanced merely by providing low levels of bio-available calcium and phosphate ions, in conjunction with minimal amounts of fluoride. Sub-ppm levels of fluoride (<1 ppm) act as a catalyst and influence reaction rates with dissolution and transformation of the various calcium phosphate mineral phases which are within tooth structure and resident within plaque adjacent to tooth surfaces.[24]

Considering further the importance of salivary levels of calcium and phosphate, the critical pH does not have a fixed value but rather is inversely proportional to the calcium and phosphate concentrations in the solution. In other words, under low calcium concentrations, remineralization is a chemical impossibility.[25]

It is now recognized that there are significant individual variations in pH, buffer capacity and salivary concentrations of calcium and phosphate. Within the one patient, variation over time in the concentration of calcium and of phosphate, pH, the hydroxyapatite ion product and the buffer capacity has been reported.[26] These changes impact directly on the likelihood of mineral loss and gain, in terms of both dental erosion and dental caries.[27]

Saliva, enamel, bone, cementum, dentine and milk contain closely related phosphoproteins which bind and stabilize calcium and phosphate, orchestrating the behaviour of these ions in a pH dependant fashion. In fact, statherins in saliva, casein phosphoproteins in Recaldent products, and phosphoproteins in tooth structure share remarkable similarity. When hard tissues are demineralized, the phosphoproteins which remain influence the ability of this tissue to remineralize.[28] We will return later to explore the mechanisms of Recaldent™, but first must revisit the traditional agents which have been used in attempts to promote remineralization.

Mineral or ionic technologies: Fluoride

Fluoride works primarily via topical mechanisms which include (1) inhibition of demineralization at the crystal surfaces inside the tooth, (2) enhancement of remineralization at the crystal surfaces (giving an acid resistant surface to the reformed crystals), and, at high concentrations, (3) inhibition of bacterial enzymes. Low levels of fluoride in saliva and plaque help prevent and reverse caries by inhibiting demineralization and enhancing remineralization. On the other hand, high levels of surface fluoride can increase resistance to carious lesion formation and to dental erosion.[29] Numerous laboratory studies have shown that low levels of fluoride, typical of those found after many hours in resting plaque and saliva, and resulting from the regular use of fluoride dentifrices, can have a profound effect on enamel demineralization and remineralization.[30]

Fluoride present in the oral fluids alters the continuously occurring dissolution and reprecipitation processes at the tooth-oral fluid interface. It is now recognized that demineralization of enamel is inhibited by concentrations of fluoride in the sub-ppm range, and that remineralization of incipient caries lesions is accelerated by trace amounts of fluoride.[31] High concentration fluoride therapies lead to deposition of surface fluoride, typically in the form of calcium fluoride on the surface, with little or no penetration of this material into the enamel.

It must also be remembered that the reactivity of fluoride on sound and carious enamel differs considerably. Carious enamel acquires more fluoride, acquires it more quickly, and itself acts as a source of retained fluoride in comparison with the more limited reactivity of sound enamel.[32]

Surface aggregates or globules of calcium fluoride form on the enamel surface immediately after topical fluoride gel treatments. The minimum concentration of fluoride required for calcium fluoride depend on whether calcium is available from plaque fluid or only through dissolution of enamel. Calcium fluoride then acts as a reservoir of fluoride. The rate of fluoride release is enhanced at lower pH levels and is controlled by phosphate concentra-

tions at high pH levels. More calcium fluoride is formed with increasing fluoride concentration, longer exposure times for topical products, and when fluoride products have low pH.[33]

Calcium fluoride is stable in saliva at neutral pH, owing to surface adsorption of phosphate and formation of a solubility-limiting phase. Extended exposure of saliva may even cause formation of a fluorapatite layer on the calcium fluoride crystals, which would further limit their dissolution. In any event, a pH less than 5 causes loss of the adsorbed phosphate, and triggers a slow dissolution of the calcium fluoride.[34] To increase its surface area, nano-sized particles of calcium fluoride have been prepared. These have a diameter of some 41 nm, with a surface area of some 46.3 square metres/gram. Under laboratory conditions, the high surface area gives greater fluoride release, which could contribute to enhanced remineralization.[35] Note that these particles are many times larger than those in Recaldent (CPP-ACP or CPP-ACFP), where the nanoclusters are some 2 nm in dimension.

