Contemporary technologies for remineralization therapies: A review

Laurence J. Walsh

In the last decade there has been a veritable explosion of interest in technologies which may have value for remineralization of enamel and dentine, or for desensitization of exposed dentine affected by dental erosion.[1] 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

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.[2] Fluorapatite is less acid soluble than hydroxyapatite, which in turn is less soluble than carbonated apatites.[3,4] Because of this chemical inhomogeneity of enamel, the process of enamel remineralization is rather complex. While a ratio of 10 calcium ions to 6 phosphate ions to 2 fluoride or hydroxyl ions (or one carbonate ion) appears suitable, there is evidence which supports 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.[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] 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.[7]

Laurence J. Walsh, BDSc, PhD, DDSc, FFOP(RCPA)
School of Dentistry, The University of Queensland
Brisbane, Australia

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. Enhanced remineralization of white spot lesions by stimulated salivary flow (e.g. from chewing a sugar-free gum) illustrates 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.[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]

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

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]

laboratory bench models exclude the involvement of saliva, and create nonsensical interpretations from the standpoint of clinical practice. Laboratory testing protocols using ionic solutions have significant limitations, most particularly related to their inability to simulate the complex biological processes involved.[15,16]

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.

For Recaldent™ and other agents 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 lack a complete repertoire of proteins, since studies which exclude salivary proteins will underestimate the true remineralizing actions of this agent.[17-19] It is 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 explore the demin-remin balance in human subjects without actually causing caries in the natural dentition of those subjects.[20]

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.[21,22]

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 by providing low levels of bio-available calcium and phosphate ions, in conjunction with minimal amounts of fluoride (<1 ppm).[24] Conversely, under low calcium concentrations, remineralization is a chemical impossibility.[25] There are significant inter-individual and time-related variations in pH, buffer capacity, and salivary concentrations of calcium and phosphate.[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]

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. Remineralization of incipient

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]

caries lesions is accelerated by trace amounts of fluoride.[31,32] High concentration fluoride therapies lead to deposition of aggregates of calcium fluoride on the surface, which then acts as a reservoir of fluoride. The rate of fluoride release is enhanced at lower pH levels.[33] A pH less than 5 causes loss of adsorbed phosphate, and triggers a slow dissolution of the calcium fluoride.[34,36] To increase its surface area, nano-sized particles of calcium fluoride have been prepared, with a diameter of some 41nm.[35] Such particles are many times larger than those in Recaldent™ (CPP-ACP or CPP-ACFP), where the nanoclusters are only 2 nm in diameter.

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 $Ca3(PO_4)_2$, and 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 (2mg/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 less soluble than alpha TCP, and thus in an unmodified form is less likely to provide bio-available calcium. It 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 have to be kept very low, in the order of less than 1%. Alternatively, TCP can be combined with a ceramic such as titanium dioxide, or other metal oxides, to limit the interaction between calcium and phosphate, and make the material more stable in solution or suspension.[42,43]

Particles of TCP or TCP alloys can be coated with sodium lauryl sulphate (SLS) or other surfactants, or with carboxylic acids (such as fumaric acid), polymers and copolymers, by pulverizing the TCP or TCP alloy together with the coating material a planetary ball mill for several days.[43] It has been suggested that the organic coating prevents undesirable interactions with fluoride, but may dissolves away when particles contact saliva. This is the basis for the 3M Espe ClinPro™ fluoride dentifrices. According to the manufacturer, this organically modified TCP technology should operate best as a remineralizing agent at neutral or slightly alkaline pH. 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, or whether any subsurface remineralization occurs. The manufacturer has provided some data on fluoride release, using the FDA method designed to assess fluoride dentifrices. The assay is however inappropriate for making any comparisons with topically applied protein-based systems such as GC MI Paste Plus™ (Tooth Mousse Plus). An independent assessment of the soluble fluoride, phosphate and calcium release properties of ClinPro Tooth Crème, GC MI Paste Plus/Tooth Mousse Plus, and 19 other commercially available topically delivered dental products with added calcium was presented at the September 2009 IADR Pan Asian Pacific Federation meeting.[45] Calcium in all 21 the products was found to have low water solubility except for MI Paste

Table 3
Summary of current technology as at February 2009

Material	Publication	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	Systematic reviews
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 clinical trials. See reference 90 for a discussion of levels of evidence.

Plus, which contained 321.8 ± 2.6 μmol water soluble calcium per gram of crème, a level which was 14 times greater than that of ClinPro Tooth Crème and other products. MI Paste Plus also contained the highest amount of water soluble phosphate (245.7 ± 2.7 μmol/g). The high water solubility of the calcium, phosphate and fluoride in MI Paste Plus was attributed to the presence of the casein phosphopeptides.

This recent work reinforces the point that bioavailable calcium is the key limiting factor in remineralization, not fluoride. This is logical given the molecular and atomic ratios within various apatities. For example, with fluorapatite, the ratio is 10 calcium, 6 phosphate and 2 fluoride ions, of the 42 atoms in the molecule. 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 lacks the detergent sodium lauryl sulfate formally found in dentifrices. 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 Proenamel™ and GC MI Paste/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]

NovaMin™

NovMin™ is a bioactive glass containing calcium sodium phosphosilicate, and comprises 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5. 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] Recent data for bioavailable calcium and phosphate suggest only a low bioavailability from NovaMin.[45]

Enamelon™

Enamelon consists of unstabilized calcium and phosphate salts with sodium fluoride. The calcium salts are 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 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 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.

The manufacturer of this product claims that its "Liquid Calcium" formula delivers fluoride along with soluble calcium and phosphate. A simple assessment of the fluoride level of Enamelon (and other products) may correlate with their remineralizing actions because of the limiting factor of calcium.[56]

Amorphous calcium phosphate (ACP)

This macromolecule was developed by the American Dental Association Health Foundation. 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 thought that ACP hydrolyzes under physiological temperatures at a pH of 7.4 to form octacalcium phosphate and an intermediate, and then surface apatite. If this did occur, it would not constitute remineralization of enamel subsurface (white spot) lesions, since these require penetration of calcium and other ions into the subsurface. The surface actions of ACP would, paradoxically, reduce surface porosity and thus render such sites less likely to undergo subsurface reminerlization.

The predominantly surface action of ACP does however explain its desensitizing effects,[58,59] and how it can fill in surface defects in tooth enamel, and cause cosmetic improvements in dimpled, abraded or etched tooth enamel.[60,61] For these reasons, ACP has been included in prophylaxis pastes and in bleaching gels. It would however be incorrect to attribute remineralizing claims to this material. One ACP-containing material, Discus Dental NiteWhite ™ claims to "rebuild tooth enamel, making teeth stronger and less susceptible to caries."[62] There is no published evidence in the current dental literature to support claims of subsurface remineralization or reversal of white spot lesions.

The stability of ACP in dental products is an issue. Single phase ACP systems are formulated without water, to keep the ACP from reacting to form apatite. An alternative approach is to separate the calcium and phosphate components, and mix these during dispensing immediately prior to use, using a dual dispensing system, similar to that described for Enamelon™.[63]

Dicalcium phosphate dehydrate (DCPD)

This material has been used in some fluoride dentifrices to attempt to enhance on the remineralizing effects of the fluoride component. Inclusion of DCPD in a dentifrice increases the levels of free calcium ions in plaque fluid, and these remain elevated for up to 12 hours after brushing, when compared to conventional silica dentifrices.[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.[65] Unfortunately, with the exception of Recaldent™ technology, other approaches have not been particularly successful at delivering water-soluble bio-available calcium.[1, 45] A further problem is that adding calcium compounds directly into gels, dentifrices, and chewing gums causes unfavorable interactions with fluoride compounds in the same products, and reduces the palatability of these dental products because inorganic calcium salts 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 Recaldent,[1] it is not readily possible to gain dramatic increases in calcium levels in the most erosive foods and beverages.

Recaldent (CPP-ACP nanocomplexes): a protein technology

Other than fluoride, this is the most extensively researched remineralization technology, with more than 50 published studies in the dental literature, including 20 on the widely known topical tooth crème GC Tooth Mousse™/MI Paste™, with a number of large scale randomized controlled clinical trials and several systematic reviews published over the past 2 years.