Fluoride is subsequently released from globules of calcium fluoride with decreasing pH, as the phosphate groups bound to the surface of the globules are protonated by acids produced by the dental plaque biofilm.[36] While salivary fluoride concentrations are high initially, they then decrease exponentially to very low concentrations within a few hours. In laboratory studies where there is no saliva or plaque present and prolonged contact with remineralizing agents is assured, artificial solutions containing calcium and phosphate, and fluoride (at levels of 1 ppm) can result in mineral gain in natural and laboratory-created white spot carious lesions over a 4 week period.[37] This, however, is not a realistic manner in which to test for the true remineralizing capabilities of a particular agent or formulation.

Beta Tricalcium phosphate (TCP)

Tricalcium phosphate has the chemical formula $Ca_3(PO_4)_2$. It has a 1.5 calcium to phosphate ratio, which is lower than the 1.67 of hydroxyapatite and fluorapatite. TCP exists in two forms, alpha and beta. Alpha TCP is formed when human enamel is heated to high temperatures. It is a relatively insoluble material in aqueous environments (2 mg/100 mL in water).[38,39] Crystalline beta TCP can be formed by combining calcium carbonate and calcium hydrogen phosphate, and heating the mixture to over 1000 degrees Celsius for 1 day, to give a flaky, stiff powder. The average size of the TCP particles can then be adjusted by milling them. Typically, particles range from 0.01 to 5 microns in size. Beta TCP is even less soluble than alpha TCP, and thus in an unmodified form is even more unlikely to provide bioavailable calcium.

Nevertheless, TCP has been studied as a possible addition to enamel matrix protein derivative in treatment of intrabony defects, where it could substitute for natural bone mineral. Beta TCP is used in products such Cerasorb®, Bio-Resorb® and Biovision®.[40] TCP has also been considered as one possible means for enhancing levels of calcium in plaque and saliva. Some small effects on free calcium and phosphate levels in plaque fluid and in saliva have been found when an experimental gum with 2.5% alpha TCP by weight was chewed, when compared to a control gum without added TCP.[41] A major problem with such uses of TCP is the formation of calcium-phosphate complexes, or if fluorides are present, formation of calcium fluoride, which would inhibit remineralization by lowering the levels of bioavailable calcium and fluoride. For this reason, TCP levels would have to be kept very low, in the order of less than 1%.

To address such problems, TCP can be combined with a ceramic such as titanium dioxide, or other metal oxides. This is thought to limit the interaction between calcium and phosphate, and make the material more stable in solution or suspension. An alloy of TCP and a metal oxide can be created by mechanical alloying, during which fracturing and the cold welding of particles occurs. There is some laboratory evidence using demineralized bovine enamel pH cycling models which show increased surface microhardness after treatment with TCP-titania alloys.[42,43]

A further development of the concept is to coat particles of TCP or TCP alloys with sodium lauryl sulphate (SLS) or other surfactants, or with carboxylic acids such as

fumaric acid), or polymers and copolymers could be used. Such organic coatings can be applied by pulverizing the TCP or TCP alloy together with the coating material in a planetary ball mill for several days, a process termed "mechanochemical ball milling".[43,44] The concept is that the organic coating on the TCP "functionalizes" the TCP, and prevents undesirable interactions with fluoride. The organic component subsequently dissolves away when placed in saliva, to leave the particles active. This is the basis for Clinpro Tooth Crème, a fluoride dentifrice which has been introduced recently by 3M ESPE. It is thought that this organically modified TCP technology will operate best at neutral or slightly alkaline pH. As with TCP alloys, there is some laboratory evidence using bovine enamel models which show increased surface microhardness, and fluoride incorporation into the outer layers of the enamel.[44] It is not yet known what effects are achieved in the enamel subsurface, the region of greatest interest in terms of remineralization therapies.