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™).

This protein nanotechnology combines specific phosphoproteins from bovine milk with forming nanoparticles of amorphous calcium phosphate (ACP). The precise ratio is 144 calcium ions plus 96 phosphate ions and 6 peptides of CPP.

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. 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.

Recaldent works effectively as a remineralizing agent at acidic pH levels (down to 4.0) as well as in the neutral and alkaline range.[1,66] The present author was involved in developing a number of the clinical protocols for using these tooth crèmes in clinical dental practice, for treating white spot lesions, fluorosis, orthodontic decalcification, enamel dysmineralization, and sensitive dentine.[67] Current work is exploring how Recaldent can be used to modify dental plaque ecology, given that CPP bind to certain plaque bacteria and also localize ACP within dental plaque biofilms.[68]

There is extensive clinical as well as laboratory evidence for the effects of Recaldent 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 provides 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 maintain 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 such as MI Paste and Tooth Mousse[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 Recaldent products. 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 MI paste/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 was 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]

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 MI Paste/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, 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 both long term large scale clinical trials and randomized controlled 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.

References

1. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J. 2008;53(3):268-73.
2. Elliott JC. Structure, crystal chemistry and density of enamel apatites. Ciba Found Symp. 1997;205:54-67.
3. Shellis RP, Wahab FK, Heywood BR. The hydroxyapatite ion activity product in acid solutions equilibrated with human enamel at 37 degrees. Caries Res. 1993;27(5):365-72.
4. Shellis RP, Wilson RM. Apparent solubility distributions of hydroxyapatite and enamel apatite. J Colloid Interface Sci. 2004;278(2):325-32.
5. Moreno EC, Aoba T. Comparative solubility study of human dental enamel, dentin, and hydroxyapatite. Calcif Tissue Int. 1991;49(1):6-13.
6. Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J. 2008;53(3):286-91.
7. Tanaka M, Matsunaga K, Kadoma Y. Correlation in inorganic ion concentration between saliva and plaque fluid. J Med Dent Sci. 2000r;47(1):55-9.
8. Edgar WM, Higham SM, Manning RH. Saliva stimulation and caries prevention. Adv Dent Res. 1994;8(2):239-45.
9. Edgar WM, Higham SM. Role of saliva in caries models. Adv Dent Res. 1995;9(3):235-8.
10. Hannig M, Fiebiger M, Güntzer M, Döbert A, Zimehl R, Nekrashevych Y. Protective effect of the in situ formed short-term salivary pellicle.Arch Oral Biol. 2004;49(11):903-10.
11. Wiegand A, Bliggenstorfer S, Magalhaes AC, Sener B, Attin T. Impact of the in situ formed salivary pellicle on enamel and dentine erosion induced by different acids. Acta Odontol Scand. 2008;66(4):225-30.
12. Lamkin MS, Oppenheim FG. Structural features of salivary function.Crit Rev Oral Biol Med. 1993;4(3-4):251-9.
13. Lamkin MS, Arancillo AA, Oppenheim FG. Temporal and compositional characteristics of salivary protein adsorption to hydroxyapatite. J Dent Res. 1996;75(2):803-8.
14. Van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Res. 2004;38(3):247-53.
15. White DJ. The application of in vitro models to research on demineralization and remineralization of the teeth. Adv Dent Res. 1995;9(3):175-93.
16. Roberts AJ. Role of models in assessing new agents for caries prevention--non-fluoride systems. Adv Dent Res. 1995;9(3):304-11.
17. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. J Oral Sci. 2007;49(2):115-20.
18. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent. 2008;36(1):74-9.
19. Giulio AB, Matteo Z, Serena IP, Silvia M, Luigi C. In vitro evaluation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) effect on stripped enamel surfaces. A SEM investigation. J Dent. 2009;37(3):228-32.
20. Zero DT. In situ caries models. Adv Dent Res. 1995;9(3):214-30.
21. Kavanagh DA, Svehla G. Variation of salivary calcium, phosphate and buffering capacity in adolescents.Arch Oral Biol. 1998;43(12):1023-7.
22. Anderson P, Hector MP, Rampersad MA. Critical pH in resting and stimulated whole saliva in groups of children and adults. Int J Paediatr Dent. 2001;11(4):266-73.
23. Aiuchi H, Kitasako Y, Fukuda Y, Nakashima S, Burrow MF, Tagami J.Relationship between quantitative assessments of salivary buffering capacity and ion activity product for hydroxyapatite in relation to cariogenic potential. Aust Dent J. 2008;53(2):167-71.
24. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent. 2004;28(3):203-14.
25. Dawes C. What is the critical pH and why does a tooth dissolve in acid? J Can Dent Assoc. 2003;69(11):722-4.
26. Larsen MJ, Jensen AF, Madsen DM, Pearce EI. Individual variations of pH, buffer capacity, and concentrations of calcium and phosphate in unstimulated whole saliva. Arch Oral Biol. 1999;44(2):111-7.
27. Zero DT, Lussi A. Erosion - chemical and biological factors of importance to the dental practitioner. Int Dent J. 2005;55(4 Suppl 1):285-90.
28. Clarkson BH, Chang SR, Holland GR. Phosphoprotein analysis of sequential extracts of human dentin and the determination of the subsequent remineralization potential of these dentin matrices. Caries Res. 1998;32(5):357-64.
29. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol. 1999;27(1):31-40.
30. Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int Dent J. 2004;54(5 Suppl 1):304-9.
31. ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med. 1991;2(3):283-96.
32. White DJ, Nancollas GH. Physical and chemical considerations of the role of firmly and loosely bound fluoride in caries prevention. J Dent Res. 1990;69 Spec No:587-94.
33. Takagi S, Liao H, Chow LC. Effect of tooth-bound fluoride on enamel demineralization/ remineralization in vitro. Caries Res. 2000;34(4):281-8.
34. Rølla G. On the role of calcium fluoride in the cariostatic mechanism of fluoride. Acta Odontol Scand. 1988;46(6):341-5.
35. Sun L, Chow LC. Preparation and properties of nano-sized calcium fluoride for dental applications.Dent Mater. 2008;24(1):111-6.
36. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention.Eur J Oral Sci. 1997t;105(5 Pt 2):461-5.
37. Iijima Y, Takagi O, Ruben J, Arends J. In vitro remineralization of in vivo and in vitro formed enamel lesions. Caries Res. 1999;33(3):206-13.
38. Aminzadeh A, Shahabi S, Walsh LJ. Raman spectroscopic studies of CO2 laser-irradiated human dental enamel. Spectrochim Acta A Mol Biomol Spectrosc. 1999;55A(6):1303-8.
39. Feuerstein O, Mayer I, Deutsch D. Physico-chemical changes of human enamel irradiated with ArF excimer laser. Lasers Surg Med. 2005;37(3):245-51.
40. Döri F, Arweiler N, Gera I, Sculean A. Clinical evaluation of an enamel matrix protein derivative combined with either a natural bone mineral or beta-tricalcium phosphate. J Periodontol. 2005;76(12):2236-43.
41. Vogel GL, Zhang Z, Carey CM, Ly A, Chow LC, Proskin HM. Composition of plaque and saliva following a sucrose challenge and use of an alpha-tricalcium-phosphate-containing chewing gum. J Dent Res. 1998;77(3):518-24.
42. Karlinsey RL. US Provisional patent 20070178220. Materials and methods for manufacturing amorphous tricalcium phosphate and metal oxide alloys of amorphous tricalcium phosphate and methods of using the same
43. Karlinsey RL, Mackey AC. Solid-state preparation and dental application of an organically modified calcium phosphate. J Material Sci

2009; 44(1):346-349.

44. Karlinsey RL. US Provisional patent 20080187500. Hybrid organic/inorganic chemical hybrid systems, including functionalized calcium phosphate hybrid systems, and a solid-state method for producing the same.