It is also not yet known what the potential is for TCP alloys or organically modified TCP in terms of remineralization. The manufacturer has provided some data on fluoride release, using a method designed to assess fluoride dentifrices. This shows some fluoride release occurs from the dentifrice. The assay is however inappropriate for making any comparisons with topically applied protein-based systems such as GC Tooth Mousse Plus™. Independent assessment of the fluoride and calcium release properties of Clinpro Tooth Crème is necessary to validate the "in house" data, but even more so to use in vivo assessments of the available ion levels in saliva and plaque fluid obtained after realistic exposure times for the various agents. It would be of interest to determine how much of the material is retained rather than expectorated after rinsing the dentifrice from the mouth at the completion of toothbrushing.

The manufacturer's web site[45] claims that Clinpro Tooth Creme "strengthens teeth better than leading brands, reverses white spots better than leading brands, and delivers more fluoride to the tooth than other tested leading brands". While the idea of using TCP as a delivery system for calcium and phosphate ions is certainly interesting from the scientific standpoint, there are as yet no publications in the refereed literature to support the specific claims made regarding remineralization of subsurface lesions or reversal of white spot lesions, since the published data are at this time limited to surface microhardness measurements. Surface fluoride levels bear no relationship to internal remineralization, i.e. remineralization of enamel subsurface lesions, or to regression of white spot lesions. It is well known in the dental literature that high fluoride concentrations deposit surface calcium fluoride but have little effect on subsurface lesions. In fact, low fluoride contents are recognized as being important for mineral crystallite growth, and this is consistent with the molecular and atomic ratios within various apatities. For example, with fluorapatite, the ratio is 10 calcium, 6 phosphate and 2 fluoride ions, such that only 2 of the 42 atoms in the molecule are fluorine atoms. Large fluoride uptakes by tooth enamel are not required for remineralization or for reductions in caries incidence.[46]

Pronamel

Despite its name, Pronamel™ is not considered a remineralizing agent per se, and it does not contain any calcium compounds. It is a relatively new addition to the Sensodyne™ family of fluoride dentifrices and is targeted to help with the problem of dental erosion. It contains 5% potassium nitrate to help relieve tooth sensitivity, has a neutral pH and a low abrasivity, and the detergent sodium lauryl sulfate normally found in dentifricesis absent. The fluoride component is sodium fluoride, giving 0.15% w/v fluoride ion, or 1500 ppm, an increase of 50% above conventional dentifrices.

There are two published studies on this product, both of which are studies of dental erosion conducted in the laboratory setting. In the first, the protective effect of incubation in a toothpaste slurry before acid challenge of human enamel slabs was examined. While pre-incubation did have a protective effect, this did not differ amongst the five brands tested.[47]

The second laboratory study also focussed on dental erosion and compared Pronamel and GC Tooth Mousse. Both were applied for 15 minutes before enamel specimens were exposed to an erosive challenge of 0.2% citric acid for 1 hour. The lack of saliva and moisture in the experimental protocol renders the latter product at a distinct disadvantage and favours a high fluoride toothpaste because of its deposition of calcium fluoride, as discussed previously. Nevertheless, both agents reduced enamel loss and offered a degree of protection from erosion.[48]

Bioactive glass containing calcium sodium phosphosilicate

NovMin™ is a bioactive glass which in aqueous solutions comprises 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5. Of these components, ionic forms of calcium and phosphorus may potentially contribute to remineralization. The manufacturer claims that "When microscopic particles of NovaMin are exposed to water, they release mineral ions that become available for the natural remineralization process. The ions form hydroxyapatite crystals, a form of hard and strong mineral in teeth".

There is some evidence of desensitizing actions of NovaMin as seen in a 6-week clinical trial,[49] and some evidence regarding reductions in plaque index and gingival index,[50] however at the time of writing there is no other published information from refereed journals regarding this material, although a number of unpublished reports are provided by the manufacturer on its web site,[51] which are focussed on its effects as a desensitizing agent.

One of these unpublished studies describes a laboratory study employing enamel slabs and pH cycling which compared two dentifrices, both containing 1100 ppm fluoride, but with the NovaMin test product containing 5% by weight NovaMin bioactive glass particles in place of an equivalent amount of silica abrasive in the control. There was improved performance of the NovaMin product in mineral gain compared with the control.

NovaMin has been incorporated into a number of products, including dentifrices and gels. One of these, Oravive Tooth Revitalizing Paste™, is a dentifrice which is explicitly free of fluoride.[52] It is unclear at present what proportion of the released calcium and phosphate ions are bio-available, and how they interact with fluoride and with salivary components. As a bioactive glass, it is thought that NovaMin particles may adhere to the teeth, but this has yet to be demonstrated formally as a mechanism for sustained release.

Unstabilized calcium and phosphate salts with sodium fluoride

The manufacturer of this product described a "Liquid Calcium" formula which was claimed to deliver fluoride along with soluble calcium and phosphate. The calcium salts were separated from the phosphate salts and sodium fluoride by a plastic divider in the centre of the toothpaste tube. There is a modest evidence base for Enamelon™ with five laboratory studies, three rat caries trials, and four clinical trials. There is evidence of a caries inhibitory action of Enamelon dentifrice in a rat dental caries model.[53]

Clinical studies have indicated that the incidence of root surface caries in radiotherapy patients using Enamelon dentifrice over 12 months was superior to a conventional fluoride dentifrice and was comparable to that of daily use of stannous fluoride gel in trays.[54,55] Some clinical benefits on desensitization of sensitive cervical dentine over an 8-week period have also been reported, compared to a conventional dentifrice containing sodium fluoride without calcium and phosphate.

An inherent technical issue with Enamelon is that calcium and phosphate are not stabilized, allowing the two ions to combine into insoluble precipitates before they come into contact with saliva or enamel. This is unlike Recaldent which has the casein phosphoproteins to stabilize calcium and phosphate.

A further point of interest is that fluoride availability may differ markedly in dentifrices with similar sources/concentrations of fluoride, providing different levels of remineralization of enamel. In other words, a simple assessment of the fluoride level of Enamelon (and other products) may not be a simple way of assessing their benefit as potential remineralizing therapies.[56]

Amorphous calcium phosphate

This material is a macromolecule developed by the American Dental Association Health Foundation at their Paffenbarger facility in Maryland, USA. It is prepared using low temperature methods, and can be modified to create hybrids which contain silica or zirconia.[57]

When applied topically, it is believed that products with ACP can deposit ACP onto the tooth surface or into microscopic surface defects, altering the smoothness and luster of the enamel surface. It is believed that the ACP hydrolyzes under physiological temperatures at a pH of 7.4 to form octacalcium phosphate and an intermediate, and then apatite. If successful, a thin surface coating of hydroxyapatite over the original tooth surface may be achieved. This is purely a surface phenomenon, and fundamentally different from remineralization of enamel subsurface lesions, which requires penetration of ions into the enamel surface. The surface effect explains why ACP has desensitizing actions.[58,59]

It now appears that a number of calcium-containing toothpastes have the ability to occlude or fill in surface defects in tooth enamel, and cause some cosmetic improvements in dimpled, abraded or etched tooth enamel.[60,61] This surface effect would, paradoxically, reduce surface porosity and thus render them incapable of achieving deep penetration of mineral into subsurface defects and white spot lesions.

ACP has been included in prophylaxis pastes and in bleaching gels, with advertising claims made regarding its benefits as a surface polishing agent and as a desensitizer respectively.[62] The stability of ACP in such products is an issue, with single phase ACP systems formulated without water, to keep the ACP reacting to form apatite. This is because ACP is the least stable calcium phosphate compound, and apatites are the most stable. An alternative approach which has been tried is to separate the calcium and phosphate components and mix these during dispensing immediately prior to use, as described above for Enamelon.[63] The challenge here is controlling the process using pH, for example through the bicarbonate and phosphate buffer systems or via dissolved carbon dioxide gas. If the pH is raised above 4.5, stability of calcium and phosphate ions reduces dramatically, and ACP will precipitate. This could be undertaken by mixing acidic calcium solutions with basic phosphate and carbonate solutions using a dual dispensing system.