45. Cai F, Yuan Y, Reynolds C, Reynolds EC. Water soluble calcium, phosphate and fluoride of various dental products. 2nd Meeting of IADR Pan Asian Pacific Federation (PAPF) and the 1st Meeting of IADR Asia/Pacific Region (APR) (Sept. 22-24, 2009). J Dent Res. 2009; 88(Spec Iss B): 57. www.dentalresearch.org.

46. Ingram GS, Agalamanyi EA, Higham SM. Caries and fluoride processes. J Dent. 2005;33(3):187-91.

47. Lussi A, Megert B, Eggenberger D, Jaeggi T. Impact of different toothpastes on the prevention of erosion. Caries Res. 2008;42(1):62-7.

48. Rees J, Loyn T, Chadwick B. Pronamel and tooth mousse: an initial assessment of erosion prevention in vitro. J Dent. 2007;35(4):355-7.

49. Du Min Q, Bian Z, Jiang H, Greenspan DC, Burwell AK, Zhong J, Tai BJ. Clinical evaluation of a dentifrice containing calcium sodium phosphosilicate (novamin) for the treatment of dentin hypersensitivity. Am J Dent. 2008;21(4):210-4.

50. Tai BJ, Bian Z, Jiang H, Greenspan DC, Zhong J, Clark AE, Du MQ. Anti-gingivitis effect of a dentifrice containing bioactive glass (NovaMin) particulate. J Clin Periodontol. 2006;33(2):86-91.

51. http://www.novamin.com/advanced-science.html

52. http://www.oravive.com/oravive_revitalizing_paste.php

53. Thompson A, Grant LP, Tanzer JM. Model for assessment of carious lesion remineralization, and remineralization by a novel toothpaste. J Clin Dent. 1999;10(1 Spec No):34-9.

54. Papas A, Russell D, Singh M, Stack K, Kent R, Triol C, Winston A. Double blind clinical trial of a remineralizing dentifrice in the prevention of caries in a radiation therapy population. Gerodontology. 1999;16(1):2-10.

55. Papas A, Russell D, Singh M, Kent R, Triol C, Winston A. Caries clinical trial of a remineralising toothpaste in radiation patients. Gerodontology. 2008;25(2):76-88.

56. Hara AT, Kelly SA, González-Cabezas C, Eckert GJ, Barlow AP, Mason SC, Zero DT. Influence of fluoride availability of dentifrices on eroded enamel remineralization in situ. Caries Res. 2009;43(1):57-63.

57. Skrtic D, Antonucci JM, Eanes ED, Brunworth RT. Silica- and zirconia-hybridized amorphous calcium phosphate: effect on transformation to hydroxyapatite. J Biomed Mater Res. 2002;59(4):597-604.

58. Tung MS, Eichmiller FC. Dental applications of amorphous calcium phosphates. J Clin Dent. 1999;10(1 Spec No):1-6.

59. Tung MS, Eichmiller FC. Amorphous calcium phosphates for tooth remineralization. Compendium Contin Educ Dent 2004; 25(9) (Suppl 1): 9-13.

60. Charig A, Winston A, Flickinger M. Enamel mineralization by calcium-containing-bicarbonate toothpastes: assessment by various techniques. Compend Contin Educ Dent. 2004;25(9 Suppl 1):14-24.

61. Litkowski LJ, Quinlan KB, Ross DR, Ghassemi A, Winston A, Charig A, Flickinger M, Vorwerk L. Intraoral evaluation of mineralization of cosmetic defects by a toothpaste containing calcium, fluoride, and sodium bicarbonate. Compend Contin Educ Dent. 2004;25(9 Suppl 1):25-31.

62. http://www.discusdental.com/products/whitening/nite_white_landing.php

63. Schemehorn BR, Orban JC, Wood GD, Fischer GM, Winston AE. Remineralization by fluoride enhanced with calcium and phosphate ingredients. J Clin Dent. 1999;10(1 Spec No):13-6.

64. Sullivan RJ, Charig A, Blake-Haskins J, Zhang YP, Miller SM, Strannick M, Gaffar A, Margolis HC. In vivo detection of calcium from dicalcium phosphate dihydrate dentifrices in demineralized human enamel and plaque. Adv Dent Res. 1997;11(4):380-7.