Dicalcium phosphate dehydrate

This material has been used in some fluoride dentifrices to attempt to enhance on the remineralizing effects of the fluoride component. Examination of plaque fluid indicates that inclusion of DCPD increases the levels of free calcium ions in plaque fluid, which is normally undersaturated with respect to DCPD, thus allowing DCPD to dissolve in the mouth. There is evidence of an elevated calcium level in plaque fluid 12 hours after brushing with such a dentifrice, when compared to conventional silica dentifrices, and of some incorporation of this calcium into the outer enamel.[64]

Other calcium compounds

Because an inverse relationship exists between plaque calcium concentrations and dental caries risk, a range of other calcium compounds have been added to oral care products in an attempt to promote remineralization. In fact, elevated plaque calcium levels have the potential to elevate plaque fluoride levels, a second parameter also linked to reduced dental caries experience.[65]

Calcium peroxide has been added to bleaching gels, whilst dicalcium phosphate, calcium carbonate, calcium chloride, calcium gluconate, calcium glycerophosphate and calcium lactate have been added to dentifrices, gels and chewing gums. Of these, calcium lactate and calcium gluconate are the most soluble in water, with solubilities of 9.3 and 3.0 g/100mL, respectively. The key problems encountered have been the limited bio-availability of calcium, interactions with fluoride compounds in the products, and the poor solubility and palatability of these calcium compounds, since inorganic calcium salts typically taste chalky or astringent.

Similar comments apply to the incorporation of calcium compounds into drinks to reduce their erosive potential. Some calcium salts have been added to erosive drinks to increase calcium levels and reduce surface softening caused by these beverages, but other than by adding CPP-ACP it is not readily possible to gain dramatic increases in calcium levels in the most erosive foods and beverages because of the inherent instability of calcium compounds.

Protein technologies: Recaldent (CPP-ACP nanocomplexes)

Other than fluoride, this is the most extensively researched technology, with more than 45 published studies in the literature, including 18 on the widely known topical tooth crème GC Tooth Mousse/MI Paste™.

This nanotechnology combines specific phosphoproteins from bovine milk with forming nanoparticles of amorphous cal-

cium phosphate (ACP). The precise ratio is 144 calcium ions plus 96 phosphate ions and 6 peptides of CPP. The nano-complexes form over a pH range from 5.0 to 9.0. Under neutral and alkaline conditions, the casein phosphopeptides stabilize calcium and phosphate ions, forming metastable solutions that are supersaturated with respect to the basic calcium phosphate phases. The amount of calcium and phosphate bound by CPP increases as pH rises, reaching the point where the CPP have bound their equivalent weights of calcium and phosphate.

Within milk, the casein phosphopeptides stabilize calcium and phosphate ions through the formation of complexes. The calcium phosphate in these complexes is biologically available for intestinal absorption, and the same concept has now been applied to create materials with bio-available calcium and phosphate in the appropriate form and molecular ratio for remineralization of subsurface lesions in enamel. Clusters of phosphorylated seryl residues are responsible for the interaction which occurs in bovine milk between the caseins and calcium phosphate, and this in turn results in the formation of casein micelles.[66]

Being in essence a protein-mineral rather than a mineral technology is an important factor which puts this technology into a separate class. The ACP forms around the proteins and thus is structurally quite different to the ACP material developed by the American Dental Foundation. Compared to plain, alloyed or organically modified TCP materials, its particles are much smaller and of different composition, and it works effectively at acidic pH levels (down to 4.0) as well as in the neutral and alkaline range.

The casein phosphopeptides (CPP) are produced from a tryptic digest of the milk protein casein, then aggregated with calcium phosphate and purified by ultrafiltration. Under alkaline conditions the calcium phosphate is present as an alkaline amorphous phase complexed by the CPP. This technology was developed by Eric Reynolds and co-workers at the University of Melbourne, and has since been incorporated into chewing gums (such as Recaldent gum™ and Trident White™) and tooth crèmes (GC Tooth Mousse and MI Paste). A formulation with incorporated fluoride to a level of 900 ppm (GC Tooth Mousse Plus™, MI Paste Plus™) is also available. The present author was involved in developing a number of the clinical protocols for using these tooth crèmes in clinical dental practice.[67]

In Recaldent, the casein derived phosphopeptides stabilize the ACP, binding to forming clusters of ACP in metastable solution, preventing their growth to the critical size required for nucleation and precipitation. CPP also localizes the ACP in dental plaque biofilms.[68]

There is extensive clinical as well as laboratory evidence for the effects of this material as a remineralizing agent, as well as a truly anti-cariogenic agent, with the latter being demonstrated in both animal and *in situ* human caries models. The material is pH responsive, with increasing pH increasing the level of bound ACP and stabilizing free calcium and phosphate, so

that spontaneous precipitation of calcium phosphate does not occur. This is also inherently an anti-calculus action.[69]

CPP-ACP provides a highly effective means for elevating calcium levels in dental plaque fluid, something which is desirable for enhancing remineralization, but is difficult to achieve by using calcium in other forms.[70] In fact, in a mouthrinse study which compared CPP-ACP and solutions of calcium phosphate, only the CPP-ACP-containing mouthrinse significantly increased plaque calcium and inorganic phosphate levels.[71]

The delivery of simultaneous calcium, fluoride and phosphate using Recaldent products which include fluoride provides an effective means of controlling the process of fluoride levels in dental plaque. These levels influence the behaviour of bacteria as well as contributing to remineralization.

The anti-caries action of Recaldent involves actions other than suppressing demineralization and enhancing remineralization. There is increasing evidence that Recaldent may influence the properties and behaviour of dental plaque through (1) binding to adhesin molecules on mutans streptococci and thus impairing their incorporation into dental plaque, (2) elevating plaque calcium ion levels to inhibit plaque fermentation; and (3) providing protein and phosphate buffering of plaque fluid pH, to suppresses overgrowth of aciduric species under conditions where fermentable carbohydrate is in excess.

The extent of remineralization seen with Recaldent does not significantly correlate with levels of CPP-bound ACP or the degrees of saturation for hydroxyapatite, octacalcium phosphate, or ACP. Rather, there is a strong correlation between remineralization and the concentration of the neutral ion pair $CaHPO_4$. By stabilizing calcium phosphate in solution, the CPP maintains high-concentration gradients of calcium and phosphate ions and ion pairs into subsurface lesions, an effect which explains the high rates of enamel subsurface remineralization which can be achieved when these products are used in solutions, gums, lozenges and crèmes.[72]

CPP-ACP incorporated into chewing gum, lozenges and mouthrinses has been shown to re-mineralize enamel subsurface lesions in numerous human in situ studies.[73] Enhanced remineralization of enamel subsurface lesions has also been shown when CPP-ACP is added to bovine milk at levels of 2.0 or 5.0 g/liter. At an intake level, 200 mL of milk once daily for each weekday over three consecutive weeks, gains in mineral content of 70 and 148%, respectively occurred, relative to the normal milk control.[74]

Current treatment protocols using Recaldent tooth crèmes[67] recognize the importance of the neutral ion species gaining access to the subsurface lesion through a porous enamel surface. This is the reason why arrested white spot lesions should have a surface etching treatment before remineralization with products such as GC Tooth Mouse or Tooth Mousse Plus. Such a treatment, either alone or combined with gentle pumicing, will remove approximately 30 microns of surface enamel, but will not cause further mineral loss from the subsurface zone of the white spot lesion.[75]

Unlike fluoride treatments with conventional dentifrices (1,000 ppm) which deposit surface mineral but do not eliminate a white spot lesion,[76] Recaldent has been shown to cause regression of lesions, with a large scale 2-year clinical trial with 2720 adolescent subjects demonstrating regression of proximal carious lesions on sequential standardized digital bitewing radiographs. Those chewing the CPP-ACP gum were also less likely to show caries progression of approximal caries relative to a control sugar-free gum.[77]

CPP-ACFP nanocomplexes

Casein phosphopeptides containing the cluster sequence-Ser(P)-Ser(P)-Ser(P)-Glu-Glu- bind fluoride as well as calcium and phosphate, and thus can also stabilize calcium fluoride phosphate as soluble complexes. These complexes are designated CPP-ACFP. Studies of such nano-complexes based on the casein alpha-S1 peptide fragment 59-79 have revealed a particle size of some 2 nm and stoichiometry of one peptide to 15 calcium, 9 phosphate and 3 fluoride ions.[78]