65. Lynch RJ, ten Cate JM. The anti-caries efficacy of calcium carbonate-based fluoride toothpastes. Int Dent J. 2005;55(3 Suppl 1):175-8.

66. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. J Biol Chem. 2005;280(15):15362-9.

67. Walsh LJ. Tooth Mouse: anthology of applications. 2007, Singapore: GC Asia Pte Ltd.

68. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptides in oral health - chemistry and clinical applications. Curr Pharm Des. 2007;13(8):793-800.

69. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist. 1998;18(1):8-16.

70. Magalhães AC, Furlani Tde A, Italiani Fde M, Iano FG, Delbem AC, Buzalaf MA. Effect of calcium pre-rinse and fluoride dentifrice on remineralisation of artificially demineralised enamel and on the composition of the dental biofilm formed in situ. Arch Oral Biol. 2007;52(12):1155-60.

71. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res. 2003;82(3):206-11.

72. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res. 1997;76(9):1587-95.

73. Manton DJ, Walker GD, Cai F, Cochrane NJ, Shen P, Reynolds EC. Remineralization of enamel subsurface lesions in situ by the use of three commercially available sugar-free gums. Int J Paediatr Dent. 2008;18(4):284-90.

74. Walker G, Cai F, Shen P, Reynolds C, Ward B, Fone C, Honda S, Koganei M, Oda M, Reynolds E. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. J Dairy Res. 2006;73(1):74-8.

75. Peariasamy K, Anderson P, Brook AH. A quantitative study of the effect of pumicing and etching on the remineralisation of enamel opacities. Int J Paediatr Dent. 2001;11(3):193-200.

76. Al-Khateeb S, Exterkate R, Angmar-Månsson B, ten Cate JM. Effect of acid-etching on remineralization of enamel white spot lesions. Acta Odontol Scand. 2000;58(1):31-6.

77. Morgan MV, Adams GG, Bailey DL, Tsao CE, Fischman SL, Reynolds EC. The anticariogenic effect of sugar-free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiography. Caries Res. 2008;42(3):171-84.

78. Cross KJ, Huq NL, Stanton DP, Sum M, Reynolds EC. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle-alpha(S1)-casein(59-79) by stabilized amorphous calcium fluoride phosphate nanocomplexes. Biomaterials. 2004;25(20):5061-9.

79. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008 ;87(4):344-8.

80. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Aust Dent J. 2008;53(1):34-40.

81. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res. 2008;42(2):88-97.

82. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res. 2004;38(6):551-6.

83. Hara AT, Karlinsey RL, Zero DT. Dentine remineralization by simulated saliva formulations with different Ca and Pi contents. Caries Res. 2008;42(1):51-6.

84. Vlacic J, Meyers IA, Walsh LJ. Laser-activated fluoride treatment of enamel as prevention against erosion. Aust Dent J. 2007;52(3):175-80.

85. Vlacic J, Meyers IA, Kim J, Walsh LJ. Laser-activated fluoride treatment of enamel against an artificial caries challenge: comparison of five wavelengths. Aust Dent J. 2007;52(2):101-5

86. Vlacic J, Meyers IA, Walsh LJ. Photonic conversion of hydroxyapapite to fluorapatite: a possible mechanism for laser-activated fluoride therapy. J Oral Laser Appl. 2008;8(2): 95-102.

87. Vlacic J, Meyers IA, Walsh LJ. Combined CPP-ACP and photoactivated disinfection (PAD) therapy in arresting root surface caries: a case report. Br Dent J. 2007;203(8):457-9.

88. Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health. 2006;6 (Suppl 1):S9-S22.

89. Huq L, Cross KJ, Ung M, Reynolds EC. A review of protein structure and gene organization for proteins associated with mineralised tissue and calcium phosphate stabilization encoded on human chromosome 4. Arch Oral Biol. 2005; 50:599-609.

90. Walsh LJ. Evidence-based practice: here to stay. Australas Dent Pract. 2009; 40(2): 146-152.