Clinical studies of mouthrinses and dentifrices containing CPP-ACP and fluoride have provided interesting insights into the synergy between these. For example, addition of CPP-ACP to a fluoride mouthrinse increases the incorporation of fluoride into dental plaque biofilm. A dentifrice containing CPP-ACP with fluoride provides remineralization which is superior to both CPP-ACP alone and to conventional and high fluoride dentifrices.[79] This synergy between CPP-ACP and fluoride had been identified in laboratory studies using GC Tooth Mousse, which showed that Tooth Mousse (without fluoride) remineralized initial enamel lesions better when applied as a topical coating after the use of a fluoride dentifrice.[80] In the absence of such "environmental" fluoride, the predominant mineral that will be formed in enamel subsurface lesions during remineralization with CPP-ACP will be hydroxyapatite.

It is now known that CPP can stabilize high concentrations of calcium, phosphate and fluoride ions at all pH values from 4.5 up to 7.0, and is able to remineralize enamel subsurface lesions observed at all pH values in this range, with a maximal effect at pH 5.5.[81] In fact, at pH values below 5.5, CPP-ACFP produces greater remineralization than CPP-ACP, and the major product formed when remineralization is undertaken with CPP-ACFP is fluorapatite, which is highly resistant to acid dissolution. In either event it appears that mineral formation is optimized, since acid challenge of lesions after remineralization with CPP-ACP or CPP-ACFP gives demineralization underneath the remineralized zone, indicating that the remineralized mineral was more resistant to subsequent acid challenge.[82]

Table 3. Summary of current technology as at February 2009

Material	Publications	Level of evidence
ClinPro Tooth Crème™	1	Preliminary
Novamin™	1	Preliminary
DCPD	2	Preliminary
Pronamel™	2	Preliminary
Enamelon™	3	Preliminary
ACP	4	Preliminary
Various Ca compounds	10	Preliminary
CPP-ACP/ACFP	45	Large RCTs
Fluoride	>5000	Systematic reviews

The publications column refers to the number of relevant MEDLINE listed refereed journal papers relating to the technology. The highest levels of evidence are randomized controlled clinical trials (RCTs) and systematic reviews of such trials. See reference 90 for a discussion of levels of evidence.

Remineralization of dentine

While this paper has focussed on remineralization of enamel, it is noteworthy that interest is increasing in treatments which can remineralize carious or eroded dentine. The presence of phosphoproteins in the normal protein composition of dentine, its more complex structure and greater water content make dentine a rather more challenging substrate to control for systematic scientific study. A particular problem is that some laboratory studies omit saliva and thus remove the important contributions of pellicle and of salivary phosphoproteins such as statherin to the process.[83] This makes data gained by studying the application of simple solutions of calcium and phosphate compounds onto dentine slabs impossible to apply into the clinical setting.

Recent work has shown that fluorapatite, rather than calcium fluoride, is formed within dentine by application of neutral sodium fluoride gels followed immediately by laser treatment, a process now termed "photonic conversion".[84-86] It has also been shown that CPP-ACP (GC Tooth Mousse) can arrest incipient root surface caries lesions and can have a hardening effect, illustrating once again the value of such approaches in patient care.[87]

Conclusions

Looking at the evidence base available at the time of writing (February 2009), it is clear that, other than for fluoride, the strongest level of clinical evidence for remineralization is for the casein phosphopeptide-based Recaldent technology, with more than 40 relevant publications and long term large scale clinical trials to support its efficacy. This technology fulfils the characteristics of an ideal novel remineralizing agent identified by Zero in 2006.[88] This is perhaps not surprising given its ontogeny, particularly its similarity to other proteins which stabilize calcium and phosphate in body fluids (Table 2).[89]

The evidence base for other novel methods (summarized in Table 3) can perhaps best be summarized as "preliminary", since at this time they are interesting from the scientific standpoint but have little in the way of laboratory, human in situ, or clinical trial data to support their use, and certainly cannot be promoted as being equal or superior to either fluoride or Recaldent. It is important for dental professionals to be aware that it takes significant time to establish the bona fides of a new technology,[90] and that a "watching brief" is necessary in this rapidly professing area of dental science.

About the author

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